Natural antifouling compounds: effectiveness in preventing invertebrate settlement and adhesion

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Abstract

Biofouling is a major economic issue regarding maritime industries that raise also important environmental concern. International legislation is restricting the use of biocidal-based antifouling (AF) coatings, and increasing efforts have been applied in the search for environmental-friendly AF agents. A wide diversity of natural AF compounds has been described for their ability to inhibit the settlement of macrofouling species. However poor information on the specific AF targets was available before the application of different molecular approaches both on invertebrate settlement strategies and bioadhesives characterization and also on the mechanistic effects of natural AF compounds. This review focuses on the relevant information about the main invertebrate macrofoulers settlement and bioadhesive mechanisms, which might help in the understanding of the reported effects, attributed to effective and non-toxic natural AF compounds towards this macrofouling species. Also aim to contribute to the elucidation of promising biotechnological strategies in the development of natural effective environmentally friendly AF paints.

Keywords: biofouling; natural antifouling compounds; larval settlement; invertebrate adhesive mechanisms; antifouling modes of action

Introduction

The establishment of new benthic biological communities in aquatic environments either in living or non-living substrata (biofouling) generally involves a sequence of succession started by the accumulation of a biochemical proteinaceous conditioning followed by bacteria, unicellular and multicellular eukaryote colonization (Wahl, 1989). The initial step of microbial biofilm formation (microfouling) is known to regulate the subsequent colonization of macroalgal spores and invertebrate larvae (macrofouling) (Pawlik, 1992). Biofilm properties, including physical characteristics, biotic composition and produced chemical signals have been reported to act as either a stimulatory or inhibitory stimulus for the settlement of a particular macrofouling community (Dobretsov et al., 2006, Hellio et al., 2005, Pawlik, 1992, Qian et al., 2007). Nature provides good models of antifouling (AF) by a combination of this chemical cues and also physical properties including surface roughness and fluid hydrodynamics. Mussels, crabs, sharks, among others, possess exterior surfaces that are able to inhibit epibiosis and biofouling (Hadfield and Paul, 2001, Magin et al., 2010). In addition, some species of macroalgae (Daoud et al., 2011, Eashwar et al., 2008, Hellio et al., 2002), sponges (Engel and Pawlik, 2000, Hellio et al., 2005, Sjogren et al., 2008), soft corals (Slattery et al., 1995, Nagabhushanam et al., 1995), ascidians (Cima and Ballarin, 2012, Menin et al., 2008, Teo and Ryland, 1995) have also the ability to prevent epibiosis, by their own chemical cues, which may range from small-molecule secondary metabolites to high-molecular weight extracellular polymers (Hadfield, 2011b, Fusetani, 2011).Along with natural surfaces, all submerged artificial structures as ships, pipelines, oil platforms, bridge pillars, fishing devices subjected to biofouling suffer adverse impacts. Particularly on ships, higher fuel consumption and decreased speed and range

are attributed to increased frictional drag (Schultz et al., 2011, Yebra et al., 2004, Schultz, 2007), and thus biofouling control is mandatory for maritime industries. The majority of antifouling paints currently in use are based on biocidal agents that induce general toxic responses in the marine environment associated with heavy metal toxicity, antibiotic toxicity, among others. Considering this, a need to develop alternative non-toxic and environmentally friendly AF agents arise in line with the EU Biocidal Product Regulation (EU) 528/2012, which led to increase the investigation on the field of natural AF compounds. A wide range of natural products have been screened for their potential to substitute the efficient but extremely toxic tributyltin (TBT), now banned in 27 countries (IMO, 2008). Some alternative booster biocides have also been introduced and believed to be less harmful for the environment, however significant environmental risks were also identified (Konstantinou and Albanis, 2004, Thomas and Brooks, 2010). A wide range of natural AF compounds from diverse source species has been identified lately by their ability to inhibit the settlement of macrofouling species (Fusetani, 2011). Recent investigations on this topic permit to recognize that microorganisms in particular are promising potential sources of non-toxic or less-toxic AF compounds, as they produce a wide-range of promise bioactive metabolites and also have the advantage of being easy to culture and to produce in large scale in short periods of time, easily ensuring product supplies renovation for commercialization (Burgess et al., 2003, Dahms et al., 2006, Dobretsov et al., 2006, Gademann, 2007, Qian et al., 2007, Tan et al., 2010, Dobretsov et al., 2013a).

However, AF compounds identification is often based on a single and general endpoint/mechanism of action, showing a narrow spectrum performance towards the biofouling community (different species and different life stages), compromising their effectiveness and their incorporation in AF paintings. Regarding the variety of adhesion

mechanisms and settlement strategies among biofouling organisms, several general modes of action of natural products are described including repellants, toxins, surface energy modifiers, nervous pathway interference (both anesthetics and neurotransmitters) and inhibitors of growth, attachment, adhesion or metamorphosis (Clare, 1996, Rittschof, 2000).However, the challenge remains in the identification of molecular mechanisms underlying the bioadhesion of a majority of biofouling organisms and the potential common effects of natural AF compounds. Increasing efforts and up-to-date techniques have been lately applied on this subject to identify and characterize different settlement and metamorphic transition processes as well as characterize bioadhesives (Chandramouli et al., 2012a, Chandramouli et al., 2012b, Gantayet et al., 2013, Thiyagarajan et al., 2009, Williams and Degnan, 2009, Zhang et al., 2010b). Also, the effects and modes of action of a range of AF natural compounds have been tested and identified against different biofouling species (Fusetani, 2011, Qian et al., 2013). Thus, there is a need to find common denominators mediating effectiveness in attachment that could be controlled by a specific/ a combination of mode(s) of action.

In this context, this review highlights the recent produced knowledge on identification and characterization of the main invertebrate macrofoulers settlement strategies and bioadhesive mechanisms, and also on the reported effects and modes of action described for effective and non-toxic natural AF compounds isolated from a variety of organisms towards invertebrate macrofouling species. This review aims to contribute to the identification of promise strategies to select broad-range natural AF compounds suitable for the development of effective environmentally friendly AF paints.

Diversity of invertebrate macrofouling adhesive strategies

Innate settling criteria

Despite biofouling adverse effects start with the formation of biofilms, the most disturbing component of this event is the colonization of hard foulers that constitute the macrofouling. Bryozoans, molluscs, barnacles, polychaetes and tunicates constitute the most dominant groups, which larval stages are induced to settle on selected underwater surfaces. Invertebrate larvae are able to actively select by prospection the most attractive place to adhere regarding many aspects as surface topology, wettability, chemistry, light exposure, streaming conditions, substrate color, among others(Aldred et al., 2006, Carl et al., 2012, Di Fino et al., 2014, Dobretsov et al., 2013b). This selection is specific on the species and is based on a combination of surface characteristics, also including biological cues (Kristensen et al., 2008). These biological settlement signals might involve both conspecific cues and extracellular polymeric substances (EPS) provided by bacteria. The constitution of previous formed biofilms will attract specific macrofouling species and repel others that in the same way might be attracted by other biofilm properties or even by biofilm-free surfaces(Qian et al., 2007, Hadfield, 2011a, Wahl et al., 2012). Such responses are well-documented in the main macrofouling species such as the bryozoan *Bugula neritina* (Dahms et al., 2004, Dobretsov and Qian, 2006), the polychaete *Hydroides elegans*(Lau et al., 2003, Harder et al., 2002, Chung et al., 2010, Shikuma et al., 2014),the mussels from the genus *Mytilus* (Bao et al., 2007, Carl et al., 2012, Satuito et al., 1995, Toupoint et al., 2012, Yang et al., 2008)and barnacle *Balanus amphitrite*(Harder et al., 2001, Zardus et al., 2008).In this context quorum sensing (QS) signals, responsible for biofilm formation, propagation and maturation in a density dependent cell-to-cell communication and gene regulation process, have been also found to have also a role in the regulation of settlement of

macrofoulers (Dobretsov et al., 2011, Dobretsov et al., 2009). Evidences show that the inhibition/induction of settlement is dependent on the nature of the bacterial biofilms regarding the production/ absence of proteolytic enzymes (Dobretsov et al., 2007).

Conspecific cues also play a crucial role as settlement inducers. Conspecific density and gregarious preferences are common characteristics among macrofouling species including mussels (Kobak, 2001, Ompi, 2011, Vooys, 2003), polychaetes (Qian, 1999)and barnacles (Aldred and Clare, 2008),however, the nature and potential of the responsible pheromones is underexplored in many of these species, except for barnacles which have been focus of extensive investigation. One of the described biogenic cues responsible for the gregarious settlement and species recognition during settlement in barnacles is a contact pheromone known as the settlement-inducing protein complex (SIPC)(Dreanno et al., 2006a, Dreanno et al., 2007, Elbourne and Clare, 2010). SIPC-like proteins are cuticular glycoproteins of high molecular mass (76-98 KDa), with lentil lectin (LCA)-binding sugar chains showing sequence similarities to a2-macroglobulin (A2M) protein family(Dreanno et al., 2006b, Matsumura et al., 1998).Based on identification and characterization of settlement-inducing proteins with similar molecular weight than SIPC, recent investigations considered SIPC as a component of the arthropod in protein complex (APC), the first peptide signal molecule attributed to promote gregarious settlement (Khandeparker and Anil, 2011, Knight-Jones, 1953). Other peptides have been identified as contributors including a glycyl-glycyl-L-arginine waterborn cue (GGR) which was described as a settlement stimulant in *B. Amphitrite* (Browne and Zimmer, 2001), and a different 32KDa protein showing the same properties (Endo et al., 2009). SIPC glycan profiling evidenced high mannose glicans content in the n-glycosation sites of SIPC gene encoding protein, supporting an interaction with mannose-binding lectins and exogenous mannose, thus increasing

settlement in *B. amphitrite* cypris larvae(Pagett et al., 2012). The effectiveness of EPS-like containing sugars as D-xylose, D-mannose and D-glucose in triggering barnacles settlement was also previously demonstrated(Khandeparker and Anil, 2011), however Pagett and co-workers (2012) concluded that it might be mannose, as a terminal monosaccharide, that contribute to the settlement of cyprids.

Settlement strategies

While the innate criteria that allow invertebrate larvae surface discrimination (settlement signals) seem to be more consistent among macrofouling species, the methods of settlement and metamorphosis are quite variable.

Bryozoans as *B. Neritina* form flexible bushy colonies as adults that release competent larvae (sexually produced zooids) that readily attach and metamorphose to start a new colony with growth capacity by asexual budding (Mihm et al., 1981). Larvae first attaches temporarily, after selection of the preferred substrate using its outer body surface and vibrative plume as sensitive parts, and then secretes a bioadhesive derived from the pyriform groove enriched in mucopolyssacharides (mucin)(Loeb and Walker, 1977).*B. neritina* larval attachment shows preferences by subtidal habitats and biofilm-associated substrates, although clean surfaces are also targets for this species(Dobretsov and Qian, 2006, Dahms et al., 2004). The final larval stage (late pre-ancestrula) permanently attaches using a different adhesive secreted by the internal sac, which contains proteic secretory material comprised in delimited granules (0.3μm)(Reed and Woollacott, 1983). A basal adhesion disc was recently described in pre-ancestrula stages constituted by elongated and interlocked fibrous cells, which seem to provide mechanical support during permanent settlement(Wong et al., 2012).Recent studies

using comparative proteomic and phosphoproteomic analysis of *B. neritina* larval stages demonstrated that larval metamorphosis might be mediated by simple changes in proteins phosphorylation status. After metamorphosis activation by environmental cues, several proteins turn down-regulated to permit sequential metamorphic changes (Thiyagarajan et al., 2009). Gene sequencing of different metamorphic pre-ancestrula stages permitted to map signal transduction pathways including Wnt/β-catenin pathway (important role in metamorphosis control), the mitogen-activated protein kinase (MAPK) pathway (regulatory role previously demonstrated in other invertebrate and vertebrate metamorphic species) and cell apoptosis pathway suggesting apoptosis as a mechanism of tissue reorganization in *B. neritina*(Wang et al., 2010, Wong et al., 2012). Further studies on proteome expression patterns during metamorphosis revealed down-regulation of proteins involved in energetic metabolism, structure, actin and tubulin depolarization, and up-regulation of transcription and transduction proteins. Also enabled the identification of key proteins such as histones, collagens, and the pheromone tempt in (known to have a role in breeding aggregations), illustrating major alterations in different characteristics during metamorphosis(Zhang et al., 2010a). Modifications of protein glycosylation pattern during larval transition stages were also demonstrated in *B. neritina,* predicted as a way to respond to oxidative stress situations and energetic unbalances (Chandramouli et al., 2012b).

Mussels and mussel final larvae (plantigrade) have the ability to select the settlement surface actively by exposing their foot and crawling and attachment is made by the production of byssal threads. Each new thread is formed in the ventral groove of the mussels foot and expands distally into adhesive plaques which mediate byssal thread attachment to a hard substratum (Warner and Waite, 1999). Threads are produced by the byssal apparatus,which is composed by different secretory glands responsible by the

production of different structural element of the byssus. The collagen gland produces byssal collagen core, the accessory gland is responsible for the proteinaceous cortex, phenol glands produce the proteinaceous adhesive and other glandular system is known to produce a sulphur-rich mucopolysaccharide(Coyne et al., 1997). The distribution of these proteins contribute to a structural differentiation of the byssus in terms of mechanical properties allowing resistance and an efficient attachment to high-energy surfaces and also a certain plasticity to reattach to new substrates by cutting off the bundle of byssal threads at the proximal stem (Coyne and Waite, 2000, Waite et al., 2005).

In contrast, barnacle cyprids, the final larval stage before settlement, have specialized antennulae with mecano- and chemoreceptors to do surface prospection, first exploring with deposition of a temporary proteinaceous glue (footprints) and then attaching permanently using an efficient adhesive cement (Aldred and Clare, 2008). The nature of these footprints was found to have homologies with the SIPC cuticular protein also functioning as a conspecific cue to other cyprids (Dreanno et al., 2007). The cement is a multi-protein complex produced in cement glands. Two types of secretory cells are described based on distinct histochemical and morphological characters apparently linked to different secretory processes:α cells which are histochemically positive for proteins, phenols including phenol oxidase enzyme; and β cells only histochemically positive under bromophenol blue staining(Odling et al., 2006, Walker, 1971).There are also two different types of cement in relation to protein composition: the primary cement is the one used for the initial attachment; the secondary cement is secreted by adult barnacles during wound healing or during partial reattachment. Thus, the primary cement has more adhesive properties, while secondary functions more as true cement, however, both share a similar amino acid composition(Aldred and Clare,

2008). To reach cyprid stage competent to settle, as a crustacean *B. Amphitrite* larval metamorphosis pass through 6 naupllia free-swimming stages. This metamorphosis processes were found to be regulated by different proteins expression and phosphorylation status, similarly to what was demonstrated for the bryozoan *B. neritina*, and despite their relative phylogenetic distance within invertebrates. In fact, different proteomic profiles were identified among the distinct larval stages permitting the identification of different processes occurring on each stage: significantly up-regulated proteins in cyprids were found to be involved in energy and metabolism functions, nervous system and signal transduction pathways (Chen et al., 2014). Proteome glycosylation pattern changes were also identified in *B. amphitrite* during larval development (Chandramouli et al., 2012b).A transcriptomic approach on *B. amphitrite* larval stages also permitted to identify several differentially expressed genes and functional groups including vitellogenin associated with late naupliar stages (may function as an energy source for non-feeding cyprids); mannose receptors that might be responsible for the advanced sensory system of cyprids; cement-proteins in cyprids involved in the adhesive secretion; receptor tyrosine kinases having a role in signal perception during cyprids settlement(Chen et al., 2011). This new molecular evidences suggest that crucial changes occurring in the late larval planktonic stages might condition the achievement of competent cyprids.

Among tubeworms, sabellariids (sandcastle worms) as *Phragmatopoma californica* also use proteinaceous cement for fixation as barnacles. This species do not represent an important biofouler, however presents useful features for studying wet adhesion(Sun et al., 2007). Their inhabiting tube is constructed with natural particulates as sand and shell particles, which are bonded together with tubeworm cement secreted from thoracic cement glands. Selected particles are transported through the tentacles to

the building organ located near the tubeworm mouth, joint with cement and placed at the end of the tube. The cement solidifies *via* covalent crosslinking through oxidative coupling of DOPA, locking in the final solid foam structure of the cement, while providing its crucial cohesive strength(Stevens et al., 2007, Stewart et al., 2004). Although sabellariids use available natural particles to construct its own tube, serpulid marine tubeworms as *Hydroides elegans*, the most associated with biofouling, developed a distinct fixation strategy by synthetizing their own complete mineralized structure(Nedved and Hadfield, 2008, Stewart et al., 2004).In this case, the secretion they produce is of inorganic-organic nature and its primary function is to be used in the construction of their own inhabiting tubes. The tubes and cement are composed of Mg-calcite, aragonite, or a combination of the two $CaCO_3$ polymorphs, and also soluble and insoluble organic matrices(SOM and IOM) composed of carboxylated and sulphated polysaccharides and striated and smooth collagen. After trochophore larvae prospection and selection of an EPS-enriched substrate that is known to help in $CaCO_3$ mineralization (Chung et al., 2010), metamorphosis occurs and tube production starts. In the process of tube formation, calcareous granules and organic components are secreted by calcium-secreting glands located anteriorly on the peristomium near the base of the collar that shapes the new produced material to form and quickly develop the inhabiting tubes. Epithelial mucocytes near the glandular openings also secrete additional organic components (Tanur et al., 2010, Chan et al., 2012). Organic sheets within CaCO3-mineralized layers of the shell and organic tube lining- containing collagen located on the inner wall of the tube shell, functions as a scaffold for biomineralization (Arias and Fernandez, 2008).

Adhesive mechanisms – new insights and recent approaches

Along with innate criteria and settlement strategies that show several common patterns among taxa, bioadhesive molecules responsible for the attachment to underwater surfaces also tend to have several base connecting points. First, its main peptidic constitution and also the high contents of specific amino acids as lysine (which provides more basic isoelectric points), glycine and serine (yielding conformational flexibility)(Callow et al., 2000). Also, modified composition of adhesive proteins either by the extensive use of amino acid post-translational modifications (PTMs) as in case of mussel byssus or by the simple employ of polar residues in barnacles. However, up-to-date identification and characterization of molecular adhesive mechanisms in different species have been demonstrating that the type of proteins is expected to condition the adhesion mechanisms. Barnacles and mussels have received particular attention in this context and more and more research studies have been conducted to pursue a full characterization of adhesion (Bandara et al., 2013, Kamino, 2013, Waite et al., 2005). Also, recent research has been focus in the detection and mapping of signal transduction pathways, which may play major roles during larval metamorphosis in macrofouling species.

Mussels

Byssal threads differentiated structure is primarily attributed to the occurrence of different collagenous proteins known as PreCol variants, with both adhesive and cohesive properties that ensure byssus mechanical properties asshock absorber(Coyne and Waite, 2000). In addition, protein families generically denominated *Mytilus* foot proteins (Mfps) were also found in the constitution of the adhesive plaque and threads

as both protective cuticular poteins (Mfp-1), matrix proteins (Mfp-2 and Mfp-4) and also adhesive proteins (Mfp-3 and Mfp-5) (Papov et al., 1995, Waite and Qin, 2001). Mefps are polar molecules all characterized by its post- or co-translational modified aminoacids such as the catecholic 3,4-dihydroxyphenylalanine (DOPA) present at around 20 mol% in Mfp-3 and Mfp-5 as adhesion promoter, 4-hydroxylated arginines and phosphorylated serines (Lin et al., 2007, Waite and Qin, 2001, Zhao et al., 2006). Similar proteins were also identified in other mussel species as *Dreissena polymorpha (*Gantayet et al., 2013).More recently, a new non-collagenous family of proteins was identified and characterized in byssus. A glycine (Gly)-, tyrosine (Tyr)- and asparagine (Asn)-rich protein family, named as thread matrix proteins (TMPs). TMPs are intimately associated with the preCOLs within the secretory granules and are present throughout the byssal threads, conferring new side chains and backbone structure heterogeneity given by the Asn residues deamidation, providing a viscoelastic matrix around collagenous fibers (Sagert and Waite, 2009).Thus, progresses have been made in the understanding of mussels adhesion mechanism, which is determined by the structure and interactions of mussel adhesion proteins (Table 1), and strongly dependent on different variables such as DOPA content, redox chemistry, metal interactions, and a synergisms among parameters (Hwang et al., 2010, Lauren and Wilker, 2007).

Tubeworms

Sabellariids (sandcastle tubeworms) composite tube building is mediated by amultipart adhesive cement, constituted by different reactive components, each one located in separate condensed granules of two types: homogenous and heterogenous. Three general protein families were first identified in *P. californica* bioadhesive, namely PC1, PC2 and PC3 (Table 1). The first two have basic pIs (around 10) and contain 10% DOPA, 30-45% Gly, 2-4% Cys and and Tyr residues modified to DOPA. PC3 is a

family of at least seven acidic variants with very high levels of Pho-Ser peptide repeats (60-90%) (Waite et al., 1992, Zhao et al., 2005). Ser-encoding PCR primers permitted to clone two close-related PC-3: PC-3A, highly rich in Ser residues (4-13) and punctuated with single Tyr residues; and and PC-3B, with shorter polySer-Tyr segments and with a non-repetitive C-terminal domain. Two more polybasic proteins, PC-4 and PC-5, rich in Lys and His residues, were recently identified (Wang and Stewart, 2012, Wang and Stewart, 2013). Immunolabeling techniques permitted to demonstrate that PC-2 and PC-5 are located in the homogenous granules whereas PC-1, PC3A,B and PC-4 are within the heterogenous granules(Wang and Stewart, 2012).This proteinaceous cement enrichment with DOPA and Pho-Ser residues is responsible for surface coupling and quinone-tanning of the cement during hardening, similarly to what happens in mussel byssus system(Stewart et al., 2004, Waite et al., 1992). Recent findings suggest that sabellariids cement contain both Mg^{2+} and Ca^{2+}, and also Cys-DOPA residues suggesting the occurrence of intermolecular crosslinking and also metal-inducing bonding as responsible for cement adhesive properties.The granular distinct contents of opposite charged polyelectrolytes, fuse together only outside the secretion organ(Sun et al., 2007). The combination of the physicochemical properties of these cement proteins, including aminoacid composition, occurrence of divalent cation species along with the environmental pH-shift between secretory gland and surrounding seawater permits to form a highly hydrophobic complex coacervate, which guarantees tubeworm wet adhesion(Wang and Stewart, 2013).

Serpulid tubeworms (e.g. *Hydroides* sp.) cement is composed of $CaCO_3$ polymorphs, and also soluble and insoluble organic matrices (SOM and IOM) composed of carboxylated and sulphated polysaccharides and striated and smooth collagen. SOM is enriched with Asp (8.4%), Glu (10.2%), Gly (14.5%) and Pro (21.4%) residues.

Sequences of acidic (Asp and Glu) and neutral (Gly) aminoacids have a role in Ca^{2+} binding and mineralization by the exposure of negatively charged aminoacids. Pro residues that show a high representativeness have a function on collagen stabilization and conformation(Tanur et al., 2010).

Barnacles

Similarly to mussel and tubeworm bioadhesives, barnacles cement adhesive properties are attributed to its main protein content (approximately 90% by weight) (Walker, 1972), however no DOPA residues were found so far in its proteins constitution contributing to adhesion, in contrast to mussel byssus and tubeworm cement (Kamino, 2013). The first reports on the primary structure of barnacles adhesive proteins were performed in *Megabalanus rosa* and five cement proteins have been identified, namely *Megabalanus rosa* cement protein Mrcp-100k, Mrcp-68k, Mrcp-52k (Kamino et al., 2000), Mrcp-20k (Kamino, 2001) and Mrcp-19k (Urushida et al., 2007) (Table 1). The first three Mrcps are larger and characterized by their insoluble nature and notable hydrophobicity. No homologies were found in published databases to highlight their function, however their presence seem to be essential for stabilizing the cement complex underwater and seem to constitute the bulk of the cement adhesive(Kamino et al., 2000). Mrcp-20k is characterized by multiple repeated Cys residues and charged amino acids including Asp (11.5%), Glu (10.4%) and His (10.4%). Mrcp-19k is heavily biased with Gly, Thr, Ser, Ala, Lys and Val (66%). Both Mrcp-20k and Mrcp-19k seem to have a role in preventing random aggregation of Mrcp-100k and Mrcp-52k during transport via the cement duct and surface coupling with substratum, with Mrcp-20k being more specific to calcified materials and Mrcp-19k being more eclectic to different surface constituents and presumably responsible for the very first contact with substratum(Mori et al., 2007). Post-translational modifications were not found in these

adhesive proteins, conversely to adhesive proteins from mussels, which are heavily modified to enable underwater attachment. In this case, evidences demonstrate that barnacles can deal with the adhesion challenge by using only simple proteins constituted by standard amino acids (Urushida et al., 2007, Kamino, 2013). Homologous genes of Mrcp-19k were isolated from *Balanus albicostatus* (Balcp19k) and *Balanus improvisus* (Bicp19k) with the same prevalence of the six amino acids, Ser, Thr, Ala, Gly, Val and Lys (69–80%mol) showing strong similarity to that of *M. rosa*, and thus suggesting an important common function for this protein in barnacle first contact bioadhesion (Urushida et al., 2007, (Liang et al., 2014).Two homologues of the Mrcp-20k, Bamcp20k-1 and Bamcp20k-2 respectively, were also recently cloned from *Balanus amphitrite*(Chen et al., 2011). Their different localization in secretory cells (Bamcp20k-1 at α cells and Bamcp20k-2 at β cells) and differential solubility (Bamcp20k-2 is insoluble in PBS buffer) suggest a distinct role in the process of adhesion(He et al., 2013). The insolubility of Bamcp20k-2 is attributed to crosslinking by disulfide bonds as the PI value for these homologue protein was found to be more acidic than Bamcp20k-1.

Modes of action and effectiveness of natural AF products

Along with the scientific development on the bioadhesive mechanisms of invertebrate biofouling species, progresses have been also achieved in the search, characterization and development of alternative AF products. To reach more environmentally friendly alternatives, research is focusing on natural compounds used for chemical defense and /or metabolic processes in a variety of marine organisms (Fusetani, 2011). A wide diversity of natural AF compounds has been purified so far

including mainly lactones, furanes, peptides, phenols, carotenoids, alkaloids and terpenoids. Furan and lactone rings have been considered important functional groups for antifouling activity and indications point that not only the functional group but also their lipophilicity are essential for the bioactivity (Xu et al., 2010, Clare et al., 1999). Advantages have been recently taken from bioactive compounds extracted from microorganisms as bacteria, cyanobacteria, fungi and also seaweeds, from which a wide diversity of metabolites, autoinducers and toxins are produced and large biomass amounts are easier to obtain (Dobretsov et al., 2013a).

The challenge for the selection of promise AF candidates might include a mechanism-oriented approach permitting to select broad-spectrum effect non-toxic products that do not act as biocidal agents. In line with the Biocidal Product Regulation (EU) 528/2012, a clear description of the mode of action, biological targets and environmental fate of new products is now required to their introduction into the market. Thus, recent research approaches on this matter are focusing on the identification of AF mechanisms (Qian et al., 2013). This led to recognize that AF compounds acting on more specific targets show less toxic responses and thus are more effective in biofouling inhibition. The evaluation of the AF effectiveness versus toxicity is generally considered on screening studies for new natural AF compounds using the effective concentration causing 50% of AF activity (EC_{50}) and the 50% lethal concentration (LC_{50}). A standard requirement of EC_{50}<25µg/mL was established by the U.S. Navy program as an efficacy level for natural antifouling agents (Thiyagarajan et al., 2004). The quotient given by those indexes (LC_{50}/EC_{50}), known as the therapeutic ratio, is generally considered as an index of potential in which values higher than 15 indicate a promise non-toxic AF agent, and much higher values lead to consider suitable AF candidates (Qian et al., 2010b). The most commonly used AF bioassays for this

purpose is by using the anti-settlement activity of representatives of the macrofouling community to reach a broad range effect AF compound. Thus, the screening bioassays for suitable AF candidates might include a measure of whole-organism effectiveness vs toxicity (therapeutic ratio) and also provide evidences of the mode of action, target functions, processes or signaling pathways somehow involved in the settlement and/or on bioadhesive mechanims of different macrofouling species. Ultimately, this knowledge could be very important in the development/improvement of new and faster AF screening bioassays.

Considering this, an overview of the recently identified natural AF candidates towards macrofouling, its suitability regarding effectiveness vs toxicity (LC50/EC50 ratio) and the attributed mechanims of AF action regarding the main macrofouling species are presented in this review (Table 2).

Anti-bioadhesion

Inhibition of macrofouling adhesion has been described as possibly induced by target non-biocidal mechanisms. This inhibition may act by simply modifying the attachment surface, by removing superficial inductive proteinaceous clues or detergent-preventing adhesion; by inhibiting biofilms occurrence by different mechanisms which are crucial for the subsequent macroadhesion; and by the inhibition of macrofoulers adhesive production itself, release/cleavage of proteinaceous adhesives, reduce adhesive strength, induce repellent compounds production (Dobretsov et al., 2007, Qian et al., 2013). Some potential natural AF candidates from a wide diversity of source species have been found to produce anti-settlement bioactivity towards invertebrate macrofoulers attributed to several of these anti-bioadhesion targets (Table 2).

A proteolytic enzyme extracted from the bacteria *Pseudoalteromonas issachenkonii* UST041101-043 showed relevant bioactivity (EC_{50}= 0.001 μg/ml) towards anti-settlement of the bryozoans *Bugula neritina*, and *Schizoporella* sp. and also the barnacle *B. amphitrite* attributed to the removal of proteinaceous clues apparently essential for the successional colonization of biofouling species (Dobretsov et al., 2007). The interference in bacterial community is also described as a target in the prevention of macrofouling adhesion, either by the selection of a particular biofilm constitution or by simply inhibiting biofilms propagation (quorum sensing inhibition). The sponge *Acanthella cavernosa* was found to produce compounds (namely kalihinol A and 10-B-formamidokalihinol A) able to induce anti-settlement of *B. amphitrite* cyprids with high AF potential (LC50/EC50 ratio around 200 and 2000, respectively), given its ability to mediate the growth of specific biofilms (Yang et al., 2006). Diketopiperazines and benzene-type secondary metabolites extracted from the bacterium *Pseudomonas rhizospherae* act as quorum sensing inhibitors preventing the adhesion of *B. neritina* and *B. amphitrite* larvae, some with promise therapeutic effect (LC50/EC50 =17;22) concerning anti-settlement activity (Qi et al., 2009). Also, diketopiperazines extracted from *Streptomyces fungicidicus* show bioactivity in preventing *B. amphitrite* cyprids attachment (Li et al., 2006), enhancing the potential of this type of compounds for AF purposes. The anti-settlement activity by detergent prevention of biofilm adhesion and lysis of microorganism was also pointed for the compound polymeric alkylpyridinium salts (Poly-APS) extracted from the marine sponge *Reniera sarai* (Turk et al., 2007). Other studies provided evidence that Poly-APS also exert anti-settlement activity in *B. amphitrite* mediated by a neurotransmission disturbance (acetylcholinesterases inhibition). Poly-APS also showed AF potential

given its effectiveness and low toxicity (EC50= 0.27 μg/ml; LC50/EC50= 111) (Faimali et al., 2003b, Sepcic and Turk, 2006).

Several biofilms themselves may act as anti-bioadhesives. Active compounds (Poly-ethers A and B) extracted of the bacterium *Winogradskyella poriferorum*, known as a biofilm inhibitor, showed bioactivity towards larval settlement inhibition in *B. amphitrite* and *H. elegans,* with high AF potential (LC50/EC50 ratio between 40 and 50) towards two different macrofouling species (Dash et al., 2009, Dash et al., 2011). Other anti-epibiotic compounds have also been described as potential AF compounds from other source taxa, as sponges, macroalgi and fungi. Hydroquinone-C acetate and dihydrofurospongin II from the sponges *Ircinia spinosa* and *Cacosponjia scalaris*, respectively, induced larval settlement inhibition in *B. amphitrite*, and also cytochalasin-analogues extracted from the soft-coral associated fungus *Aspergillus elegans* (Zheng et al., 2013, Hellio et al., 2005). Different compounds (isorhodoptilometrin, citreorosein, emodin and 6,8,5'6'- tetrahydroxy-3'-methylflavone) extracted from the marine gorgonian coral-associated fungus *Penicillium* sp. SCSGAF0023 also inhibited the larval settlement of *B. amphitrite* and showed AF potential based on the low EC50 levels (6.1-17.9) (Bao et al., 2013). The macroalga *Delisea pulchra* produce bioactive halogenated furanones, with efficient antibacterial activity and also anti-settlement activity towards *B. amphitrite* (de Nys et al., 1995). Analogues of these furanone compounds were used successfully as bioactive ingredient of "Netsafe" and "Pearlsafe" antifouling paints (Raveendran and Mol, 2009). 3-chloro-2,5-dihydroxybenzyl alcohol extracted from the fungus *Ampelomyces* sp., with recognized anti-bacterial activity, also inhibited larval settlement in *B. neritina* and *H. elegans,* however only showing AF effectiveness against *B. neritina* (LC50/EC50=

88.9), and some precocious toxicity to *H. elegans* (LC50/EC50= 3.77) (Kwong et al., 2006). This precludes its classification as a broad-spectrum promise AF candidate.

Some natural compounds have been also described only for its potential to inhibit invertebrate species attachment. Capsaicin-analogue compounds from the chili pepper plants *Capsicum frutenscens* were able to inhibit mussels *(Dreissena polymorpha)* attachment (Angarano et al., 2007). These compounds were recently found to induce blocking of bacteria surface attachment sites (Peng et al., 2012), and thus this might be at least one of the target mechanisms contributing to the anti-settlement capacity of capsaicins. A few other compounds extracted from a cyanobacteria (*Lyngbya majuscula*), including isolmajusculamide, majusculamide A and dolastatin 16 revealed high bioactivity against *B. amphitrite* larval settlement and great AF potential with terapeutic ratios of 77, 111 and 6666, respectively (Tan et al., 2010). Despite this apparent potential and other advantages related with their microorganism nature, cyanobacterial is still a quite unexplored group regarding promise AF compounds towards macrofouling species, and at the best of our knowledge this study applying *L. majuscula* is the single study testing the potential of cyanobacterial-produced metabolites in anti-settlement bioassays. Some other compounds were identified as potential AF compounds against larval settlement extracted from fungus species. The alkaloid penispirolloid A presented a promise EC50 (2.4 μg/ml) towards the anti-settlement activity of *B. neritina* (He et al., 2012)*;* cochliomycin A, zeaenol *and* LL-Z1640-1 were extracted from *Cochliobolus lunatus* and tested as *B. amphitrite* larval settlement inhibitors with promise EC50 values of 1.2, 5 and 5.3 μg/ml, respectively(Shao et al., 2011b); aspergilone A from *Aspergillus* sp. also showed a promise EC50 value of approximately 7 μg/ml for the same endpoint (Shao et al., 2011a). From sponges, *Acanthella cavernosa* produce other kind of compounds

(sesquiterpenes) with potent bioactivity towards *B. amphitrite* settlement (EC50= 0.14-7.2 µg/ml) and isocianide 1, a very promise compound towards the same bioactivity with very competitive levels conferring effectiveness of this natural compound (EC50= 0.046 µg/ml and LC50/EC50 higher than 650) (Nogata and Kitano, 2006, Nogata et al., 2003). Extracted from *Dysidea avara,* the modified metabolite 4'-propylthioavarone was considered efficient with a therapeutic ratio higher than 40 (Tsoukatou et al., 2007). Plants also contributed to the knowledge on this field of promise natural AF compounds with several identified bioproducts including flavone and isoflavone derivatives from terrestrial plants extracts, genistein from soybeans and recently piperamides from the species *Piper betle* (Table 2) (Huang et al., 2014, Zhou et al., 2009b). In this last particular case, two compounds (piperoleine B and piperine) were selected as potent settlement inhibitors in three macrofouling species, *B. amphitrite*, *H. elegans* and *B. neritina* showing strong broad-spectrum AF effectiveness, both in terms of EC50 values (ranging from 0.7 to 1.6 µg/ml) and therapeutic ratios (Table 2). Promise compounds have been also extracted from soft corals including from the species *Leptogorgia virgulata* (pukalide) and *Renilla reniformis* (renillafoulin A) with very low EC50 values (0.05 and 0.02, respectively) towards the anti-settlement of *B. amphitrite* cypris (Keifer et al., 1986, Rittschof et al., 1985).

More specific targets of anti-settlement bioactivity have been proposed related with mechanisms of adhesion. Inhibition of mussels efficient attachment, for instance, was attributed to an interference with redox reactions occurring during the formation of the byssus in *D. polymorpha* exposed to L-dopa and phenolic anti-oxidants from plant extracts. These compounds showed high effectiveness as AF products (EC50=0.4-29; LC50/EC50= 125) (Cope et al., 1997), and this bioactivity seems to deal with the main constitution of byssal threads, heavily composed by dopa-containing proteins.

The inhibitory effect of some potential AF compounds towards the activity of key enzymes related with the metabolism of adhesion has also been described. The enzyme phenoloxidase, responsible for a step in the production of mussels byssal thread plaques, by an oxidation process to convert phenols into 0-quinones, was found to be inhibited by meroditerpenoids from the macroalga *Cystoseira baccata* (Mokrini et al., 2008), bastandins from the sponge *Ianthella basta* (Bayer et al., 2011), n-substitued imides (Zentz et al., 2002), and other natural organic extracts derived from macroalgae (Cope et al., 1997, Hellio et al., 2000), with concomitant inhibition of mussels (*Mytillus edulis*) attachment (Table 2). Chitinase activity, responsible for the hydrolyze of chitin into oligomers of *N*-acetyl-d-glucosamine, important in the moult cycle of barnacle cyprids, was inhibited by styloguanidines extracted from the marine sponge *Stylotella aurantium*, acting in preventing barnacles cyprids adhesion (Kato et al., 1995).

New source species were also searched for potential natural AF bioproducts in preliminary studies using biological extracts. Attachment inhibition ability was reported for biofilm exudates of *Baccilus* and *Macrococcus* towards the attachment of the mussel *Aulacomya maoriana* (Alfaro et al., 2011). Organic extracts of the sea hare *Notarchus leachii cirrosus* and the bryozoan *B. neritina*, usually tested as a target biofouling species, revealed bioactivity towards settlement inhibition of another species of barnacles, *Balanus albicostatus* (EC50 around 20 μg/ml) (Feng et al., 2011). These kind of studies based on biological extracts showing promise AF activity are a good bottom line for bioassay-guided approach to reach pure AF compounds.

Neurotransmission disruption

Compounds that somehow exert neurotransmission disruption have also been found to induce anti-settlement of invertebrate larvae, as a number of neurotransmitters

and their derivatives are settlement and metamorphosis inducers of several marine invertebrates (Faimali et al., 2003a). During the exploration period larvae are known to follow particular cues (settlement signals) to select the substrate and metamorphose. These cues originate cascade signaling pathways in which will intervene a range of molecules including neurotransmitters (Gohad et al., 2012). Studies on the anti-settlement potential of vertebrates self-stable a_2-adrenoreceptor agonists used as veterinary sedative agents (medetomidine) have revealed positive results against *B. improvisus* cyprids settlement, attributed to activation of octopamine receptors with concomitant hyperactivity in locomotor response (Dahlstrom et al., 2000, Lind et al., 2010). Also, other imidazoline-type compounds (Dahlström et al., 2005), cetirizine, mizolastine (antagonists of histamine) (Zhou et al., 2009a), and several signaling influencing compounds (Rittschof et al., 2003, Jin et al., 2014) were found to inhibit larval settlement in *B.* amphitrite. The dopamine receptor antagonist SCH23390 was also found to inhibit barnacles settlement by interfering with the successful release of the adhesive cement (Martensson and Gunnarsson, 2005). Serotonergic neurons are also believed to be involved in cyprids settlement as serotonin is known to mediate signal transduction inducing larval settlement and serotonin-uptake blockers produce the inverse response in cyprids successful attachment (Yamamoto et al., 1996). L-tryptophan, and dopamine neurotransmitters were also found to promote larval searching behavior and settlement in *B. amphitrite* (Kon-ya et al., 1995, Yamamoto et al., 1999). Concerning compounds from natural sources, 2,5,6-tribromo-1-methyl gramine, a competitive serotonin antagonist extracted from the bryozoan *Zoobotryon pellucidum* showed effective bioactivity towards the anti-settlement of *B. amphitrite* (EC50=0.015) and also *M. edulis* (Kon-ya et al., 1994). The analogue compound 5,6-

dichlorogramine was already tested as coating in an acryl board surface submerged in seawater, and its AF activity was validated for at least two months (Omae, 2003).

The enzyme acetylcholinesterase (AChE), responsible for modulating motor and cognitive functions in synaptic neurons by the maintenance of the neurotransmitter acetylcholine, is also believed to play a fundamental role in the permanent attachment of cyprids (Faimali et al., 2003a). If so, compounds able to inhibit AChE at very low concentrations might be promise AF compounds. It is the case of poly-APS extracted from the sponge *R. sarai,* previously mentioned as effective towards *B. amphitrite.* Thus, all this indirect anti-adhesion mechanisms based on neurotransmitters are suitable to be applied as biochemical screening methods for natural AF compounds.

Metabolic-signaling pathways inhibition

As the modes of action compromising bioadhesion are generally tested alone (just anti-settlement response) or in synergy of few of them (i.e. anti-settlement and a biochemical endpoint), poor information about the antifouling targets are reached. However, biochemical responses, molecular changes and metabolic-signaling pathways alterations occur behind general mechanisms effectively contributing to the inhibitory effect. The recent characterization studies on gene sequencing, proteome and phosphoproteome of biofouling invertebrate larvae during settlement and metamorphosis provided a base to explore the molecular effects of promise AF compounds towards this species (Chen et al., 2014, Han et al., 2013, Wang et al., 2010, Zhang et al., 2010a, Zhang et al., 2010b). As a consequence, research efforts have been made recently to reach more specific AF targets (Table 2).

Butenolide and analogue compounds from the bacteria *Streptomyces albidoflavus* have been deeper investigated as a promise natural AF compound. First

described as a potent and broad-range effective larval settlement inhibitor for *B. amphitrite* (EC50= 0.518 μg/ml; LC50/EC50≈ 97), *B. neritina* (EC50= 0.199 μg/ml; LC50/EC50≈ 251) and *H. elegans* (EC50= 0.0168 μg/ml; LC50/EC50≈ 119)(Xu et al., 2010), butenolides were found to affect abundance and phosphorylation status of structural proteins, mitochondrial peptidases, Ca^{2+}binding proteins and molecular chaperones in *B.neritina* (Qian et al., 2010a), and influenced the primary metabolism of energy production (ACAT1 and ACADVL) in *B. amphitrite* and *B. neritina* (Zhang et al., 2012b). These altered mechanisms were proposed as targets for the anti-settlement potential of these compounds at the observed low concentrations. Alterations in the redox-regulatory mechanims of settlement were demonstrated in *B. amphitrite* cyprids exposed to poly-ether B extracted from the bacterium *Winogradskyella poriferorum* (Dash et al., 2012), compound that was previously selected as a promise AF agent towards this species and *H. elegans* (Dash et al., 2011). Also, the compound methyltetradecanoid acid (12-MTA) extracted from the bacterium *Streptomyces* sp. showed anti-settlement potential towards *H.elegans* larvae (EC50= 0.6 μg/ml; LC50/EC50≈ 133), concomitant with alteration in the expression of genes important for larval settlement (Xu et al., 2009). Meleagrin extracted from the fungus *Penicillium* sp. influenced protein expression involved in metabolic pathways, also in extra-cellular matrix receptor interactions, actin cytoskeleton reactions and inhibition of cypris major protein of *B. amphitrite* associated with settlement inhibition (Han et al., 2013). Compounds extracted from sponges with previously described AF potential were also studied for specific targets. It is the case of Isocianide 1 extracted from *Acanthella cavernosa* that promotes binding and alteration of specific proteins including a 30KDa voltage-dependent anion channels protein, a phosphorylation precursor (NADH-ubiquinone oxireductase) and cytochrome P450 in both *B. amphitrite* and *B. neritina*

(Zhang et al., 2012a); bastadins from *Ianthella basta* was previously reported to inhibit the activity of phenoloxidase and efficient attachment in mussels, was also found to inhibit *B. improvisus* larval settlement attributed to induced alterations on intracellular Ca^{2+} levels (Ortlepp et al., 2007).

All this mechanistic and molecular responses give valuable insights to the understanding of how bioactive compounds act in the target organisms and which repercussions it may cause to macrofouling organisms populations and also for the surrounding environment, in the case of its application as a natural AF agent. As so there is a need to invest in further studies on specific modes of action of natural bioactive compounds using comparative genomic and proteomic approaches, taking advantages of the recent base knowledge on transcriptomic and proteomic profiling of the main macrofouling species. This knowledge is also of great importance in the improvement of screening techniques towards new bioactive compounds, as novel molecular bioassays or biomarkers based on this specific molecular AF targets might be developed.

An overview of the current scenario of natural AF candidates shows that a large amount of compounds have been described as anti-bioadhesives based only on anti-settlement activity, to less of them have been designated a more specific mode of action and to very few compounds have been attributed AF target(s). From these last well-studied compounds few have been included in AF paints as active ingredients mainly due to their poor yields which raises commercial supply issues (Fusetani, 2011, Qian et al., 2013). Some alternatives have been developed to overcome this question including the search for AF candidates from easy culturable natural resources and/or by the chemical synthesis of promise compounds, like for example isocyanides (Kitano et al., 2011). Also, in the pursuit for potent AF compounds, structural optimization studies

based on the structure-activity relationships (SAR) have been applied to promise candidates to identify the elements responsible for bioactivity. In fact, this review shows that despite the wide variety of compounds identified from very different source species, similar or even the same anti-settlement targets are often reached for unrelated compounds. This suggests that common structural elements/functional groups might be responsible for recognized AF activity. In the case of the compound butenolide, for instance, recent studies showed that furan and furanone functional groups are responsible for AF bioactivity (Li et al., 2012). Thus, all these research approaches are of great importance for the identification of natural eco-friendly AF compounds suitable to be included in AF coatings and eligible to be commercialized unreservedly.

Conclusions

A considerable amount of diverse natural AF compounds have been isolated, characterized, and considered effective and non-toxic based on toxicological assumptions. However there is still a gap to fill in the research for their mode of action, specific targets, presumable environmental fate and thus their potential to be incorporated on effective AF paints. This necessity enhanced efforts in developing more knowledge on molecular identity of biofouling species, particularly regarding larval settlement and bioadhesive mechanisms via transcriptome description (Chen et al., 2011), gene expression (Lin et al., 2013) and proteomic profiling (Han et al., 2013, Chen et al., 2014, Thiyagarajan et al., 2009, Zhang et al., 2010b) as described in this review. This recent knowledge provides valuable tools to future comparative studies on molecular expression towards promise AF compounds. The molecular evidences of biological changes during larval transition stages and concerning bioadhesive molecules should help in the understanding of AF compounds the modes of action and specific

targets. This potential is starting to be exploited in some recent studies, in which specific molecular targets were identified as being responsible for the AF bioactivity potential. This seems to indicate that the strategies for screening potential AF products might include the evaluation of AF specific targets along with the elucidation of the anti-settlement efficacy vs toxicity in the main macrofouling species. Regarding the promise compounds with already identified AF targets, research efforts would focus now on increasing commercial suitability and potency by chemical synthesis and structural optimization for maximizing bioactivity.

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References

Aldred, N. & Clare, A.S. (2008). The adhesive strategies of cyprids and development of barnacle-resistant marine coatings. *Biofouling,* 24**:** 351-63. DOI 10.1080/08927010802256117

Aldred, N., Ista, L.K., Callow, M.E., Callow, J.A., Lopez, G.P. & Clare, A.S. (2006). Mussel (Mytilus edulis) byssus deposition in response to variations in surface wettability. *J Roy Soc Interface,* 3**:** 37-43. DOI 10.1098/rsif.2005.0074

Alfaro, A.C., Young, T. & Ganesan, A.M. (2011). Regulatory effects of mussel (Aulacomya maoriana Iredale 1915) larval settlement by neuroactive

compounds, amino acids and bacterial biofilms. *Aquaculture,* 322**:** 158-168. DOI DOI 10.1016/j.aquaculture.2011.08.038

Angarano, M.B., Mcmahon, R.F., Hawkins, D.L. & Schetz, J.A. (2007). Exploration of structure-antifouling relationships of capsaicin-like compounds that inhibit zebra mussel (Dreissena polymorpha) macrofouling. *Biofouling,* 23**:** 295-305. DOI 10.1080/08927010701371439

Arias, J.L. & Fernandez, M.S. (2008). Polysaccharides and proteoglycans in calcium carbonate-based biomineralization. *Chemical reviews,* 108**:** 4475-82. DOI 10.1021/cr078269p

Bandara, N., Zeng, H.B. & Wu, J.P. (2013). Marine mussel adhesion: biochemistry, mechanisms, and biomimetics. *J Adhes Sci Technol,* 27**:** 2139-2162. DOI Doi 10.1080/01694243.2012.697703

Bao, J., Sun, Y.L., Zhang, X.Y., Han, Z., Gao, H.C., He, F., Qian, P.Y. & Qi, S.H. (2013). Antifouling and antibacterial polyketides from marine gorgonian coral-associated fungus Penicillium sp SCSGAF 0023. *J Antibiot,* 66**:** 219-223. DOI Doi 10.1038/Ja.2012.110

Bao, W.Y., Satuito, C.G., Yang, J.L. & Kitamura, H. (2007). Larval settlement and metamorphosis of the mussel Mytilus galloprovincialis in response to biofilms. *Mar Biol,* 150**:** 565-574. DOI Doi 10.1007/S00227-006-0383-4

Bayer, M., Hellio, C., Marechal, J.P., Frank, W., Lin, W.H., Weber, H. & Proksch, P. (2011). Antifouling Bastadin Congeners Target Mussel Phenoloxidase and Complex Copper(II) Ions. *Mar Biotechnol,* 13**:** 1148-1158. DOI Doi 10.1007/S10126-011-9378-3

Browne, K.A. & Zimmer, R.K. (2001). Controlled Field Release of a Waterborne Chemical Signal Stimulates Planktonic Larvae to Settle. *The Biological Bulletin,* 200**:** 87-91.

Burgess, J.G., Boyd, K.G., Armstrong, E., Jiang, Z., Yan, L.M., Berggren, M., May, U., Pisacane, T., Granmo, A. & Adams, D.R. (2003). The development of a marine natural product-based antifouling paint. *Biofouling,* 19**:** 197-205. DOI Doi 10.1080/0892701031000061778

Callow, J.A., Stanley, M.S., Wetherbee, R. & Callow, M.E. (2000). Cellular and molecular approaches to understanding primary adhesion in Enteromorpha: an overview. *Biofouling,* 16**:** 141-150. DOI 10.1080/08927010009378439

Carl, C., Poole, A.J., Sexton, B.A., Glenn, F.L., Vucko, M.J., Williams, M.R., Whalan, S. & De Nys, R. (2012). Enhancing the settlement and attachment strength of pediveligers of Mytilus galloprovincialis by changing surface wettability and microtopography. *Biofouling,* 28**:** 175-86. DOI 10.1080/08927014.2012.662676

Chan, V.B., Li, C., Lane, A.C., Wang, Y., Lu, X., Shih, K., Zhang, T. & Thiyagarajan, V. (2012). CO(2)-driven ocean acidification alters and weakens integrity of the calcareous tubes produced by the serpulid tubeworm, Hydroides elegans. *PloS one,* 7**:** e42718. DOI 10.1371/journal.pone.0042718

Chandramouli, K.H., Reish, D. & Qian, P.-Y. (2012a). Gel-Based and Gel-Free Identification of Proteins and Phosphopeptides during Egg-to-Larva Transition in Polychaete Neanthes arenaceodentata. *PloS one,* 7**:** e38814.

Chandramouli, K.H., Zhang, Y., Wong, Y.H. & Qian, P.-Y. (2012b). Comparative Glycoproteome Analysis: Dynamics of Protein Glycosylation during Metamorphic Transition from Pelagic to Benthic Life Stages in Three Invertebrates. *Journal of Proteome Research,* 11**:** 1330-1340.

Chen, Z.F., Matsumura, K., Wang, H., Arellano, S.M., Yan, X.C., Alam, I., Archer, J.a.C., Bajic, V.B. & Qian, P.Y. (2011). Toward an Understanding of the

Molecular Mechanisms of Barnacle Larval Settlement: A Comparative Transcriptomic Approach. *PloS one,* 6. DOI DOI 10.1371/journal.pone.0022913

Chen, Z.F., Zhang, H., Wang, H., Matsumura, K., Wong, Y.H., Ravasi, T. & Qian, P.Y. (2014). Quantitative Proteomics Study of Larval Settlement in the Barnacle Balanus amphitrite. *PloS one,* 9**:** e88744. DOI 10.1371/journal.pone.0088744

Chung, H.C., Lee, O.O., Huang, Y.L., Mok, S.Y., Kolter, R. & Qian, P.Y. (2010). Bacterial community succession and chemical profiles of subtidal biofilms in relation to larval settlement of the polychaete Hydroides elegans. *Isme J,* 4**:** 817-828. DOI Doi 10.1038/Ismej.2009.157

Cima, F. & Ballarin, L. (2012). Immunotoxicity in ascidians: Antifouling compounds alternative to organotins III - The case of copper(I) and Irgarol 1051. *Chemosphere,* 89**:** 19-29. DOI DOI 10.1016/j.chemosphere.2012.04.007

Clare, A.S. (1996). Marine natural product antifoulants: Status and potential. *Biofouling,* 9**:** 211-229.

Clare, A.S., Rittschof, D., Gerhart, D.J., Hooper, I.R. & Bonaventura, J. (1999). Antisettlement and Narcotic Action of Analogues of Diterpene Marine Natural Product Antifoulants from Octocorals. *Mar Biotechnol (NY),* 1**:** 427-0436.

Cope, W.G., Bartsch, M.R. & Marking, L.L. (1997). Efficacy of candidate chemicals for preventing attachment of zebra mussels (Dreissena polymorpha). *Environmental Toxicology and Chemistry,* 16**:** 1930-1934. DOI Doi 10.1897/1551-5028(1997)016<1930:Eoccfp>2.3.Co;2

Coyne, K.J., Qin, X.X. & Waite, J.H. (1997). Extensible collagen in mussel byssus: A natural block copolymer. *Science,* 277**:** 1830-1832. DOI DOI 10.1126/science.277.5333.1830

Coyne, K.J. & Waite, J.H. (2000). In search of molecular dovetails in mussel byssus: from the threads to the stem. *The Journal of experimental biology,* 203**:** 1425-31.

Dahlström, M., Lindgren, F., Berntsson, K., Sjögren, M., Mårtensson, L.G.E., Jonsson, P.R. & Elwing, H. (2005). Evidence for different pharmacological targets for imidazoline compounds inhibiting settlement of the barnacle Balanus improvisus. *Journal of Experimental Zoology Part A: Comparative Experimental Biology,* 303A**:** 551-562. DOI 10.1002/jez.a.163

Dahlstrom, M., Martensson, L.G.E., Jonsson, P.R., Arnebrant, T. & Elwing, H. (2000). Surface active adrenoceptor compounds prevent the settlement of cyprid larvae of Balanus improvisus. *Biofouling,* 16**:** 191-+.

Dahms, H.U., Dobretsov, S. & Qian, P.Y. (2004). The effect of bacterial and diatom biofilms on the settlement of the bryozoan Bugula neritina. *J Exp Mar Biol Ecol,* 313**:** 191-209. DOI Doi 10.1016/J.Jembe.2004.08.005

Dahms, H.U., Ying, X. & Pfeiffer, C. (2006). Antifouling potential of cyanobacteria: a mini-review. *Biofouling,* 22**:** 317-327. DOI Doi 10.1080/08927010600967261

Daoud, G.I., Veron, B., Beach, E., Kudyk, J., Banaigs, B., Patel, A.V., Pope, J., Hayes, P.K. & Hellio, C. (2011). Biological Activity and Chemical Fingerprinting of Microalgal Extracts for Antifouling Applications. *European Journal of Phycology,* 46**:** 60-60.

Dash, S., Chandramouli, K.H., Zhang, Y. & Qian, P.Y. (2012). Effects of poly-ether B on proteome and phosphoproteome expression in biofouling Balanus amphitrite cyprids. *Biofouling,* 28**:** 405-415. DOI Doi 10.1080/08927014.2012.679731

Dash, S., Jin, C.L., Lee, O.O., Xu, Y. & Qian, P.Y. (2009). Antibacterial and antilarval-settlement potential and metabolite profiles of novel sponge-associated marine

bacteria. *J Ind Microbiol Biot,* 36**:** 1047-1056. DOI DOI 10.1007/s10295-009-0588-x

Dash, S., Nogata, Y., Zhou, X.J., Zhang, Y.F., Xu, Y., Guo, X.R., Zhang, X.X. & Qian, P.Y. (2011). Poly-ethers from Winogradskyella poriferorum: Antifouling potential, time-course study of production and natural abundance. *Bioresource Technol,* 102**:** 7532-7537. DOI DOI 10.1016/j.biortech.2011.05.034

De Nys, R., Steinberg, P.D., Willemsen, P., Dworjanyn, S.A., Gabelish, C.L. & King, R.J. (1995). Broad-Spectrum Effects of Secondary Metabolites from the Red Alga Delisea-Pulchra in Antifouling Assays. *Biofouling,* 8**:** 259-271.

Di Fino, A., Petrone, L., Aldred, N., Ederth, T., Liedberg, B. & Clare, A.S. (2014). Correlation between surface chemistry and settlement behaviour in barnacle cyprids (Balanus improvisus). *Biofouling,* 30**:** 143-152. DOI 10.1080/08927014.2013.852541

Dobretsov, S., Abed, R.M. & Teplitski, M. (2013a). Mini-review: Inhibition of biofouling by marine microorganisms. *Biofouling,* 29**:** 423-41. DOI 10.1080/08927014.2013.776042

Dobretsov, S., Abed, R.M.M. & Voolstra, C.R. (2013b). The effect of surface colour on the formation of marine micro and macrofouling communities. *Biofouling,* 29**:** 617-627.

Dobretsov, S., Dahms, H.U. & Qian, P.Y. (2006). Inhibition of biofouling by marine microorganisms and their metabolites. *Biofouling,* 22**:** 43-54. DOI Doi 10.1080/08927010500504784

Dobretsov, S. & Qian, P.Y. (2006). Facilitation and inhibition of larval attachment of the bryozoan Bugula neritina in association with mono-species and multi-species biofilms. *J Exp Mar Biol Ecol,* 333**:** 263-274. DOI Doi 10.1016/J.Jembe.2006.01.019

Dobretsov, S., Teplitski, M., Bayer, M., Gunasekera, S., Proksch, P. & Paul, V.J. (2011). Inhibition of marine biofouling by bacterial quorum sensing inhibitors. *Biofouling,* 27**:** 893-905. DOI Doi 10.1080/08927014.2011.609616

Dobretsov, S., Teplitski, M. & Paul, V. (2009). Mini-review: quorum sensing in the marine environment and its relationship to biofouling. *Biofouling,* 25**:** 413-427. DOI 10.1080/08927010902853516

Dobretsov, S., Xiong, H.R., Xu, Y., Levin, L.A. & Qian, P.Y. (2007). Novel antifoulants: Inhibition of larval attachment by proteases. *Mar Biotechnol,* 9**:** 388-397. DOI DOI 10.1007/s10126-007-7091-z

Dreanno, C., Kirby, R.R. & Clare, A.S. (2006a). Locating the barnacle settlement pheromone: spatial and ontogenetic expression of the settlement-inducing protein complex of Balanus amphitrite. *P Roy Soc B-Biol Sci,* 273**:** 2721-2728. DOI Doi 10.1098/Rspb.2006.3649

Dreanno, C., Kirby, R.R. & Clare, A.S. (2007). Involvement of the barnacle settlement-inducing protein complex (SIPC) in species recognition at settlement. *J Exp Mar Biol Ecol,* 351**:** 276-282. DOI DOI 10.1016/j.jembe.2007.07.003

Dreanno, C., Matsumura, K., Dohmae, N., Takio, K., Hirota, H., Kirby, R.R. & Clare, A.S. (2006b). An alpha(2)-macroglobulin-like protein is the cue to gregarious settlement of the barnacle Balanus amphitrite. *P Natl Acad Sci USA,* 103**:** 14396-14401. DOI Doi 10.1073/Pnas.0602763103

Eashwar, M., Nallathambi, T. & Kuberaraj, K. (2008). On microalgal settlements and the sluggish development of marine biofouling in Port Blair waters, Andamans. *Biofouling,* 24**:** 67-73. DOI 10.1080/08927010701799480

Egan, S., James, S. & Kjelleberg, S. (2002). Identification and characterization of a putative transcriptional regulator controlling the expression of fouling inhibitors in Pseudoalteromonas tunicata. *Appl Environ Microb,* 68**:** 372-378. DOI Doi 10.1128/Aem.68.1.372-378.2002

Elbourne, P.D. & Clare, A.S. (2010). Ecological relevance of a conspecific, waterborne settlement cue in Balanus amphitrite (Cirripedia). *J Exp Mar Biol Ecol,* 392**:** 99-106. DOI DOI 10.1016/j.jembe.2010.04.013

Endo, N., Nogata, Y., Yoshimura, E. & Matsumura, K. (2009). Purification and partial amino acid sequence analysis of the larval settlement-inducing pheromone from adult extracts of the barnacle, Balanus amphitrite (=Amphibalanus amphitrite). *Biofouling,* 25**:** 429-434. DOI 10.1080/08927010902875113

Engel, S. & Pawlik, J.R. (2000). Allelopathic activities of sponge extracts. *Mar Ecol Prog Ser,* 207**:** 273-281. DOI Doi 10.3354/Meps207273

Faimali, M., Falugi, C., Gallus, L., Piazza, V. & Tagliafierro, G. (2003a). Involvement of acetyl choline in settlement of Balanus amphitrite. *Biofouling,* 19 Suppl**:** 213-20. DOI 10.1080/0892701021000044228

Faimali, M., Sepcic, K., Turk, T. & Geraci, S. (2003b). Non-toxic antifouling activity of polymeric 3-alkylpyridinium salts from the Mediterranean sponge Reniera sarai (Pulitzer-Finali). *Biofouling,* 19**:** 47-56. DOI Doi 10.1080/0892701021000036966

Feng, D.Q., Ke, C.H., Lu, C.Y. & Li, S.J. (2011). Antifouling activity of marine sessile organisms from China against barnacle settlement. *Journal of the Marine Biological Association of the United Kingdom,* 91**:** 1073-1079. DOI Doi 10.1017/S0025315410002195

Fusetani, N. (2011). Antifouling marine natural products. *Natural product reports,* 28**:** 400-10. DOI 10.1039/c0np00034e

Gademann, K. (2007). Cyanobacterial natural products for the inhibition of Biofilm formation and biofouling. *Chimia,* 61**:** 373-377. DOI DOI 10.2533/chimia.2007.373

Gantayet, A., Ohana, L. & Sone, E.D. (2013). Byssal proteins of the freshwater zebra mussel, Dreissena polymorpha. *Biofouling,* 29**:** 77-85. DOI Doi 10.1080/08927014.2012.746672

Gohad, N.V., Aldred, N., Orihuela, B., Clare, A.S., Rittschof, D. & Mount, A.S. (2012). Observations on the settlement and cementation of barnacle (Balanus amphitrite) cyprid larvae after artificial exposure to noradrenaline and the locations of adrenergic-like receptors. *J Exp Mar Biol Ecol,* 416–417**:** 153-161. DOI http://dx.doi.org/10.1016/j.jembe.2012.02.013

Hadfield, M.G. (2011a). Biofilms and Marine Invertebrate Larvae: What Bacteria Produce That Larvae Use to Choose Settlement Sites. *Annu Rev Mar Sci,* 3**:** 453-470. DOI DOI 10.1146/annurev-marine-120709-142753

Hadfield, M.G. (2011b). Biofilms and Marine Invertebrate Larvae: What Bacteria Produce That Larvae Use to Choose Settlement Sites. *Annual Review of Marine Science,* 3**:** 453-470. DOI doi:10.1146/annurev-marine-120709-142753

Hadfield, M.G. & Paul, V.J. (2001). Natural chemical cues for settlement and metamorphosis of marine invertebrate larvae. *Marine chemical ecology CRC Press, Boca Raton, FL***:** 431-461.

Han, Z., Sun, J., Zhang, Y., He, F., Xu, Y., Matsumura, K., He, L.S., Qiu, J.W., Qi, S.H. & Qian, P.Y. (2013). iTRAQ-Based Proteomic Profiling of the Barnacle Balanus amphitrite in Response to the Antifouling Compound Meleagrin. *Journal of Proteome Research,* 12**:** 2090-2100. DOI Doi 10.1021/Pr301083e

Harder, T., Lau, S.C.K., Dahms, H.U. & Qian, P.Y. (2002). Isolation of bacterial metabolites as natural inducers for larval settlement in the marine polychaete Hydroides elegans (Haswell). *Journal of Chemical Ecology,* 28**:** 2029-2043. DOI Doi 10.1023/A:1020702028715

Harder, T.N., Thiyagarajan, V. & Qian, P.Y. (2001). Effect of cyprid age on the settlement of balanus amphitrite darwin in response to natural biofilms. *Biofouling,* 17**:** 211-219. DOI 10.1080/08927010109378480

He, F., Liu, Z., Yang, J., Fu, P., Peng, J., Zhu, W.M. & Qi, S.H. (2012). A novel antifouling alkaloid from halotolerant fungus Penicillium sp OUCMDZ-776. *Tetrahedron Lett,* 53**:** 2280-2283. DOI Doi 10.1016/J.Tetlet.2012.02.063

He, L.S., Zhang, G. & Qian, P.Y. (2013). Characterization of Two 20kDa-Cement Protein (cp20k) Homologues in Amphibalanus amphitrite. *PloS one,* 8. DOI 10.1371/journal.pone.0064130

Hellio, C., Berge, J.P., Beaupoil, C., Le Gal, Y. & Bourgougnon, N. (2002). Screening of marine algal extracts for anti-settlement activities against microalgae and macroalgae. *Biofouling,* 18**:** 205-215. DOI Doi 10.1080/08927010290010137

Hellio, C., Bourgougnon, N. & Le Gal, Y. (2000). Phenoloxidase (EC 1.14.18.1) from the byssus gland of Mytilus edulis: Purification, partial characterization and application for screening products with potential antifouling activities. *Biofouling,* 16**:** 235-244.

Hellio, C., Tsoukatou, M., Marechal, J.P., Aldred, N., Beaupoil, C., Clare, A.S., Vagias, C. & Roussis, V. (2005). Inhibitory effects of Mediterranean sponge extracts and metabolites on larval settlement of the barnacle Balanus amphitrite. *Mar Biotechnol,* 7**:** 297-305. DOI DOI 10.1007/s10126-004-3150-x

Huang, X.-Z., Xu, Y., Zhang, Y.-F., Zhang, Y., Wong, Y.H., Han, Z., Yin, Y. & Qian, P.-Y. (2014). Nontoxic piperamides and their synthetic analogues as novel antifouling reagents. *Biofouling***:** 1-9. DOI 10.1080/08927014.2014.889688

Huang, Y.L., Li, M., Yu, Z.L. & Qian, P.Y. (2011). Correlation between pigmentation and larval settlement deterrence by Pseudoalteromonas sp sf57. *Biofouling,* 27**:** 287-293. DOI 10.1080/08927014.2011.562978

Hwang, D.S., Zeng, H., Masic, A., Harrington, M.J., Israelachvili, J.N. & Waite, J.H. (2010). Protein- and metal-dependent interactions of a prominent protein in mussel adhesive plaques. *The Journal of biological chemistry,* 285**:** 25850-8. DOI 10.1074/jbc.M110.133157

Imo (2008). International Convention on the Control of Harmful Anti-fouling Systems on Ships. *International Maritime Organization,* Adoption: 5 October 2001; Entry into force: 17 September 2008.

Jin, C., Qiu, J., Miao, L., Feng, K. & Zhou, X. (2014). Antifouling activities of anti-histamine compounds against the barnacle Amphibalanus (= Balanus) amphitrite. *J Exp Mar Biol Ecol,* 452**:** 47-53. DOI http://dx.doi.org/10.1016/j.jembe.2013.12.006

Kamino, K. (2001). Novel barnacle underwater adhesive protein is a charged amino acid-rich protein constituted by a Cys-rich repetitive sequence. *Biochemical Journal,* 356**:** 503-507. DOI Doi 10.1042/0264-6021:3560503

Kamino, K. (2013). Mini-review: Barnacle adhesives and adhesion. *Biofouling,* 29**:** 735-749. DOI 10.1080/08927014.2013.800863

Kamino, K., Inoue, K., Maruyama, T., Takamatsu, N., Harayama, S. & Shizuri, Y. (2000). Barnacle cement proteins. Importance of disulfide bonds in their insolubility. *The Journal of biological chemistry,* 275**:** 27360-5. DOI 10.1074/jbc.M910363199

Kato, T., Shizuri, Y., Izumida, H., Yokoyama, A. & Endo, M. (1995). Styloguanidines, New Chitinase Inhibitors from the Marine Sponge Stylotella-Aurantium. *Tetrahedron Lett,* 36**:** 2133-2136. DOI Doi 10.1016/0040-4039(95)00194-H

Keifer, P.A., Rinehart, K.L. & Hooper, I.R. (1986). Renillafoulins, Antifouling Diterpenes from the Sea Pansy Renilla-Reniformis (Octocorallia). *Journal of Organic Chemistry,* 51**:** 4450-4454. DOI Doi 10.1021/Jo00373a020

Khandeparker, L. & Anil, A.C. (2011). Role of conspecific cues and sugars in the settlement of cyprids of the barnacle, Balanus amphitrite. *J Zool,* 284**:** 206-214. DOI DOI 10.1111/j.1469-7998.2011.00794.x

Kitano, Y., Akima, C., Yoshimura, E. & Nogata, Y. (2011). Anti-barnacle activity of novel simple alkyl isocyanides derived from citronellol. *Biofouling,* 27**:** 201-5. DOI 10.1080/08927014.2011.553282

Knight-Jones, E. (1953). Laboratory experiments on gregariousness during setting in Balanus balanoides and other barnacles. *Journal of Experimental Biology,* 30**:** 584-598.

Kobak, J. (2001). Light, gravity and conspecifics as cues to site selection and attachment behaviour of juvenile and adult Dreissena polymorpha Pallas, 1771. *J Mollus Stud,* 67**:** 183-189. DOI Doi 10.1093/Mollus/67.2.183

Kon-Ya, K., Miki, W. & Endo, M. (1995). L-Tryptophan and Related Compounds Induce Larval Settlement of the Barnacle Balanus amphitrite Darwin. *Fisheries Sci,* 61**:** 800-803. DOI 10.2331/fishsci.61.800

Kon-Ya, K., Shimidzu, N., Adachi, K. & Miki, W. (1994). 2, 5, 6-Tribromo-l-methylgramine, an Antifouling Substance from the Marine Bryozoan Zoobotryon pellucidum. *Fisheries Sci,* 60**:** 773-775.

Konstantinou, I.K. & Albanis, T.A. (2004). Worldwide occurrence and effects of antifouling paint booster biocides in the aquatic environment: a review. *Environment international,* 30**:** 235-248. DOI http://dx.doi.org/10.1016/S0160-4120(03)00176-4

Kristensen, J.B., Meyer, R.L., Laursen, B.S., Shipovskov, S., Besenbacher, F. & Poulsen, C.H. (2008). Antifouling enzymes and the biochemistry of marine settlement. *Biotechnol Adv,* 26**:** 471-481. DOI Doi 10.1016/J.Biotechadv.2008.05.005

Kwong, T.F.N., Miao, L., Li, X.C. & Qian, P.Y. (2006). Novel antifouling and antimicrobial compound from a marine-derived fungus Ampelomyces sp. *Mar Biotechnol,* 8**:** 634-640. DOI Doi 10.1007/S10126-005-6146-2

Lau, S.C.K., Harder, T. & Qian, P.Y. (2003). Induction of larval settlement in the serpulid polychaete Hydroides elegans (Haswell): Role of bacterial extracellular polymers. *Biofouling,* 19**:** 197-204. DOI Doi 10.1080/0892701031000072163

Lauren, M.H. & Wilker, J.J. (2007). Synergistic effects of metals and oxidants in the curing of marine mussel adhesive. *J Mater Sci,* 42**:** 8934-8942. DOI Doi 10.1007/S10853-007-1648-0

Li, X., Dobretsov, S., Xu, Y., Xiao, X., Hung, O.S. & Qian, P.Y. (2006). Antifouling diketopiperazines produced by a deep-sea bacterium, Streptomyces fungicidicus. *Biofouling,* 22**:** 201-208. DOI Doi 10.1080/08927010600780771

Li, Y.X., Zhang, F.Y., Xu, Y., Matsumura, K., Han, Z., Liu, L.L., Lin, W.H., Jia, Y.X. & Qian, P.Y. (2012). Structural optimization and evaluation of butenolides as potent antifouling agents: modification of the side chain affects the biological activities of compounds. *Biofouling,* 28**:** 857-864. DOI Doi 10.1080/08927014.2012.717071

Liang, C., Li, Y.Q., Hu, B.R. & Wu, W.J. (2014). Prokaryotic expression and functional characterization of the 19 kDa protein in balanus albicostatus cement.

Lin, H.-C., Wong, Y.H., Tsang, L.M., Chu, K.H., Qian, P.-Y. & Chan, B.K.K. (2013). First study on gene expression of cement proteins and potential adhesion-related genes of a membranous-based barnacle as revealed from Next-Generation Sequencing technology. *Biofouling***:** 1-13. DOI 10.1080/08927014.2013.853051

Lin, Q., Gourdon, D., Sun, C., Holten-Andersen, N., Anderson, T.H., Waite, J.H. & Israelachvili, J.N. (2007). Adhesion mechanisms of the mussel foot proteins mfp-1 and mfp-3. *Proceedings of the National Academy of Sciences,* 104**:** 3782-3786. DOI 10.1073/pnas.0607852104

Lind, U., Alm Rosenblad, M., Hasselberg Frank, L., Falkbring, S., Brive, L., Laurila, J.M., Pohjanoksa, K., Vuorenpaa, A., Kukkonen, J.P., Gunnarsson, L., Scheinin, M., Martensson Lindblad, L.G. & Blomberg, A. (2010). Octopamine receptors from the barnacle balanus improvisus are activated by the alpha2-adrenoceptor agonist medetomidine. *Mol Pharmacol,* 78**:** 237-48. DOI 10.1124/mol.110.063594

Loeb, M.J. & Walker, G. (1977). Origin, composition, and function of secretions from pyriform organs and internal sacs of four settling cheilo-ctenostome bryozoan larvae. *Mar Biol,* 42**:** 37-46. DOI 10.1007/BF00392012

Magin, C.M., Cooper, S.P. & Brennan, A.B. (2010). Non-toxic antifouling strategies. *Materials Today,* 13**:** 36-44. DOI http://dx.doi.org/10.1016/S1369-7021(10)70058-4

Martensson, L. & Gunnarsson, F. (2005). Preventing marine biofouling by inhibiting adhesive release from barnacles. United States Patent.

Matsumura, K., Nagano, M., Kato-Yoshinaga, Y., Yamazaki, M., Clare, A.S. & Fusetani, N. (1998). Immunological studies on the settlement-inducing protein complex (SIPC) of the barnacle Balanus amphitrite and its possible involvement in larva-larva interactions. *P Roy Soc B-Biol Sci,* 265**:** 1825-1830.

Menin, A., Ballarin, L., Bragadin, M. & Cima, F. (2008). Immunotoxicity in ascidians: Antifouling compounds alternative to organotins - II. The case of Diuron and TCMS pyridine. *J Environ Sci Heal B,* 43**:** 644-654. DOI Doi 10.1080/03601230802352690

Mihm, J.W., Banta, W.C. & Loeb, G.I. (1981). Effects of adsorbed organic and primary fouling films on bryozoan settlement. *J Exp Mar Biol Ecol,* 54**:** 167-179. DOI http://dx.doi.org/10.1016/0022-0981(81)90142-8

Mokrini, R., Mesaoud, M.B., Daoudi, M., Hellio, C., Marechal, J.P., El Hattab, M., Ortalo-Magne, A., Piovetti, L. & Culioli, G. (2008). Meroditerpenoids and derivatives from the brown alga Cystoseira baccata and their antifouling properties. *J Nat Prod,* 71**:** 1806-11. DOI 10.1021/np8004216

Mori, Y., Urushida, Y., Nakano, M., Uchiyama, S. & Kamino, K. (2007). Calcite-specific coupling protein in barnacle underwater cement. *FEBS J,* 274**:** 6436-46. DOI 10.1111/j.1742-4658.2007.06161.x

Nagabhushanam, R., Mary, A., Mary, V., Sarojini, R. & Fingerman, M. (1995). Bioactive compounds having potential antifouling properties from corals of the Indian ocean: A review. *Arabian Sea***:** 215-226.

Nedved, B.T. & Hadfield, M.G. (2008). Hydroides Elegans (Annelida: Polychaeta): A Model for Biofouling Research. Springer Berlin Heidelberg.

Nogata, Y. & Kitano, Y. (2006). Isocyano Compounds as Non-Toxic Antifoulants. IN Fusetani, N. & Clare, A. (Eds.) *Antifouling Compounds*. Springer Berlin Heidelberg.

Nogata, Y., Yoshimura, E., Shinshima, K., Kitano, Y. & Sakaguchi, I. (2003). Antifouling substances against larvae of the barnacle Balanus amphitrite from the marine sponge, Acanthella cavernosa. *Biofouling,* 19**:** 193-196. DOI Doi 10.1080/0892701031000065963

Odling, K., Albertsson, C., Russell, J.T. & Martensson, L.G. (2006). An in vivo study of exocytosis of cement proteins from barnacle Balanus improvisus (D.) cyprid larva. *The Journal of experimental biology,* 209**:** 956-64. DOI 10.1242/jeb.02031

Omae, I. (2003). General Aspects of Tin-Free Antifouling Paints. *Chemical reviews,* 103**:** 3431-3448. DOI 10.1021/cr030669z

Ompi, M. (2011). Settlement behaviour and size of mussel larvae from the family Mytilidae: Brachidontes erosus (Lamarck, 1819), Brachidontes rostratus (Dunker, 1857), Trichomya hirsutus (Lamarck, 1819), and Mytilus galloprovincialis Lamarck, 1819. *Journal of Coastal Development,* 13**:** 215-227.

Ortlepp, S., Sjogren, M., Dahlstrom, M., Weber, H., Ebel, R., Edrada, R., Thoms, C., Schupp, P., Bohlin, L. & Proksch, P. (2007). Antifouling activity of bromotyrosine-derived sponge metabolites and synthetic analogues. *Mar Biotechnol,* 9**:** 776-785. DOI Doi 10.1007/S10126-007-9029-X

Pagett, H.E., Abrahams, J.L., Bones, J., O'donoghue, N., Marles-Wright, J., Lewis, R.J., Harris, J.R., Caldwell, G.S., Rudd, P.M. & Clare, A.S. (2012). Structural characterisation of the N-glycan moiety of the barnacle settlement-inducing protein complex (SIPC). *Journal of Experimental Biology,* 215**:** 1192-1198. DOI Doi 10.1242/Jeb.063503

Papov, V.V., Diamond, T.V., Biemann, K. & Waite, J.H. (1995). Hydroxyarginine-containing Polyphenolic Proteins in the Adhesive Plaques of the Marine Mussel Mytilus edulis. *Journal of Biological Chemistry,* 270**:** 20183-20192. DOI 10.1074/jbc.270.34.20183

Pawlik, J.R. (1992). Chemical Ecology of the Settlement of Benthic Marine-Invertebrates. *Oceanogr Mar Biol,* 30**:** 273-335.

Peng, B.X., Wang, J.L., Peng, Z.H., Zhou, S.Z., Wang, F.Q., Ji, Y.L., Ye, Z.J., Zhou, X.F., Lin, T. & Zhang, X.B. (2012). Studies on the synthesis, pungency and anti-biofouling performance of capsaicin analogues. *Sci China Chem,* 55**:** 435-442. DOI DOI 10.1007/s11426-011-4307-x

Qi, S.H., Xu, Y., Gao, J., Qian, P.Y. & Zhang, S. (2009). Antibacterial and antilarval compounds from marine bacterium Pseudomonas rhizosphaerae. *Ann Microbiol,* 59**:** 229-233.

Qian, P.Y. (1999). Larval settlement of polychaetes. *Hydrobiologia,* 402**:** 239-253. DOI Doi 10.1023/A:1003704928668

Qian, P.Y., Chen, L. & Xu, Y. (2013). Mini-review: Molecular mechanisms of antifouling compounds. *Biofouling,* 29**:** 381-400. DOI 10.1080/08927014.2013.776546

Qian, P.Y., Lau, S.C.K., Dahms, H.U., Dobretsov, S. & Harder, T. (2007). Marine biofilms as mediators of colonization by marine macroorganisms: Implications for antifouling and aquaculture. *Mar Biotechnol,* 9**:** 399-410. DOI DOI 10.1007/s10126-007-9001-9

Qian, P.Y., Wong, Y.H. & Zhang, Y. (2010a). Changes in the proteome and phosphoproteome expression in the bryozoan Bugula neritina larvae in response to the antifouling agent butenolide. *Proteomics,* 10**:** 3435-3446. DOI Doi 10.1002/Pmic.201000199

Qian, P.Y., Xu, Y. & Fusetani, N. (2010b). Natural products as antifouling compounds: recent progress and future perspectives. *Biofouling,* 26**:** 223-234. DOI Doi 10.1080/08927010903470815

Rao, D., Webb, J.S., Holmstrom, C., Case, R., Low, A., Steinberg, P. & Kjelleberg, S. (2007). Low densities of epiphytic bacteria from the marine alga Ulva australis inhibit settlement of fouling organisms. *Appl Environ Microb,* 73**:** 7844-7852. DOI Doi 10.1128/Aem.01543-07

Raveendran, T. & Mol, V.L. (2009). Natural product antifoulants. *Current science,* 97**:** 508-520.

Reed, C.G. & Woollacott, R.M. (1983). Mechanisms of rapid morphogenetic movements in the metamorphosis of the bryozoan Bugula neritina (Cheilostomata, Cellularioidea): II. The role of dynamic assemblages of microfilaments in the pallial epithelium. *Journal of Morphology,* 177**:** 127-143. DOI 10.1002/jmor.1051770202

Rittschof, D. (2000). Natural product antifoulants: one perspective on the challenges related to coatings development. *Biofouling,* 15**:** 119-127.

Rittschof, D., Hooper, I.R., Branscomb, E.S. & Costlow, J.D. (1985). Inhibition of barnacle settlement and behavior by natural products from whip corals,Leptogorgia virgulata (Lamarck, 1815). *J Chem Ecol,* 11**:** 551-63. DOI 10.1007/BF00988567

Rittschof, D., Lai, C.-H., Kok, L.-M. & Teo, S.L.-M. (2003). Pharmaceuticals as antifoulants: Concept and principles. *Biofouling,* 19**:** 207-212. DOI 10.1080/0892701021000083769

Sagert, J. & Waite, J.H. (2009). Hyperunstable matrix proteins in the byssus of Mytilus galloprovincialis. *Journal of Experimental Biology,* 212**:** 2224-2236. DOI Doi 10.1242/Jeb.029686

Satuito, C.G., Natoyama, K., Yamazaki, M. & Fusetani, N. (1995). Induction of Attachment and Metamorphosis of Laboratory Cultured Mussel Mytilus-Edulis Galloprovincialis Larvae by Microbial Film. *Fisheries Sci,* 61**:** 223-227.

Schultz, M.P. (2007). Effects of coating roughness and biofouling on ship resistance and powering. *Biofouling,* 23**:** 331-341. DOI Doi 10.1080/08927010701461974

Schultz, M.P., Bendick, J.A., Holm, E.R. & Hertel, W.M. (2011). Economic impact of biofouling on a naval surface ship. *Biofouling,* 27**:** 87-98. DOI Doi 10.1080/08927014.2010.542809

Sepcic, K. & Turk, T. (2006). 3-Alkylpyridinium compounds as potential non-toxic antifouling agents. *Progress in molecular and subcellular biology,* 42**:** 105-24.

Shao, C.L., Wang, C.Y., Wei, M.Y., Gu, Y.C., She, Z.G., Qian, P.Y. & Lin, Y.C. (2011a). Aspergilones A and B, two benzylazaphilones with an unprecedented carbon skeleton from the gorgonian-derived fungus Aspergillus sp. *Bioorganic & Medicinal Chemistry Letters,* 21**:** 690-693. DOI Doi 10.1016/J.Bmcl.2010.12.005

Shao, C.L., Wu, H.X., Wang, C.Y., Liu, Q.A., Xu, Y., Wei, M.Y., Qian, P.Y., Gu, Y.C., Zheng, C.J., She, Z.G. & Lin, Y.C. (2011b). Potent Antifouling Resorcylic Acid Lactones from the Gorgonian-Derived Fungus Cochliobolus lunatus. *Journal of Natural Products,* 74**:** 629-633. DOI Doi 10.1021/Np100641b

Shikuma, N.J., Pilhofer, M., Weiss, G.L., Hadfield, M.G., Jensen, G.J. & Newman, D.K. (2014). Marine Tubeworm Metamorphosis Induced by Arrays of Bacterial Phage Tail-Like Structures. *Science*. DOI 10.1126/science.1246794

Sjogren, M., Dahlstrom, M., Hedner, E., Jonsson, P.R., Vik, A., Gundersen, L.L. & Bohlin, L. (2008). Antifouling activity of the sponge metabolite agelasine D and

synthesised analogs on Balanus improvisus. *Biofouling,* 24**:** 251-258. DOI Doi 10.1080/08927010802072753

Slattery, M., Mcclintock, J.B. & Heine, J.N. (1995). Chemical Defenses in Antarctic Soft Corals - Evidence for Antifouling Compounds. *J Exp Mar Biol Ecol,* 190**:** 61-77. DOI Doi 10.1016/0022-0981(95)00032-M

Stevens, M.J., Steren, R.E., Hlady, V. & Stewart, R.J. (2007). Multiscale structure of the underwater adhesive of Phragmatopoma californica: a nanostructured latex with a steep microporosity gradient. *Langmuir,* 23**:** 5045-9. DOI 10.1021/la063765e

Stewart, R.J., Weaver, J.C., Morse, D.E. & Waite, J.H. (2004). The tube cement of Phragmatopoma californica: a solid foam. *The Journal of experimental biology,* 207**:** 4727-34. DOI 10.1242/jeb.01330

Sun, C., Fantner, G.E., Adams, J., Hansma, P.K. & Waite, J.H. (2007). The role of calcium and magnesium in the concrete tubes of the sandcastle worm. *Journal of Experimental Biology,* 210**:** 1481-1488. DOI Doi 10.1242/Jeb.02759

Tan, L.T., Goh, B.P.L., Tripathi, A., Lim, M.G., Dickinson, G.H., Lee, S.S.C. & Teo, S.L.M. (2010). Natural antifoulants from the marine cyanobacterium Lyngbya majuscula. *Biofouling,* 26**:** 685-695. DOI Doi 10.1080/08927014.2010.508343

Tanur, A.E., Gunari, N., Sullan, R.M.A., Kavanagh, C.J. & Walker, G.C. (2010). Insights into the composition, morphology, and formation of the calcareous shell of the serpulid Hydroides dianthus. *Journal of structural biology,* 169**:** 145-160. DOI http://dx.doi.org/10.1016/j.jsb.2009.09.008

Teo, S.L.M. & Ryland, J.S. (1995). Potential Antifouling Mechanisms Using Toxic-Chemicals in Some British Ascidians. *J Exp Mar Biol Ecol,* 188**:** 49-62. DOI Doi 10.1016/0022-0981(95)99069-K

Thiyagarajan, V., Harder, T., Qiu, J.W. & Qian, P.Y. (2004). Energy content at metamorphosis and growth rate of the early juvenile barnacle Balanus amphitrite (vol 143, pg 543, 2003). *Mar Biol,* 144**:** 619-621. DOI Doi 10.1007/S00227-003-1262-X

Thiyagarajan, V., Wong, T. & Qian, P.-Y. (2009). 2D Gel-Based Proteome and Phosphoproteome Analysis During Larval Metamorphosis in Two Major Marine Biofouling Invertebrates. *Journal of Proteome Research,* 8**:** 2708-2719. DOI 10.1021/pr800976u

Thomas, K.V. & Brooks, S. (2010). The environmental fate and effects of antifouling paint biocides. *Biofouling,* 26**:** 73-88. DOI Doi 10.1080/08927010903216564

Toupoint, N., Mohit, V., Linossier, I., Bourgougnon, N., Myrand, B., Olivier, F., Lovejoy, C. & Tremblay, R. (2012). Effect of biofilm age on settlement of Mytilus edulis. *Biofouling,* 28**:** 985-1001. DOI Doi 10.1080/08927014.2012.725202

Tsoukatou, M., Marechal, J.P., Hellio, C., Novakovic, I., Tufegdzic, S., Sladic, D., Gasic, M.J., Clare, A.S., Vagias, C. & Roussis, V. (2007). Evaluation of the activity of the sponge metabolites avarol and avarone and their synthetic derivatives against fouling micro- and macroorganisms. *Molecules,* 12**:** 1022-34.

Turk, T., Frangez, R. & Sepcic, K. (2007). Mechanisms of toxicity of 3-alkylpyridinium polymers from marine sponge Reniera sarai. *Marine Drugs,* 5**:** 157-167. DOI Doi 10.3390/Md504157

Urushida, Y., Nakano, M., Matsuda, S., Inoue, N., Kanai, S., Kitamura, N., Nishino, T. & Kamino, K. (2007). Identification and functional characterization of a novel barnacle cement protein. *FEBS J,* 274**:** 4336-46. DOI 10.1111/j.1742-4658.2007.05965.x

Vooys, C.G.N. (2003). Effect of a tripeptide on the aggregational behaviour of the blue mussel Mytilus edulis. *Mar Biol,* 142**:** 1119-1123. DOI 10.1007/s00227-003-1039-2

Wahl, M. (1989). Marine epibiosis. I. Fouling and antifouling: some basic aspects. *Mar Ecol Prog Ser,* 58**:** 175-189.

Wahl, M., Goecke, F., Labes, A., Dobretsov, S. & Weinberger, F. (2012). The second skin: Ecological role of epibiotic biofilms on marine organisms. *Frontiers in Microbiology,* 3. DOI 10.1371/journal.pone.0027387

Waite, J.H., Andersen, N.H., Jewhurst, S. & Sun, C. (2005). Mussel Adhesion: Finding the Tricks Worth Mimicking. *The Journal of Adhesion,* 81**:** 297-317. DOI 10.1080/00218460590944602

Waite, J.H., Jensen, R.A. & Morse, D.E. (1992). Cement Precursor Proteins of the Reef-Building Polychaete Phragmatopoma-Californica (Fewkes). *Biochemistry,* 31**:** 5733-5738. DOI Doi 10.1021/Bi00140a007

Waite, J.H. & Qin, X.X. (2001). Polyphosphoprotein from the adhesive pads of Mytilus edulis. *Biochemistry,* 40**:** 2887-2893. DOI Doi 10.1021/Bi002718x

Walker, G. (1971). A study of the cement apparatus of the cypris larva of the barnacle Balanus balanoides. *Mar Biol,* 9**:** 205-212. DOI 10.1007/BF00351380

Walker, G. (1972). The Biochemical Composition of the Cement of two Barnacle Species, Balanus Hameri and Balanus Crenatus. *Journal of the Marine Biological Association of the United Kingdom,* 52**:** 429-435. DOI doi:10.1017/S0025315400018786

Wang, C.S. & Stewart, R.J. (2012). Localization of the bioadhesive precursors of the sandcastle worm, Phragmatopoma californica (Fewkes). *The Journal of experimental biology,* 215**:** 351-61. DOI 10.1242/jeb.065011

Wang, C.S. & Stewart, R.J. (2013). Multipart copolyelectrolyte adhesive of the sandcastle worm, Phragmatopoma californica (Fewkes): catechol oxidase catalyzed curing through peptidyl-DOPA. *Biomacromolecules,* 14**:** 1607-17. DOI 10.1021/bm400251k

Wang, H., Zhang, H., Wong, Y.H., Voolstra, C., Ravasi, T., B. Bajic, V. & Qian, P.-Y. (2010). Rapid transcriptome and proteome profiling of a non-model marine invertebrate, Bugula neritina. *PROTEOMICS,* 10**:** 2972-2981. DOI 10.1002/pmic.201000056

Warner, S.C. & Waite, J.H. (1999). Expression of multiple forms of an adhesive plaque protein in an individual mussel, Mytilus edulis. *Mar Biol,* 134**:** 729-734. DOI DOI 10.1007/s002270050589

Williams, E.A. & Degnan, S.M. (2009). Carry-over effect of larval settlement cue on postlarval gene expression in the marine gastropod Haliotis asinina. *Molecular Ecology,* 18**:** 4434-4449. DOI DOI 10.1111/j.1365-294X.2009.04371.x

Wong, Y.H., Wang, H., Ravasi, T. & Qian, P.Y. (2012). Involvement of Wnt signaling pathways in the metamorphosis of the bryozoan Bugula neritina. *PloS one,* 7**:** e33323. DOI 10.1371/journal.pone.0033323

Xu, Y., He, H.P., Schulz, S., Liu, X., Fusetani, N., Xiong, H.R., Xiao, X. & Qian, P.Y. (2010). Potent antifouling compounds produced by marine Streptomyces. *Bioresource Technol,* 101**:** 1331-1336. DOI DOI 10.1016/j.biortech.2009.09.046

Xu, Y., Li, H.L., Li, X.C., Xiao, X. & Qian, P.Y. (2009). Inhibitory Effects of a Branched-Chain Fatty Acid on Larval Settlement of the Polychaete Hydroides elegans. *Mar Biotechnol,* 11**:** 495-504. DOI DOI 10.1007/s10126-008-9161-2

Yamamoto, H., Shimizu, K., Tachibana, A. & Fusetani, N. (1999). Roles of dopamine and serotonin in larval attachment of the barnacle, Balanus amphitrite. *Journal*

of experimental zoology, 284**:** 746-758. DOI Doi 10.1002/(Sici)1097-010x(19991201)284:7<746::Aid-Jez4>3.0.Co;2-J

Yamamoto, H., Tachibana, A., Kawaii, S., Matsumura, K. & Fusetani, N. (1996). Serotonin involvement in larval settlement of the barnacle, Balanus amphitrite. *Journal of experimental zoology,* 275**:** 339-345. DOI Doi 10.1002/(Sici)1097-010x(19960801)275:5<339::Aid-Jez2>3.3.Co;2-G

Yang, J.L., Satuito, C.G., Bao, W.Y. & Kitamura, H. (2008). Induction of metamorphosis of pediveliger larvae of the mussel Mytilus galloprovincialis Lamarck, 1819 using neuroactive compounds, KCl, NH4Cl and organic solvents. *Biofouling,* 24**:** 461-470. DOI Doi 10.1080/08927010802340309

Yang, L.H., Lee, O.O., Jin, T., Li, X.C. & Qian, P.Y. (2006). Antifouling properties of 10 beta-formamidokalihinol-A and kalihinol A isolated from the marine sponge Acanthella cavernosa. *Biofouling,* 22**:** 23-32. DOI Doi 10.1080/08927010500498623

Yebra, D.M., Kiil, S. & Dam-Johansen, K. (2004). Antifouling technology - past, present and future steps towards efficient and environmentally friendly antifouling coatings. *Progress in Organic Coatings,* 50**:** 75-104. DOI DOI 10.1016/j.porgcoat.2003.06.001

Zardus, J.D., Nedved, B.T., Huang, Y., Tran, C. & Hadfield, M.G. (2008). Microbial biofilms facilitate adhesion in biofouling invertebrates. *Biol Bull-Us,* 214**:** 91-98.

Zentz, F., Hellio, C., Valla, A., De La Broise, D., Bremer, G. & Labia, R. (2002). Antifouling activities of N-substituted imides: Antimicrobial activities and inhibition of Mytilus edulis phenoloxidase. *Mar Biotechnol,* 4**:** 431-440. DOI DOI 10.1007/s10126-002-0004-2

Zhang, H., Wong, Y.H., Wang, H., Chen, Z., Arellano, S.M., Ravasi, T. & Qian, P.-Y. (2010a). Quantitative Proteomics Identify Molecular Targets That Are Crucial in Larval Settlement and Metamorphosis of Bugula neritina. *Journal of Proteome Research,* 10**:** 349-360. DOI 10.1021/pr100817v

Zhang, Y., Xu, Y., Arellano, S.M., Xiao, K. & Qian, P.-Y. (2010b). Comparative proteome and phosphoproteome analyses during cyprid development of the barnacle Balanus (= Amphibalanus) amphitrite. *Journal of proteome research,* 9**:** 3146-3157.

Zhang, Y.F., Kitano, Y., Nogata, Y., Zhang, Y. & Qian, P.Y. (2012a). The Mode of Action of Isocyanide in Three Aquatic Organisms, Balanus amphitrite, Bugula neritina and Danio rerio. *PloS one,* 7. DOI 10.1371/journal.pone.0045442

Zhang, Y.F., Zhang, H.M., He, L.S., Liu, C.D., Xu, Y. & Qian, P.Y. (2012b). Butenolide Inhibits Marine Fouling by Altering the Primary Metabolism of Three Target Organisms. *Acs Chem Biol,* 7**:** 1049-1058. DOI 10.1021/Cb200545s

Zhao, H., Robertson, N.B., Jewhurst, S.A. & Waite, J.H. (2006). Probing the adhesive footprints of Mytilus californianus byssus. *Journal of Biological Chemistry,* 281**:** 11090-11096. DOI DOI 10.1074/jbc.M510792200

Zhao, H., Sun, C., Stewart, R.J. & Waite, J.H. (2005). Cement proteins of the tube-building polychaete Phragmatopoma californica. *The Journal of biological chemistry,* 280**:** 42938-44. DOI 10.1074/jbc.M508457200

Zheng, C.J., Shao, C.L., Wu, L.Y., Chen, M., Wang, K.L., Zhao, D.L., Sun, X.P., Chen, G.Y. & Wang, C.Y. (2013). Bioactive Phenylalanine Derivatives and Cytochalasins from the Soft Coral-Derived Fungus, Aspergillus elegans. *Marine Drugs,* 11**:** 2054-2068. DOI Doi 10.3390/Md11062054

Zhou, X., Xu, Y., Jin, C. & Qian, P.Y. (2009a). Reversible anti-settlement activity against Amphibalanus (=Balanus) amphitrite, Bugula neritina, and Hydroides elegans by a nontoxic pharmaceutical compound, mizolastine. *Biofouling,* 25**:** 739-47. DOI 10.1080/08927010903154724

Zhou, X.J., Zhang, Z., Xu, Y., Jin, C.L., He, H.P., Hao, X.J. & Qian, P.Y. (2009b). Flavone and isoflavone derivatives of terrestrial plants as larval settlement inhibitors of the barnacle Balanus amphitrite. *Biofouling,* 25**:** 69-76. DOI 10.1080/08927010802455941