Bioactive agents from marine mussels and their effects on human health

Ulrike Grienke[a], Joe Silke[b], Deniz Tasdemir[a]*

[a] School of Chemistry, National University of Ireland, Galway (NUIG), University Road, Galway, Ireland

[b] Marine Institute, Rinville, Oranmore, Co. Galway, Ireland

* To whom the correspondence should be addressed. Phone: +353-91-492450. Fax: +353-91-495576. E-mail: deniz.tasdemir@nuigalway.ie

Abstract

The consumption of marine mussels as popular seafood has increased steadily over the past decades. Awareness of mussel derived molecules that promote health has contributed to extensive research efforts in that field. This review highlights the bioactive potential of mussel components from species of the genus *Mytilus* (*e.g. M. edulis*) and *Perna* (*e.g. P. canaliculus*). In particular, bioactivity related to three major chemical classes of mussel primary metabolites, *i.e.* proteins, lipids, and carbohydrates, is evaluated. Within the group of proteins the focus is mainly on mussel peptides *e.g.* those obtained by bio-transformation processes such as fermentation. In addition, mussel lipids, comprising polyunsaturated fatty acids (PUFAs), are discussed as agents well-known for the prevention and treatment of rheumatoid arthritis (RA). Within the third group of carbohydrates, mussel polysaccharides are investigated. Furthermore, the importance of monitoring the mussel as food material in respect to contaminations with natural toxins produced by microalgae is discussed.

Keywords

Abbreviations

AA, amino acid; AMP, antimicrobial peptide; ASP, amnesic shellfish poisoning; AZP, azaspiracid shellfish poisoning; CFP, ciguatera fish poisoning; COX, cyclooxigenase; DHA, docosapentaenoic acid; DSP, diarrhetic shellfish poisoning; EPA, eicosapentaenoic acid; HAB, harmful algal bloom; LO, lipoxygenase; NSP, neurotoxic shellfish poisoning; PSP, paralytic shellfish poisoning; PUFA, polyunsaturated fatty acid

1.   Introduction

It is predicted that, approximately 2,210,000 species exist in the ocean, from which only around 190,000 species have been catalogued so far (Mora et al., 2011). The phylum Mollusca represents one of the largest and most diverse groups of marine animals. The Bivalvia, a large class with around 20,000 species (Chapman, 2009) within Mollusca, includes some of the best known invertebrates such as clams, oysters, scallops, and mussels and is represented at all depths and in all marine environments. Molluscan shells, including those from bivalves, have been used as tools, containers, fetishes, and decorations since ancient times. Large populations, particularly those living in coastal areas, *e.g.* aboriginal groups, have relied on these animals for a substantial portion of their diet (Brusca & Brusca, 1990). Apart from the New Zealand green-lipped mussel, few reports available in the public domain deal with the traditional use of mussels against diseases. The sauce from a decoction of *M. edulis*, for instance, is traditionally used in China for its immune strengthening properties and to treat liver and kidney dysfunctions as well as impotence and menoxenia (Li & Ding, 2006). Nowadays, molluscan shellfish including bivalves are harvested commercially and are of considerable relevance for aquaculture industries worldwide. Farmed marine mussels from the *Mytilidae* family, comprising genera such as *Mytilus* and *Perna*, are popular in human diet, providing high levels of proteins, omega-3 polyunsaturated fatty acids (PUFAs), iodine, and carbohydrates.

Considering the close relation between food and health, bioactive mussel components have proven to play a vital role for the development of functional foods, defined as food with specific beneficial health effect beyond simple nutrition, or nutraceuticals, describing a union between nutrition and pharmaceutics (Bernal et al., 2011; Haller, 2010; Lordan, Ross, & Stanton, 2011). Moreover, relatively high volumes of mussel wastes in aquaculture and processing, prompted researchers to focus on this underexplored source for bioactives (Harnedy & FitzGerald, 2012; Kim & Mendis, 2006). Over the past decades, bioactive properties of mussel components have been investigated by many researchers and several dietary supplements containing mussel extracts have been brought to the market. For example Lyprinol[®], a dietary supplement product containing the lipid extract of the green-lipped mussel, *Perna canaliculus*, is sold almost worldwide as anti-

inflammatory and anti-arthritic remedy. Hence, the importance of marine mussels as source for bioactive substances such as *e.g.* antimicrobial, anti-inflammatory as well as anti-cancer agents is increasing rapidly. In this review article, we focus on mussel primary metabolites comprising peptides, lipids, and carbohydrates considering their bioactive properties as well as different classes of shellfish toxins and their impact on human health.

1.1.   Literature search and data evaluation concerning mussel bioactives

This review covers literature up to January 2013 and is based on the combination of surveys in three scientific databases, *i.e.* SciFinder Scholar (Chemical Abstracts Service - http://www.cas.org/products/sfacad/index.html), ISI Web of Knowledge (Thomson Reuters - http://www.webofknowledge.com), and Scopus (Reed Elsevier - http://www.scopus.com). The two most abundant mussel genera, *i.e. Mytilus* and *Perna,* were applied as key words and the retrieved references were further refined focusing on reported bioactivity. Figure 1 gives an overview on the number of publications dealing with mussel bioactives corresponding to three major primary metabolite classes, *i.e.* proteins/peptides/amino acids, lipids, and carbohydrates, as well as miscellaneous metabolites. Furthermore, selected publications were evaluated according to the type of bioactivity in relation to metabolite classes, revealing that most studies deal with antimicrobial mussel peptides or anti-inflammatory mussel lipids (Figure 2).

Insert Figure 1 here.

Insert Figure 2 here.

1.2.   Marine mussel species of interest: Morphology, geographical distribution, and habitat

Marketed worldwide as live, frozen or processed seafood, marine mussels are native to both, northern and southern hemispheres. The mussel industry is split into two production techniques, *i.e.* bottom mussels, naturally grown on the seabed and harvested by specialised dredging equipment, and rope mussels, cultivated on rope structures in aquaculture (Gosling, 1992). In their natural

environment mussels have to adapt to parameters such as salinity, wave exposure, substrate, zone, height, temperature, and water quality. Most species tolerate a wide range of salinity. However, at very low salinities the mussel growth is limited, which leads to smaller sizes (Almada-Villela, 1984). Mussels, occurring in low and mid intertidal areas, prefer sheltered places where individuals are usually attached to hard surfaces such as rocky substrates. In order to adhere to boulders, cobbles, or pebbles they use their byssal threads which are proteinaceous silk-like fibres also known as the mussel's beard (Lee et al., 2011). The most limiting parameter for the distribution of marine mussels is the temperature, as some species prefer colder while some prefer warmer waters. Furthermore, the content of active metabolites varies with season, life cycle, and habitat (Freites, Fernandez-Reiriz, & Labarta, 2002).

Commercially most relevant marine mussel species belong to the two genera of *Mytilus* and *Perna*. *Mytilus* species occur in temperate waters of Europe, Asia, and America, whereas *Perna* species are cultured in warmer waters such as Thailand, the Philippines, China, and New Zealand (Gosling, 1992). Within the genus *Mytilus*, the marine mollusc *M. edulis*, is commonly known as blue or black mussel (Figure 3A and 3B) due to the colour of its shell (size up to 100 mm in length). It is mostly cultured in Canada, USA, Europe, and Africa. Another common edible mussel, *M. galloprovincialis*, originates from the Mediterranean Sea. Interestingly, it is not possible to distinguish *M. edulis* and *M. galloprovincialis* based solely upon morphological characteristics (Gosling, 1992). Moreover, hybrids between the blue mussel and the Mediterranean mussel, have been reported in Ireland, western France, southwest England, and north Scotland (Gosling, Doherty, & Howley, 2008). Concerning the genus *Perna*, major aquaculture mussel species include *P. viridis*, the Asian green mussel, and *P. canaliculus*, the green-lipped mussel which is endemic to New Zealand (Figure 3C and 3D). The latter one is an integral dietary part of the indigenous Maori culture (Maori name: kuku) and is the basis of an important aquaculture and processing industry serving both export and domestic markets. Besides its green lip, it is characterised by a bright green stripe around the posterior ventral margin of the shell (size up to 240 mm in length) (Wakimoto et al., 2011).

Insert Figure 3 here.

2. The bioactive potential of metabolites derived from marine mussels

Most research groups focus on the evaluation of the bioactive potential of extracts, hydrolysates, or purified components derived from whole mussel meat, single organs, cell compartments, or blood. Mussel shells, produced in large amounts as terrestrial waste from aquaculture processing plants, are composed of organo-minerals such as aragonite and calcite as well as shell matrix proteins (Gosling, 1992; Marin et al., 2007). Since there is low evidence for bioactivity reported for shell components, this review will not focus on this part of the mussel.

2.1. Bioactive proteins, peptides, and amino acids from marine mussels

2.1.1. Generation of bioactive proteinaceous metabolites

Mussels contain a large portion of muscle tissue with considerably high content of protein. Interestingly, the number of research publications reporting significant bioactivity exerted by high molecular weight proteins is very low. However, one of the rare examples for bioactive proteinaceous macromolecules is pernin (60 kDa), found in the cell-free haemolymph (plasma) of *P. canaliculus*. It is a self-aggregating glycosylated protein consisting of 497 amino acids resembling an anti-thrombin peptide known from terrestrial leeches. However, the anti-thrombin activity found for this mussel-derived protein is only weak (Scotti et al., 2001).

To exert significant bioactivity, complex protein macromolecules usually need to be split into shorter chains of amino acids (peptides) either by processing techniques such as fermentation or by gastrointestinal digestion. Since ancient times, fermentation has been used as a method for food preservation by controlling the growth and multiplication of a number of pathogens. Still today it is popular as an affordable technology to enhance food safety but also to improve digestibility, taste and flavour of foods (Motarjemi, 2002; Visessanguan et al., 2004). Fermented fish and shellfish sauces are used as nutritional condiments in various cuisines, *e.g.* in African and South East Asian countries. During fermentation, bioactive peptides or amino acids are enzymatically produced from large precursor or parent proteins which usually show only weak or no bioactivity. The underlying

biochemical process of fermentation which depends on temperature, pH, and time, is called hydrolysis or proteolysis, respectively. The cleavage of proteins is either catalysed by endogenous and/or exogenous enzymes which can be inactivated by heat treatment in order to terminate the process. Although favoured as a low cost option, the use of endogenous proteases which are already present in food matrices, such as shellfish meat, has the disadvantage of long time periods required to obtain desired bioactive peptides. The production of fermented blue mussel sauces (FBMSs) requires six to twelve months of fermentation (Jung, Rajapakse, & Kim, 2005). Usually, this so-called autoproteolysis takes place in brine or salt solution (above 20% NaCl, w/w) to avoid microbial contamination during long time periods. The downside of these fermented products is the high salt content, which is unfavourable especially for consumers with risk factors for cardiovascular diseases. However, electrodialysis can be used as a method to reduce the amount of salt (Park, Je, & Kim, 2005).

Despite higher costs, commercial enzymes are preferred to endogenous proteases, due to faster reaction rates and much shorter fermentation time frames. By the use of food-grade enzymes such as alcalase, neutrase, papain, trypsin, Protamex®, and Flavourzyme®, precursor proteins can be cleaved within an average of 30 minutes of hydrolysis (Dai et al., 2012). Moreover, the use of well-characterised commercial enzymes allows a better control over protein breakdown, molecular weight, and composition of generated peptides.

2.1.2. Purification techniques and characterisation of proteinaceous metabolites

To gain insights into putative health effects of proteinaceous metabolites, these are either investigated in the form of hydrolysate mixtures from fresh, homogenised mussels or as isolated and purified amino acids, proteins, or peptides. Besides desired bioactive peptides or amino acids, generated hydrolysates also contain non-proteinaceous components or inactive protein macromolecules. Methods of choice to purify hydrolysate mixtures and to obtain specific peptide classes according to their molecular weight include *e.g.* centrifugation or ultrafiltration using appropriate membranes. To achieve further separation these procedures are followed by gel and ion exchange chromatography techniques as well as RP-HPLC (Ngo et al., 2012; Silva, Park, &

Hubinger, 2010). However, separation is not always beneficial in respect to bioactivity. In some cases mixtures of peptides, amino acids, and sugars show higher bioactivity (*e.g.* antioxidant activity) than single purified peptides (Sarmadi & Ismail, 2010).

Some peptides, such as antimicrobial peptides (AMPs, see below) are naturally available in the mussel, hence, there is no need for time-consuming processes to break down larger proteins to obtain these bioactive peptides. As highlighted by Charlet and co-workers (1996), peptide extraction and purification protocols for AMPs generally include the suspension of homogenised mussel meat, blood or haemolymph in acidic aqueous solutions. In further bioactivity-guided steps the mixture is usually centrifuged and the supernatant is subjected to solid phase extraction followed by RP- and gel permeation HPLC (Charlet et al., 1996). In order to check the purity and/or molecular weight of peptides electrophoresis (*e.g.* SDS-PAGE) or ESI-QTOF tandem mass analyses are performed. In a final step, primarily automated Edman degradation or sequencing is used to elucidate amino acid sequences (Charlet et al., 1996; Jung & Kim, 2009). To date, the 3D structures of only very few mussel peptides have been completely resolved by NMR techniques, *i.e.* the AMPs MGD-1 (Yang et al., 2000) and mytilin B (Roch et al., 2008). Furthermore, knowledge about characteristic structural features, *e.g.* special loop regions, gives further insights into requirements necessary for observed bioactivities (Romestand et al., 2003). Despite the availability of advanced molecular techniques such as expressed sequence tag analyses and gene cloning, the identification of further bioactive mussel peptides and the investigation of their sequential properties as well as structural features still appear as new area of research.

2.1.3. Potential health benefits and biological properties of proteinaceous metabolites

In general, bioactive peptides derived from marine mussels contain 5 to 40 amino acid residues. Depending on the amino acid sequence and structural properties, major biological effects of mussel peptides include antimicrobial, antihypertensive, and anticoagulant activities (Je et al., 2005; Jung & Kim, 2009; Löfgren et al., 2008). An overview on bioactive mussel proteins/peptides/amino acids is given in Table 1.

Insert Table 1 here.

2.1.3.1. Antimicrobial peptides (AMPs)

In the field of molecular cell biology, AMPs comprise the most studied group of peptides from marine mussels. Only recently, AMPs have attracted the attention of medicinal chemists for exploitation as novel drug candidates in the treatment of infectious diseases in humans (Otero-Gonzalez et al., 2010; Sperstad et al., 2011). As mentoned above, AMPs are natural peptides (not enzymatically hydrolysed) which are expressed by the mussel itself as part of its haemolymph, the specific innate immunodefense system. AMPs might be the reason that mussels seem to be less affected by diseases compared to other bivalve molluscs (Gestal et al., 2008). AMPs show antifungal, antibacterial, or antiviral effects (Charlet et al., 1996; Mitta et al., 1999; Yasin et al., 2000) and act via binding to microorganisms, by means of electrostatic interaction with cell wall or membrane residues, promoting their elimination through different mechanisms (Jenssen, Hamill, & Hancock, 2006). These types of amphipathic, cationic peptides defined by a molecular weight <10 kDa, are also widespread among insects (Hancock & Scott, 2000; Steiner et al., 1981). According to their secondary structure, AMPs can be categorised into three major groups, *i.e.* (i) linear α-helical peptides, (ii) cysteine-rich peptides containing β-sheets and disulfide bonds, and (iii) peptides with an over-representation of certain amino acids (Yasin et al., 2000; Zasloff, 2002).

Among different species of marine mussels, AMPs have primarily been identified in two species of the *Mytilus* genus and one species of the *Perna* genus. AMPs comprise multiple isoforms in six families, *i.e.* defensins, mytimycins, myticins, mytilins, big defensins, and mytimacins, which mostly belong to the group of cysteine-rich peptides usually showing several disulfide bonds (Charlet et al., 1996; Gerdol et al., 2012; Löfgren et al., 2009; Mitta, Vandenbulcke, et al., 2000).

About 15 years ago, isolation and characterisation of AMPs from marine mussels (Table 1) began with the identification of mytimycin (6.5 kDa), defined by twelve cysteine residues, as a first solely antifungal molecule from the blood of *M. edulis* (Charlet et al., 1996; Sonthi et al., 2011). Further isolated AMPs are generally smaller (3.7 – 4.5 kDa), positively charged and amphiphilic. The two representatives defensin A and B (Table 1), for instance, were purified from *M. edulis*

comprising six cysteine residues (Charlet et al., 1996). The defensins MGD-1 and MGD-2 (Mitta, Vandenbulcke, et al., 2000; Romestand et al., 2003) and the family of myticins (A, B, and C), found in *M. galloprovincialis*, show eight cysteine residues engaged in four intramolecular disulfide bonds (Hubert, Noel, & Roch, 1996; Mitta et al., 1999). Furthermore, five AMP isoforms of mytilins were identified (A, B, C, D and G1). Mytilin A and B were first isolated from *M. edulis* (Charlet et al., 1996) and mytilin B, C, D, and G1 originate from *M. galloprovincialis* (Mitta, Vandenbulcke, et al., 2000).

Apart from mytimycin which exerts only antifungal effects, members of all *Mytilus* AMP families show a broad-spectrum activity against Gram-positive (*e.g. S. aureus*) and Gram-negative (*e.g. E. coli*) bacteria in liquid growth inhibition assays (Charlet et al., 1996; Hubert, Noel, & Roch, 1996; Mitta, Vandenbulcke, et al., 2000). As in the case of mytilin B and D (Mitta, Vandenbulcke, et al., 2000), myticin B (Mitta et al., 1999), and defensin MGD-1 (Mitta et al., 1999), these antibacterial effects are accompanied with considerable antifungal activity (*e.g.* against *Neurospora crassa* and *Fusarium oxysporum*). Mytilin A has also been tested against fish viral pathogens and protozoan parasites (Carriel-Gomes et al., 2007; Löfgren et al., 2008). However, in both cases it was only active at near cytotoxic concentrations.

Concerning AMPs originating from *Perna* species, much less information is available in the literature (Table 1). To our best knowledge, only one AMP (9.7 kDa) was reported from the gill extract of *P. viridis*, the greenshell mussel occurring in the region of Hong Kong. This peptide exhibited distinct antibacterial (against *S. aureus*) and antifungal (against *A. flavus*) activity by comparing the zone of inhibition with either erythromycin or fluconazole as positive control using a standardized single disc method (Chandran, Rameshkumar, & Ravichandran, 2009).

2.1.3.2. Antioxidant and antihypertensive peptides

Several studies on fermented or similarly processed marine mussels focus on the investigation of health beneficial bioactive properties of the derived peptides. Major effects include antihypertensive and antioxidant activity as well as radical scavenging capacity. For example, Korean researchers identified an antihypertensive and two antioxidant peptides from the sauce of

fermented *M. edulis* (Je et al., 2005; Jung, Rajapakse, & Kim, 2005; Rajapakse et al., 2005). One of these purified peptides (6.5 kDa; N-terminal amino acid sequence EVMAGNLYPG; Table 1) exhibits angiotensin I converting enzyme (ACE) inhibition with an IC_{50} value of 19.34 µg/mL (= 2.98 µM) in a spectrophotometric assay (Cushman & Cheung, 1971; Je et al., 2005). For this peptide effective blood pressure lowering effects were confirmed in an *in vivo* model using spontaneously hypertensive rats. Further two isolated peptides which have a much smaller size of 962 Da and 620 Da respectively (Table 1), were found to exhibit radical scavenging properties in a lipid peroxidation inhibitory assay. The first one (MRSP; 962 Da; sequence HFGDPFH; Table 1) was able to scavenge a variety of radicals (superoxide, hydroxyl, carbon-centred, DPPH) with IC_{50} values in a range between 21 and 96 µM, whereas no IC_{50} values were reported for the latter one (620 Da; FGHPY; Table 1). In terms of lipid peroxidation both peptides showed a strong inhibition which was higher than that observed for the natural antioxidant α-tocopherol, the reference substance (Jung, Rajapakse, & Kim, 2005; Rajapakse et al., 2005). Another potent antioxidant mussel peptide with the amino acid sequence LVGDEQAVPAVCVP, characterised by a low molecular weight of 1.6 kDa, was identified from *M. coruscus* using an *in vitro* gastrointestinal digestion system (Jung et al., 2007). This peptide showed higher protective activity against lipid peroxidation in a linoleic acid model system than vitamin C and α-tocopherol.

In another study, Dai and co-workers analysed the ACE inhibiting effect of *M. edulis* protein hydrolysates obtained by enzymatic processing using six different food-grade proteases (Dai et al., 2012). As a result, the hydrolysates produced by alcalase (peptide molecular weights <1,000 Da) revealed the highest inhibition on ACE with an IC_{50} value of 66.3 µg/mL. The contained antioxidant or antihypertensive peptides are suggested to find further applications in food preservation as alternative to synthetic additives or in pharmaceutical industries to avoid lipid peroxidation (Jung, Rajapakse, & Kim, 2005; Rajapakse et al., 2005).

2.1.3.3. Other biological effects related to marine mussel proteins and peptides

Besides AMPs, antioxidant and antihypertensive peptides, mussels provide a source for further bioactive proteinaceous compounds. For example, an oligopeptide with a potent dose-

dependent anticoagulant activity has been isolated from *M. edulis* (Jung & Kim, 2009). This oligopeptide, named *M. edulis* anticoagulant peptide (MEAP), is characterised by a molecular weight of 2.5 kDa. MEAP is able to prolong the thrombin time as well as the activated partial thromboplastin time and interact with key blood coagulation factors (Jung & Kim, 2009). Furthermore, a recent publication reports anti-inflammatory properties for extracts from *M. galloprovincialis* containing fifteen essential and non-essential amino acids quantified by GC-MS (Badiu et al., 2010). In this work, Badiu and colleagues observed enhanced dermal and epidermal neoformation in an *in vivo* model for skin burns that suggests further development of mussel derived proteic extracts for therapeutic applications.

Another part of the mussel which has already been intensively investigated in the direction of applied research is its byssus. Since mussels grow in intertidal zones they are exposed to harsh waves. These circumstances have driven the development of strong adhesive byssal threads in order to brave the elements. The adhesive power of mussels has been the subject of numerous studies trying to synthetically mimic these characteristics in polymers used for surgical applications, technology, and industry (Brubaker & Messersmith, 2012; Lee et al., 2011). Adhesive protein extracts from *M. edulis* byssus, for instance, have shown promising results as moisture-resistant biocompatible curing agents. In an experimental model, they were shown to form strong bonds between two overlapping strips of porcine small intestinal submucosa (Ninan et al., 2007). These findings will clearly drive future efforts to develop new mussel-inspired materials especially for wet adhesion necessary for surgical applications to coat, heal, or seal tissues.

2.2. Bioactive lipids and non-polar components from marine mussels

2.2.1. Isolation, purification and characterisation of lipid metabolites

Among the three major groups of mussel primary metabolites, lipids have so far shown the highest potential for the commercial development of health beneficial functional foods or dietary supplements. Mussel lipid extracts and fractions can be obtained by solvent extraction and are purified by chromatographic separation. The increasing instability during purification processes

limits the investigation of single lipid components, hence analyses mostly focus on the characterisation of lipid extracts or fractions rather than pure compounds.

Mussel lipid extracts are usually obtained by extraction of fresh or freeze-dried mussel meat. By means of enzymatic (*e.g.* using lipase or protease) or chemical (*e.g.* KOH) hydrolysis complex lipids are cleaved to obtain *e.g.* single fatty acids. In general, normal phase column chromatography is subsequently used to fractionate the crude extracts into major lipid classes, *i.e.* sterol esters, triglycerides, free fatty acids, sterols, and phospholipids. In some cases, purification and structural analysis is also pursued and achieved by chromatographic techniques such as preparative TLC (McPhee et al., 2010). GC, GC-FID, or GC-MS techniques are the most suitable methods for the characterization of fatty acids present in mussel oils. Classically, lipid classes have to be hydrolysed and methylated to obtain fatty acid methyl esters (FAMEs). Subsequently, identification of lipid components is carried out by comparison to known standards.

2.2.2. Bioactive marine oils from the New Zealand green-lipped mussel *P. canaliculus*

Several anti-inflammatory and anti-arthritic dietary supplements which contain mussel lipids are available commercially. The best known products are Seatone[®] and Lyprinol[®]. Their development was inspired by the observation of the New Zealand Maori population living in coastal areas that consume a high amount of green-lipped mussels (*P. canaliculus*) in their diet. These people develop osteoarthritis (OA) to a much lesser extent than inland Maoris. In 1976, Seatone[®] was launched as the first commercially available anti-arthritic green-lipped mussel product (McFarlane & Croft, 1980). In early years, stability problems of this freeze-dried mussel extract strongly affected the level of anti-inflammatory activity and raised concerns among consumers and industry. To overcome these issues, 3% tartaric acid was added directly after shucking the mussel as antioxidant and metal chelator which resulted in consistently high anti-inflammatory effects (Halpern, 2000; Whitehouse et al., 1997). Subsequently, the formulation method was improved and in 1998, Lyprinol[®] was brought to the market as another anti-inflammatory marine mussel product. It contains the oil of *P. canaliculus* obtained by supercritical fluid extraction (CO_2-SFE) of the stabilised, freeze-dried mussel powder formulated with olive oil and vitamin E as an antioxidant

(Singh et al., 2008; Whitehouse et al., 1997). By using this technique and CO_2 as extracting medium, the material is not exposed to high temperatures, hence a mild extraction is achieved without activating lipid degrading enzymes such as phospholipases and lipoxygenases (Wakimoto et al., 2011). Since there are no organic solvents present during this extraction process, it is considered as food-friendly.

Lipid classes present in mussel oil comprise sterol esters, triglycerides, free fatty acids (saturated and unsaturated), carotenoids, sterols and polar lipids (Sukumaran et al., 2010). In total there are 90 fatty acid components reported for Lyprinol[®] (Murphy et al., 2002). Omega-3 polyunsaturated fatty acids (PUFAs) with 13% eicosapentaenoic acid (EPA) and 21% docosapentaenoic acid (DHA) are found as major ingredients (Murphy et al., 2002). Furthermore, novel anti-inflammatory omega-3 PUFAs, *i.e.* 5,9,12,15-octadecatetraenoic acid, 5,9,12,16-nonadecatetraenoic acid, 7,11,14,17-eicosatetraenoic acid, and 5,9,12,15,18-heneicosapentaenoic acid have been purified by normal and reversed phase chromatography and identified by GC-MS (Singh et al., 2008; Treschow et al., 2007). Figure 4 gives an overview on main and novel PUFA structures present in *P. canaliculus* oils. The nomenclature of these fatty acids is based on the number of carbon atoms and the number of their double bonds. Basically PUFAs are categorised in the omega-6 and the omega-3 class. Omega-6 PUFAs are found mainly in plants and are contained in vegetable oils, whereas omega-3 PUFAs are found in fish and shellfish and occur to a lesser extent in plants. In terms of bioactivity, the omega-3 PUFAs originating from fish and shellfish sources are considered to be more efficient in their biological activity than those found in plants (Chan & Cho, 2009).

Insert Figure 4 here.

The anti-inflammatory mode of action of the green-lipped mussel oil has been linked to its ability to inhibit the production of inflammatory mediators by affecting key enzymes in the arachidonic acid (AA) cascade (Figure 5) (McPhee et al., 2007; McPhee et al., 2010). AA is metabolised via well characterised pathways including cyclooxygenase (COX) and lipoxygenase

(LO) enzymes. As a result, pro-inflammatory (*e.g.* prostaglandins), chemotactic, broncho-constricting (*e.g.* leukotrienes), or potential tumour promoting (*e.g.* 5-hydroxyeicosatetraenoic acid (5-HETE)) agents are produced. Due to their structural similarity to AA (Figure 4), mussel PUFAs like EPA and DHA can act as competitive substrates for these key enzymes and thus reduce inflammatory responses. For example, the production of prostaglandin E_2 (PGE_2) is inhibited by Lyprinol[®] with an IC_{50} of 1.2 µg/mL (Whitehouse et al., 1997). Furthermore, the efficacy of Lyprinol[®] for the treatment of chronic airway inflammation was also demonstrated in a study with patients with atopic asthma (Emelyanov et al., 2002).

Insert Figure 5 here.

As opposed to conventional therapeutic treatment options such as non-steroidal anti-inflammatory drugs (NSAIDs), the use of above discussed mussel oil products as natural remedies against arthritis causes very few side effects. Rapid-acting NSAIDs are well known for their adverse effects in the gastro-intestinal system due to non-selective inhibition of COX enzymes, related deficiency in cytoprotective prostaglandins, and subsequently reduced mucous production leading *e.g.* to the development of gastric ulcers (Toki et al., 2007). In contrast, green-lipped mussel preparations demonstrate *in vivo* gastro-protective effects (Rainsford & Whitehouse, 1980). Lyprinol[®] does neither affect platelet aggregation nor the natural resistance of the stomach mucosa in humans which is related to the involvement of the inducible COX-II rather than COX-I (Whitehouse et al., 1997). *P. canaliculus* lipid extracts inhibit COX-I and COX-II by 12% and 25%, respectively (McPhee et al., 2007). Hence, these mussel oils are mainly suitable for the treatment of chronic inflammation. This further explains that the mentioned mussel products show no or modest activity on the carrageenan-induced paw oedema assay in rats, an experiment which is typically used to evaluate acute inflammation (Whitehouse et al., 1997).

In a recent study, McPhee and co-workers (2007) compared *in vitro* COX-inhibiting effects of Lyprinol[®] and the total lipid extracts obtained from *P. canaliculus* and *M. edulis* before and after KOH-, protease-, or protease-lipase-hydrolysis (McPhee et al., 2007). The non-hydrolysed total lipid

extracts of both *P. canaliculus* as well as *M. edulis* showed a moderate COX inhibition. Related to an increase of free fatty acids, a strong COX inhibition was observed after saponifying the extracts by KOH hydrolysis. Except for higher lipid contents in *P. canaliculus*, there were no significant differences observed between the two mussel species. Interestingly, also Lyprinol[®] has demonstrated a 10-fold higher inhibition of both COX isoforms after hydrolysis (McPhee et al., 2007).

A recent study provided mechanistic insights into the anti-inflammatory effects of Lyprinol[®] in the arachidonic acid cascade. (Lee et al., 2008) Lee and co-workers (2008) investigated protein expression profiles of splenocytes in rats with adjuvant-induced arthritis (AIA). In AIA rats consuming Lyprinol[®] orally, they found that six metabolism-related proteins are down-regulated while malate dehydrogenase (MDH), a key enzyme in gluconeogenesis, is up-regulated. Hence, the level of glucose necessary for Major-Histocompatibility-Complex I (MHC-I) activation is decreased which underlines the anti-inflammatory mechanism of Lyprinol[®], since an activated MHC-I is involved in the development of autoimmune diseases. These findings indicate that Lyprinol's anti-inflammatory activity is mediated by multiple mechanisms, which are still not fully evaluated.

Since their launch in 1976, effects of nutritional green-lipped mussel supplement products on osteoarthritis (OA), rheumatoid arthritis (RA), asthma, and cancer have been studied in various clinical trials and discussed in numerous review papers (Brien et al., 2008; Doggrell, 2011; Gibson et al., 1980; Gibson & Gibson, 1998; Lau et al., 2004; Sukumaran et al., 2010). Within its main field of application, *i.e.* in patients suffering from OA or RA, outcomes of most studies have proven a reduced amount of pain and stiffness related to the intake of mussel preparations (Halpern, 2000). Apart from orally applied Lyprinol[®] capsules, also topical preparations such as skin cream containing mussel lipids seem to be effective against OA and RA (Chandler, 2005a, 2005b; Kenneth, Waite, & Downs, 2009; Mulye & Assoulin, 2012; Williams & Sansom, 2008). However, to our knowledge no comparative study has been conducted to evaluate the efficacy of orally versus topically applied mussel lipids.

2.2.3. Bioactive marine oils from *Mytilus* species

In contrast to lipids from *Perna* species, the amount of information available on mussel lipids from *Mytilus* species is comparably small. In general, *Mytilus* oils are known to contain similar major long chain omega-3 PUFAs (EA, EPA and DHA) as *Perna* oils, however, in considerably lower yields (McPhee et al., 2007; McPhee et al., 2010). Anti-inflammatory effects were found for both non-hydrolysed *M. edulis* crude lipid extracts and hydrolysed triglyceride fractions (Christie, 1982; McPhee et al., 2010). Especially after saponification of the crude extracts, containing EPA and DHA at a percentage of 37% of total fatty acids, inhibition of leukotriene production was observed in a neutrophil 5-LO assay *in vitro* and in an AIA rat model (McPhee et al., 2010). Combined with the 5-LO inhibition the total free fatty acid fractions show a selective *in vitro* inhibition of COX-II (McPhee et al., 2007). Moreover, since there were no negative side effects observed in animal models, the findings of McPhee and colleagues suggest the potential of *M. edulis* lipid extracts or fractions as anti-inflammatory agents.

Further *Mytilus* species reported to contain bioactive lipids comprise the Korean mussel, *M. coruscus* and the Mediterranean mussel, *M. galloprovincialis*. Different *M. coruscus* lipid extracts obtained by suspension in organic solvents (MeOH, chloroform, hexane) or solvent mixtures (MeOH:chloroform, MeOH:hexane) were analysed for their potential to induce apoptosis of several cancer cells including human prostate, breast, lung, and liver cancer cell lines. As a result, the hexane extract was found to possess the highest *in vitro* anti-tumour effects by inducing apoptosis of human prostate cancer cells (Kim et al., 2011). Interestingly, the most active fraction of the hexane extract of this Korean mussel was found to contain remarkably high 33.4% of EPA. In another study, Badiu and colleagues investigated the wound healing potential of *M. galloprovincialis* lipid extracts obtained from extractions with chloroform:MeOH (1:2; v/v). A positive effect on burnt skin was confirmed in an animal model using Wistar rats, suggesting further development of these extracts for skin-care products (Badiu et al., 2008).

2.2.4. Isolated single lipid components from marine mussels

Bioactivity studies on marine lipids are usually carried out on extracts or fractions rather than pure compounds. As mentioned before, this might be due to the increasing instability of fatty acids,

for instance, during isolation processes. In a very recent study, Wakimoto and colleagues (2011) detected unstable anti-inflammatory and antioxidant furan fatty acids (Figure 4) in the green-lipped mussel, *P. canaliculus*. After semisynthetic stabilisation, one of the furan fatty acid ethyl esters showed an even higher anti-inflammatory potential than EPA ethyl ester in an *in vivo* model of AIA (Wakimoto et al., 2011).

Another rare example for an isolated single lipid compound is lysolecithin. This hydrophilic phospholipide belongs to the class of phosphatidylcholines and was identified as anti-histaminic and anti-inflammatory component in *P. canaliculus* lipid fractions in the mid 1980s (Kosuge et al., 1986). However, no further studies have been undertaken to pursue these results.

2.3. Bioactive carbohydrates from marine mussels

2.3.1. Structural characteristics and analysis of mussel carbohydrates

The group of mussel carbohydrates is primarily represented by polysaccharides. These sugars are composed of monosaccharides which are connected via glycosidic bonds forming linear or branched macromolecules (average molecular weight 1.5×10^6 Da). It is distinguished between homo- (one type of monosaccharide) and heteropolysaccharides (different types of monosaccharides). In some cases sugar components are covalently linked to polypeptide side chains of cell wall proteins. These so-called glycoproteins are important for the invertebrate's immune system (Smital & Kurelec, 1998). In general, carbohydrates can be extracted from the mussel by using different organic solvents. In most cases they are obtained by hot-water extraction followed by further purification steps including anion-exchange and gel-permeation chromatography (Miller et al., 1993).

Techniques such as dialysis and lyophilisation allow a coarse fractionation of the sugar components. Isolation and purification is usually achieved by the use of molecular-sieve or affinity chromatography (Ovodova et al., 1992). An array of different techniques such as complete hydrolysis, periodate oxidation, methylation analysis, Fourier transform infrared spectroscopy (FTIR), and NMR, are applied in a final step to elucidate the structure of the carbohydrate of interest.

2.3.2. Bioactivities related to mussel carbohydrates

Among the three major bioactive primary metabolite classes from mussels, the least attention has been paid to the group of carbohydrates, hence only very few research articles are available in the literature. In 1992, Ovodova and co-workers reported a high immunomodulating activity for mytilan, a branched bioglycan isolated from *Crenomytilus grayanus*, a marine mussel originating from Japan (Ovodova et al., 1992). Mytilan is a non-covalently linked complex of 95% polysaccharide and 5% protein with lectin (carbohydrate-binding) properties. The polysaccharide as the carbohydrate part consists of α-D-glucan similar to glycogen, an equivalent to starch which is known as an energy storing molecule in plants. Sonication of mytilan resulted in a mixture of glucose, maltose, and malto-oligosaccharides. Further NMR-based analyses of mytilan revealed an even higher degree of branching than glycogen (Ovodova et al., 1992).

In another study, a glycogen was isolated from *P. canaliculus* and investigated in an *in vivo* model in rats with a carrageenin-induced footpad oedema where it was given *i.v.* and showed a dosed-dependent anti-inflammatory activity (Miller et al., 1993). However, this activity could no longer be observed after treatment with KOH or proteinase K suggesting that the anti-inflammatory properties are mediated by proteinaceous moieties associated with the macromolecule glycogen (Miller et al., 1993).

Recently, Xu and colleagues published a work on the isolation and characterisation of the antioxidant polysaccharide MP-I (composed of glucose monomers) from *M. coruscus* (Xu et al., 2008). It was found to be an α-(1→4)-D-glucan, branched with a single α-D-glucose at the C6 position every eighth residue along the main chain (Xu et al., 2008). This molecule (1.35×10^6 Da) exhibited a protective effect on acute liver injury in mice given intraperitoneally. MP-1 has been shown to inhibit lipid peroxidation which is involved in tissue damage during hepatic failure. The observed bioactivities render MP-I as beneficial marine mussel carbohydrate which may find pharmaceutical applications in humans (Xu et al., 2008). Cheng and co-workers (2010) have evaluated the impact of several extraction methods (by using water, acidic, or alkaline extracting media) on the antioxidant activity of mussel polysaccharides. A dose-dependent antioxidant activity

was detected for all mussel extracts in an *in vitro* spectrophotometric assay, with water and alkaline extracts being the most active (Cheng, Yu, & Zhang, 2010).

2.4.   Miscellaneous bioactive compounds from marine mussels

Our literature survey suggest that the most bioactive marine mussel compounds can be classified as typical primary metabolites such as the discussed proteins, lipids, or carbohydrates. However, MytiLec, a sugar-binding protein (lectin) isolated from the mussel *M. galloprovincialis*, is discussed here under miscellaneous compounds due to its carbohydrate-binding properties. Recently, this compound was discovered by Japanese researchers as a 17 kDa α-D-galactose-binding lectin comprising a novel primary structure which is expected to promote research not only in the field of glycobiology (Fujii et al., 2012), but also in pharmacology. MytiLec belongs to the humoral defense factors of the mussel similar to AMPs (Casas et al., 2011). In terms of bioactivity, it exhibits a dose-dependent cytotoxic effect on human Burkitt's lymphoma Reji cells (Fujii et al., 2012). These results give insights into possible anti-cancer applications and will also help to reveal the physiological role of this novel galactose-binding lectin.

3.   Biotoxins affecting marine mussels

Besides the beneficial effects and bioactives that mussel components may yield, it is vital to also consider potential harmful biotoxins that may be present in mussels. Mussels in common with other bivalve molluscs are filter-feeders. They can filter up to eight litres of sea water per hour whereby they derive their nutrition by passing water over their gills and extract and sort particles of food during this process (Jones, Richards, & Southern, 1992). They selectively choose filtered particles based on size and eject non-edible particles as pseudo-faeces before they enter the digestive tract. A large component of the ingested particles comprise of nutrient rich eukaryotic microalgae, mostly diatoms and dinoflagellates (Newell & Shumway, 1993). In marine environments there are a small but significant group of microalgae referred to as harmful algal bloom (HAB) species that cause injury to human health or socioeconomic interests, or to components of aquatic ecosystems (Anderson, Cembella, & Hallegraeff, 2012). Mussels are fairly non-discriminatory towards the

species of microalgae that they ingest. Their target feed can include a number of different HAB species that contain various compounds that are toxic to humans, and filter feeding shellfish including mussels are a significant source by which these toxins find a pathway through the food chain at concentrations that can cause human illness.

HAB toxins have typically been classified based on the illness that they produce in human consumers; amnesic shellfish poisoning (ASP), ciguatera fish poisoning (CFP), diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), paralytic shellfish poisoning (PSP), and azaspiracid shellfish poisoning (AZP). In addition there are other toxins that have yet to demonstrate adverse effects (acute or chronic) in humans including the Cyclic Imines Group, Pectenotoxins (PTX) Group and Yessotoxins (YTX) Group (UNESCO, 2005). Within these groups there are a large number of toxins differing in their structures, solubility and mode of action. In addition, a wide range of metabolites is found in shellfish due to biotransformation through enzymatic reactions.

The majority of human diseases associated with HAB toxins appear to be acute phenomena, although some can cause prolonged chronic disease (*e.g.* ASP). Toxins from the PSP, DSP and ASP groups are considered to have a worldwide distribution and their causative species are also known to have global distribution (Ciminiello & Fattorusso, 2004). The micro-algae responsible for the remaining toxins are less cosmopolitan and their toxins are more restricted geographically. The distribution can however at best be described as patchy in a biogeographical sense, and this is compounded by variation in biotransformation of the parent toxin into chemical analogs that may be more or less toxic as they pass through the food web. There is also evidence of transfer of HABs by anthropogenic and non-anthropogenic vectors and this can result in toxins appearing in previously toxin free areas so the potential for toxic HAB species is to be regarded as potentially worldwide (Bolch & de Salas, 2007).

The classification of toxins based on symptomology has been greatly advanced with the introduction of high-resolution analytical separation and detection technologies including HPLC combined with MS and NMR technologies. This has resulted in several prominent groups of toxins which can be summarised as (a) linear and macrocyclic polyethers, *e.g.* okadaic acid or dinophysistoxins; (b) ladder-frame polyethers, *e.g.* ciguatoxins and brevetoxins; (c) macrocyclic

imines, *e.g.*, spirolides and gymnodimine; (d) tetrahydropurines, *e.g.*, saxitoxin and analogs; and (e) toxic secondary amines, including domoic acid (Figure 6).

Insert Figure 6 here.

At present, there are no practical solutions to removing these toxins from shellfish other than allowing them to naturally depurate by metabolic processes within the shellfish growing area. This can take between several weeks to several months depending on the level of toxin and environmental parameters. The primary preventive tool for intoxications with natural toxins is the monitoring of toxin levels in algae in the harvesting areas. Based on the presence of toxins, restrictions can be placed and harvesting of shellfish forbidden if levels of toxin are too high.

4.   Conclusion and outlook

Unlike other molluscs such as oysters, marine mussels are generally resistant to diseases (Gosling, 1992). Harsh environmental conditions and challenges might be prompting the ability of the mussel to produce bioactives. Within the past decades, marine mussels, in particular species of the genera *Mytilus* and *Perna*, have been investigated for their primary metabolites. In addition as highlighted in this review, monitoring the mussel for possible contaminations with harmful biotoxins is of great importance for public health and product development. Driven by both industry and academia, prospecting a tremendous potential in the development of functional foods and nutraceuticals, research has been mainly directed towards the bioactive potential of proteins, lipids, and carbohydrates. As opposed to natural product drug discovery programmes which are usually aiming at the discovery of small molecules or secondary metabolites, the investigation of bioactive primary metabolites is a research area scattered over several scientific disciplines often accompanied by a lack of target-oriented strategies for analytics, isolation, and characterisation procedures for compounds of interest. To overcome these issues it might be of relevance to cross frontiers between chemistry and food industry in order to successfully use the gained knowledge about bioactives from marine mussels. As witnessed in a growing number of cases and especially in light of current

European legislations concerning aquaculture waste regulations a huge commercial interest facilitates such endeavours.

Since protein accounts for the major part of mussel meat it is not surprising that a significant percentage of bioactives is found among proteins, peptides and amino acids. The advantages of this are reflected in a win-win situation for both industry and academia. Considering prospering aquaculture businesses producing large volumes of mussel waste materials, the generation of bioactive proteinaceus metabolites helps to exploit these waste materials and offers sources for the development of functional foods or nutraceuticals. However, challenges such as stability problems and the finding of suitable food-grade formulation methods require interdisciplinary expertise. For instance, as part of their immune system mussels produce a wide range of AMPs which have been mostly characterised by environmental biologists to evaluate the mussel's physiological functions. Since the emergence of resistance developed by microorganisms against state of the art treatment options, AMPs from marine mussels might also be considered as valuable source for therapeutics. In aquaculture, AMPs have been proposed as natural antimicrobial agents for the treatment of infectious diseases in marine species (Balseiro et al., 2011). Moreover, AMPs show a great potential as a natural antimicrobial food additive for human consumption. However, further target-oriented research is needed to overcome negative aspects like bitter taste (Cho et al., 2004), possible interactions with other food components, or allergies (Ngo et al., 2011).

In terms of commercial applications, the investigation of mussel lipids has shown much more progress compared to mussel proteins. As exemplified by Lyprinol®, a dietary supplement product containing the oil of the New Zealand green-lipped mussel, many dietary supplement products have been brought to the market. Major health claims of these products are related to beneficial effects in the treatment of arthritic conditions. The lack of any significant adverse effects and efficient relief from pain and stiffness appear to be key factors behind the success of Lyprinol® and similar mussel oil products as readily available over-the-counter-products worldwide.

The bioactive potential of mussel carbohydrates has been explored to a lesser extent in comparison to the other two classes of metabolites, however existing studies point out promising immune-modulating or antioxidant bioactivities especially for mussel-derived polysaccharides

(Ovodova et al., 1992; Xu et al., 2008). So far no commercial applications have been reported for these bioactives. In summary, primary metabolites from marine mussels of the genus *Mytilus* and *Perna* have shown promising results. Hence, they represent invaluable sources for the development of functional foods, food ingredients, or pharmaceuticals. Further advances in processing and analytical technologies as well as a more interdisciplinary research focus are expected to promote a straightforward and targeted development of health beneficial products in the near future.

5.   Acknowledgements

6.   References

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Captions

Figure 1. Comparison of number of selected publications dealing with bioactives from marine mussels categorised in four classes, *i.e.* proteins/peptides/amino acids (AA), lipids, carbohydrates, and miscellaneous.

Figure 2. Evaluation of number of publications which report bioactive properties related to three major classes of mussel compounds. Peptides, lipids, and carbohydrates are differentiated in different shades of grey.

Figure 3. Pictures of *M. edulis* (A, whole mussel; B, shells) and *P. canaliculus* (C, whole mussel; D, shells).

Figure 4. Structures of *P. canaliculus* PUFAs (omega-3) and novel anti-inflammatory furan fatty acids in comparison to omega-6 PUFAs eicosadienoic acid (EA) and arachidonic acid (AA).

Figure 5. Down-regulation of the key enzymes in the arachidonic acid cascade by lipid extract of *P. canaliculus* (shown in red) resulting in a reduced formation of inflammatory mediators (figure modified from (Halpern, 2000). HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; LT, leukotriene; PGI$_2$, prostacyclin; TX, thromboxane.

Figure 6. Chemical structures of shellfish biotoxins: (a) okadaic acid; (b) ciguatoxin and brevetoxin A; (c) spirolide C and gymnodimine; (d) saxitoxin; and (e) domoic acid.

Table 1. Overview on bioactive proteins, peptides, and amino acids derived from marine mussels.
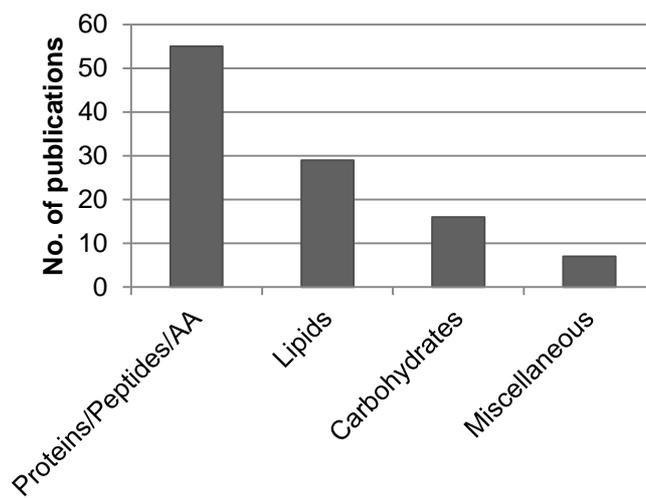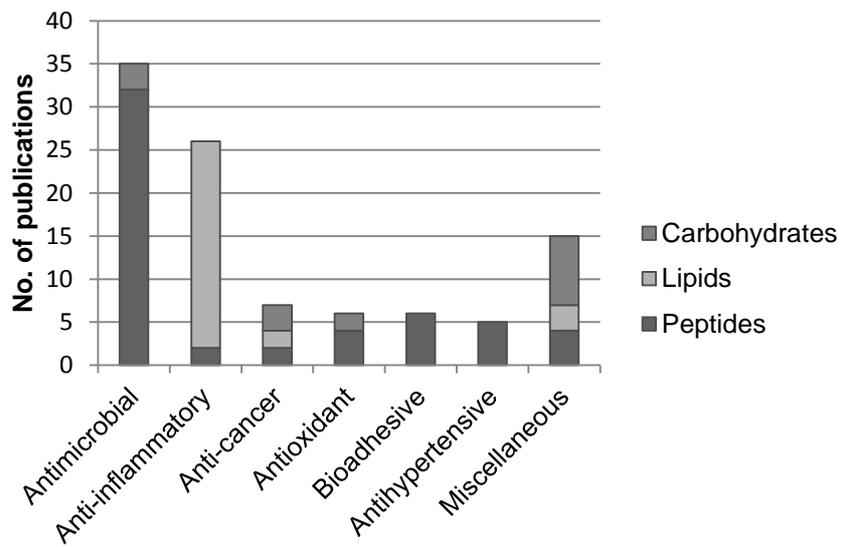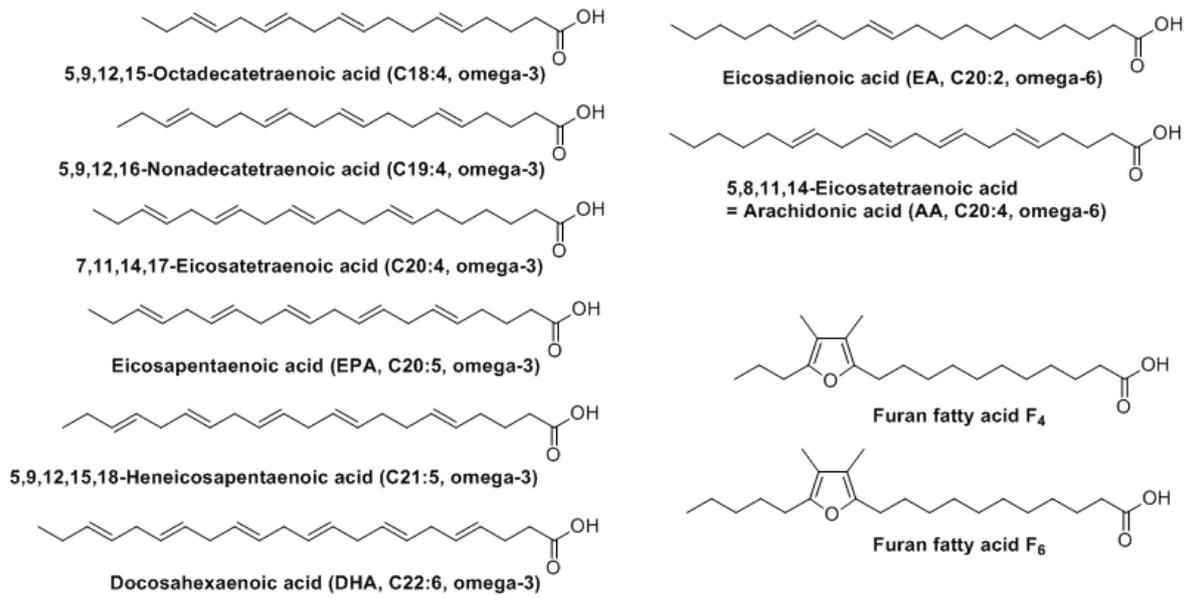
Figures, Graphics & Tables

Figure 1

Figure 2

A

C

B

D

Figure 3
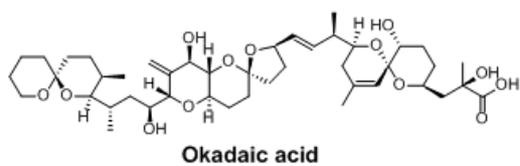
5,9,12,15-Octadecatetraenoic acid (C18:4, omega-3)

5,9,12,16-Nonadecatetraenoic acid (C19:4, omega-3)

7,11,14,17-Eicosatetraenoic acid (C20:4, omega-3)

Eicosapentaenoic acid (EPA, C20:5, omega-3)

5,9,12,15,18-Heneicosapentaenoic acid (C21:5, omega-3)

Docosahexaenoic acid (DHA, C22:6, omega-3)

Eicosadienoic acid (EA, C20:2, omega-6)

5,8,11,14-Eicosatetraenoic acid
= Arachidonic acid (AA, C20:4, omega-6)

Furan fatty acid F_4

Furan fatty acid F_6
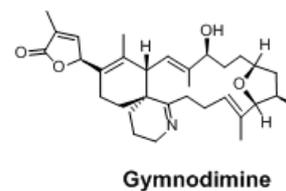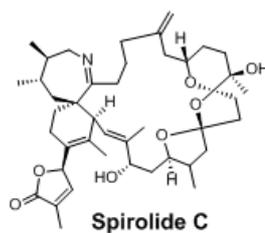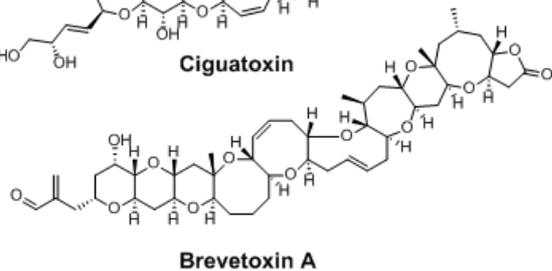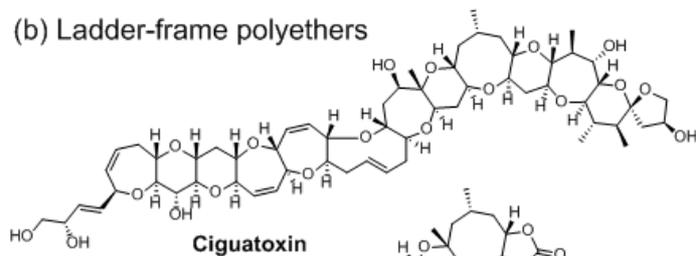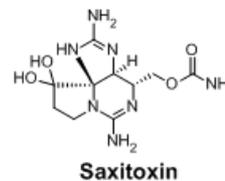
Figure 4

Figure 5

Figure 6

Table 1

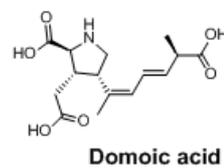| Biological activity - name of bioactive protein/peptide	Sequence and molecular weight	Mussel species	Origin/product	Reference(s)
Antioxidant				
Mussel-derived radical scavenging peptide (MRSP)	HFGDPFH (962 Da)	ME	Fermented sauce	(Rajapakse et al., 2005)
N.g.	LVGDEQAVPAVCVP (1.59 kDa)	MC	*In vitro* gastrointestinal digest	(Jung et al., 2007)
N.g.	FGHPY (620 Da)	ME	Fermented sauce	(Jung, Rajapakse, & Kim, 2005)
Antimicrobial				
Mytilus defensin A	GFGCPNDYCHRHCKSIPGRXGGYCGGXHRLRCTCYR (~ 4 kDa)	ME	Blood	(Charlet et al., 1996)
Mytilus defensin B	GFGCPNDYPCHRHCKSIPGRYGGYCGGXHRLRCTC— (~ 4 kDa)	ME	Blood	(Charlet et al., 1996)
MGD-1	GFGCPNNYQCHRHCKSIPGRCGGYCGGWHRLRCTCYRCG (4.4 kDa)	MG	Hemocytes	(Hubert, Noel, & Roch, 1996; Mitta et al., 1999; Yang et al., 2000)
MGD-2	GFGCPNNYACHQHCKSIRGYCGGYCASWFRLRCTCYRCG (4.4 kDa)	MG	Hemocytes	(Mitta, Hubert, et al., 2000; Mitta et al., 1999; Yang et al., 2000)
Mytilin A	GCASRCKAKCAGRRCKGWASASFRGRCYCKCFRC (~ 4 kDa)	ME, MG	Hemocytes	(Charlet et al., 1996; Löfgren et al., 2009; Mitta, Vandenbulcke, et al., 2000)
Mytilin B	SCASRCKGHCRARRCGYYVSVLYRGRCYCKCLRC (4.0 kDa)	ME, MG	Hemocytes	(Charlet et al., 1996; Mitta, Vandenbulcke, et al., 2000)
Mytilin C	SCASRCKSRCRARRCRYYVSVRYGGFCYCRC- (4.2 kDa)	MG	Hemocytes	(Mitta, Vandenbulcke, et al., 2000)
Mytilin D	GCASRCKAKCAGRRCKGWASASFRRRCYCKCFRC (3.9 kDa)	MG	Hemocytes	(Mitta, Vandenbulcke, et al., 2000)
Mytilin G1	VVTCGSLCKAHCTFRKCGYFMSVLYHGRCYCRCLLC (~ 4 kDa)	MG	Hemocytes	(Mitta, Vandenbulcke, et al., 2000)

Biological activity - name of bioactive protein/peptide	Sequence and molecular weight	Mussel species	Origin/product	Reference(s)
Myticin A	HSHACTSYWCGKFCGTASCTHYLCRVLHPGKMCACVHCSR (4.5 kDa)	MG	Hemocytes	(Padhi & Verghese, 2008)
Myticin B	HPHVCTSYYCSKFCGTAGCTRYGCRNLHRGKLCFCLHCSR (4.6 kDa)	MG	Hemocytes	(Padhi & Verghese, 2008)
Myticin C	QSVACRSYYCSKFCGSAGCSLYGCYLLHPGKICYCLHCSR (4.4 kDa)	MG	Hemocytes	(Balseiro et al., 2011; Padhi & Verghese, 2008)
N.g.	N.g. (9.7 kDa)	PV	Gill homogenate	(Chandran, Rameshkumar, & Ravichandran, 2009)
Anti-inflammatory				
	15 Essential and non-essential amino acids	MG	Proteic extract	(Badiu et al., 2010)
Antihypertensive				
N.g.	EVMAGNLYPG (Partial sequence – N-terminal region; 6.5 kDa)	ME	Fermented sauce	(Je et al., 2005)
Antifungal				
Mytimycin	DCCRKPFRKHCWDCTAGTPYYGYSTRNIFGCTC--- (6.5 kDa)	ME	Blood	(Charlet et al., 1996)
Anticoagulant				
N.g.	EADIDGDGQVNYEEFVAMMTSK (2.5 kDa)	ME	Edible part	(Jung & Kim, 2009)
Anti-thrombin				
Pernin	Protein composed of 497 amino acids (60 kDa)	PC	Cell-free haemolymph	(Scotti et al., 2001)
Adhesive for surgical applications				
	Adhesive protein	ME	Byssus	(Ninan et al., 2007)

ME, *M. edulis*; MC, *M. coruscus*; MG, *M. galloprovincialis*; PC, *P. canaliculus*; PV, *P. viridis; N.g., not given*