Metadata of the article that will be visualized in OnlineFirst

Please note: Images will appear in color online but will be printed in black and white.

1	Article Title	**Seaweed extracts as antimicrobial agents in aquaculture**	
2	Article Sub- Title		
3	Article Copyright - Year	**Springer Science+Business Media Dordrecht 2014 (This will be the copyright line in the final PDF)**	
4	Journal Name	Journal of Applied Phycology	
5	Corresponding Author	Family Name	**Vatsos**
6		Particle	
7		Given Name	**Ioannis N**
8		Suffix	
9		Organization	University of Nordland
10		Division	Faculty of Biosciences and Aquaculture
11		Address	Post Office Box 1490, Bodø 8049, Norway
12		e-mail	inv@uin.no
13	Author	Family Name	**Rebours**
14		Particle	
15		Given Name	**Celine**
16		Suffix	
17		Organization	Bioforsk, Norwegian Institute for Agricultural and Environmental Research
18		Division	
19		Address	Frederik A. Dahlsvei 20, Ås 1430, Norway
20		e-mail	celine.rebours@bioforsk.no
21	Schedule	Received	21 July 2014
22		Revised	10 December 2014
23		Accepted	11 December 2014
24	Abstract	In the last 20 years, there has been an increasing interest in using various seaweed extracts as prophylactic and/or therapeutic agents in aquaculture. Up until now, most studies on the direct antimicrobial effect of seaweeds have taken place in various parts of Asia, particularly in India. All groups of seaweeds exhibit significant antimicrobial properties against many infectious agents of fish and shrimp, but the genera that appear to exhibit a broader range of antibacterial properties are *Asparagopsis* spp. (red seaweed) and *Sargassum* spp. (brown seaweed). The activity can	

		be affected by many factors and the method of extraction is one of the most important ones, as the extracts that are produced using organic solvents appear more efficient. In fish, almost all published information on bacterial pathogens comes from in vitro screenings, where extracts of different seaweed species were tested against many bacterial species. On the other hand, in shrimp, the studies have been focusing on the antimicrobial effects of seaweed extracts mainly against many *Vibrio* species. Regarding the viral pathogens, in fish, there is only one published study on fish viruses (IHNV and IPNV), while in shrimp there are many studies on WSSV. There are only two published studies on fish parasites (*Ichthyophonus hoferi* and *Neobendenia* spp.) and no studies on pathogenic fish and shrimp fungi. Interestingly, there are no published studies on salmons and carps, the main fish species that are extensively farmed. When the antimicrobial properties were studied in vivo, the seaweed extracts were either incorporated directly in the feeds (dry or live) or added directly into the water in which the fish and shrimp were reared. In the last case, the water-soluble antimicrobial seaweed substances affected the communication between the bacterial pathogens, rather than their growth. The development of parasites was also affected. In addition, one study indicated that short-term immersion of shrimp in seaweed extracts appeared to have a therapeutic effect against *Vibrio parahaemolyticus*. On the other hand, incorporation of the extracts into the feeds appeared to be an effective delivery method for the prevention and treatment of different infectious diseases. Up until now, there are no complete studies on the pharmacodynamics and pharmacokinetics of seaweed extracts in fish or shrimp. However, the findings indicate that they can reduce the bacterial load within the tissues. Another issue that has not been examined yet is the applicability of using these extracts on a commercial scale. Currently, the increased extraction cost inhibits the extensive use of these extracts. Other methodologies, such the production of synthetic analogues with similar properties, may decrease the production cost. Based on the published studies, seaweed extracts exhibit promising antimicrobial properties, but further research is needed before the complete potential of seaweed extracts is assessed.
25	Keywords separated by ' - '	Seaweed - Antimicrobial - Fish - Shrimp - Aquaculture
26	Foot note information	

J Appl Phycol
DOI 10.1007/s10811-014-0506-0

5TH CONGRESS OF THE INTERNATIONAL SOCIETY FOR APPLIED PHYCOLOGY

Seaweed extracts as antimicrobial agents in aquaculture

Ioannis N Vatsos · Celine Rebours

Received: 21 July 2014 / Revised and accepted: 10 December 2014

Abstract In the last 20 years, there has been an increasing interest in using various seaweed extracts as prophylactic and/or therapeutic agents in aquaculture. Up until now, most studies on the direct antimicrobial effect of seaweeds have taken place in various parts of Asia, particularly in India. All groups of seaweeds exhibit significant antimicrobial properties against many infectious agents of fish and shrimp, but the genera that appear to exhibit a broader range of antibacterial properties are *Asparagopsis* spp. (red seaweed) and *Sargassum* spp. (brown seaweed). The activity can be affected by many factors and the method of extraction is one of the most important ones, as the extracts that are produced using organic solvents appear more efficient. In fish, almost all published information on bacterial pathogens comes from in vitro screenings, where extracts of different seaweed species were tested against many bacterial species. On the other hand, in shrimp, the studies have been focusing on the antimicrobial effects of seaweed extracts mainly against many *Vibrio* species. Regarding the viral pathogens, in fish, there is only one published study on fish viruses (IHNV and IPNV), while in shrimp there are many studies on WSSV. There are only two published studies on fish parasites (*Ichthyophonus hoferi* and *Neobendenia* spp.) and no studies on pathogenic fish and shrimp fungi. Interestingly, there are no published studies on salmons and carps, the main fish species that are extensively farmed. When the antimicrobial properties were studied in vivo, the seaweed extracts were either incorporated directly in the feeds (dry or live) or added directly into the water in which the fish and shrimp were reared. In the last case, the water-soluble antimicrobial seaweed substances affected the communication between the bacterial pathogens, rather than their growth. The development of parasites was also affected. In addition, one study indicated that short-term immersion of shrimp in seaweed extracts appeared to have a therapeutic effect against *Vibrio parahaemolyticus*. On the other hand, incorporation of the extracts into the feeds appeared to be an effective delivery method for the prevention and treatment of different infectious diseases. Up until now, there are no complete studies on the pharmacodynamics and pharmacokinetics of seaweed extracts in fish or shrimp. However, the findings indicate that they can reduce the bacterial load within the tissues. Another issue that has not been examined yet is the applicability of using these extracts on a commercial scale. Currently, the increased extraction cost inhibits the extensive use of these extracts. Other methodologies, such the production of synthetic analogues with similar properties, may decrease the production cost. Based on the published studies, seaweed extracts exhibit promising antimicrobial properties, but further research is needed before the complete potential of seaweed extracts is assessed.

Keywords Seaweed · Antimicrobial · Fish · Shrimp · Aquaculture

I. N. Vatsos (✉)
Faculty of Biosciences and Aquaculture, University of Nordland,
Post Office Box 1490, 8049 Bodø, Norway
e-mail: inv@uin.no

C. Rebours
Bioforsk, Norwegian Institute for Agricultural and Environmental Research, Frederik A. Dahlsvei 20, 1430 Ås, Norway
e-mail: celine.rebours@bioforsk.no

Introduction

With an average annual growth rate of 8.9 % since 1970, aquaculture is considered to be the fastest growing food-producing sector in the world and accounts for about 36 % of the global fish supply and almost 60 % of the global shrimp supply (FAO 2014). In terms of quantity, farming of cyprinids dominates the aquaculture production, with 25.4 million T,

while the production of salmonids and crustaceans (shrimp and prawns) contributes with 3.2 and 4.3 million T, respectively (FAO 2014). Diseases, either infectious or non-infectious, are important limiting factors that affect the production volume and consequently the production cost. In 2006, for instance, for a global production of 1.6 million T of salmon, the cost for sea lice treatments was estimated at 305 million € (Costello 2009). It has been estimated that in Norway, the top salmonid producer in the world, the cost of sea lice control is about 0.19 €kg^{-1} of salmon (Costello 2009). Furthermore, it was estimated that in 2010, over 77 million USD were spent in Norway on fish diseases management, including the implementation of legislation and support to surveillance and control programmes (The Fish Site 2010).

The development of many vaccines, mainly against fish pathogens, and the use of various antimicrobial agents have reduced the impact of many diseases. However, there is currently an increasing demand for more environment-friendly disease control schemes and many researchers have examined alternative approaches. Among these approaches, the use of various natural products that derive from different living organisms, such as plants (e.g. essential oils), animals (e.g. chitozan) and seaweeds has received a lot of attention (Romero et al. 2012).

Seaweeds, also known as macroalgae, are photosynthetic multicellular aquatic organisms that can be found in almost every aquatic environment, in all geographical areas. Humans had realized their important value as early as 14,000 years ago (Dillehay et al. 2008). The first reports of seaweeds growing on ropes used for fish farming came from Japan, about 400 years ago (Buchholz et al. 2012). A more systematic culture started in the 1950s, in order to meet the increasing demand for seaweeds as food and mostly as sources of polymers. In 2012, over 21 million tons of seaweeds were produced, over 96 % of which were cultured in Asia (FAO 2014).

Many studies on different seaweed species have confirmed their nutritional value. In particular, seaweeds are low in calories, have high content of dietary fibres, are a good source of polyunsaturated fatty acids DHA and EPA, and may contain proteins up to 44 % dry matter with an amino acid profile of interest (Holdt and Kraan 2011). The red and the green seaweeds are generally rich in carbohydrates, whereas the brown seaweeds are generally richer in soluble fibre and iodine (Gupta and Abu-Ghannam 2011a). In some cases, some essential amino acids might be limiting, as for example tryptophan, while the concentration of other amino acids, like taurine, can be high particularly in red algae (Dawczynski et al. 2007). In addition to their nutritional value, seaweeds exhibit interesting pharmacological properties, such as antioxidant, anti-inflammatory, antimicrobial and even anticancer properties (El Gamal 2010; Gupta and Abu-Ghannam 2011a; Gupta and Abu-Ghannam 2011b; Holdt and Kraan 2011; Mohamed et al. 2012). The active compounds include polysaccharides (e.g. fucoidan), various phytochemicals (e.g. phlorotannins), carotenoids, minerals, peptides and lipids (Gupta and Abu-Ghannam 2011b; Holdt and Kraan 2011). It is worth mentioning that some of these compounds, as for example phlorotannins, are not found in terrestrial plants.

The present review focuses on published studies on the direct antimicrobial properties of seaweeds and their extracts against various pathogens of farmed fish and shrimp. Many of these extracts also exhibit significant immunostimulatory (Caipang et al. 2011) and antioxidant properties (Kang et al. 2013; Wijesinghe et al. 2014), which can enhance the resistance and immune response against many infectious agents, but these will not be discussed in the present review.

Control of infectious diseases in aquaculture

In contrast to terrestrial farmed animals, most of the fish species that are farmed today have been recently domesticated from wild populations and thus they are still not well adapted to the conditions that exist in farms (Kibenge et al. 2012). Many of these conditions, such as crowding, regularly handling, improper water quality parameters and the use of artificial commercial feeds, can cause various degrees of stress to fish, which in turn can make them more vulnerable to all infectious diseases (Huntingford et al. 2006). As a rule, the most common infectious diseases that are observed in farmed aquatic animals are those associated with bacterial pathogens (about 50 %), followed by the viral, the parasitic and finally the fungal diseases (McLoughlin 2006). Differences, depending on the species and country, may exist. For instance, in farmed salmonids, bacterial diseases are not considered a major problem compared to the losses caused by viral agents, but in marine fish species bacterial diseases are far more important in terms of financial loss and frequency (Johansen et al. 2011).

The control of the infectious diseases that affect the farmed aquatic animals relies on the use of effective prophylactic as well as therapeutic measures. Numerous studies have demonstrated that the extensive use of various chemotherapeutants used for the treatment of the parasitic, bacterial and fungal diseases in aquaculture have serious impacts on the environment and increase the health risks for both humans and animals (Burridge et al. 2010). It is well established for instance that the extensive use of various chemicals induces a strong selective pressure on the pathogens, resulting in the appearance of multi-resistant strains. Subsequently, through the horizontal exchange of genetic material that occurs between bacterial species, this resistance, which is an important virulence factor for many pathogens, is transferred to other pathogens. Furthermore, the resistance to the antimicrobial agents that is developed in animal bacterial pathogens can be also transferred to human pathogens (Martinez 2009).

In aquaculture, the main routes of administration of the various chemotherapeutants are either via medicated feeds or by immersion. Both of these methods can have a direct impact on a wide range of bacterial species that live in the aquatic environment. In both cases, it is very difficult to control the leaching of the active substances to the immediate environment (Heuer et al. 2009) and thus residues of many antimicrobials are often found in the sediment under the fish and shellfish farms (Petersen et al. 2002; Romero et al. 2012). Miranda and Zemelman (2002) studied the presence of oxytetracycline-resistant bacteria in the environment of Chilean salmon farms and found that the number of oxytetracycline-resistant bacteria was significantly increased in the effluent water. The presence of these resistant bacteria was associated with previous treatments that took place in the farms. These findings are of great significance as many in vitro studies have already demonstrated the transferability of antibiotic resistance genes between fish or shrimp and human pathogens (Heuer et al. 2009). Moreover, the use of the various chemotherapeutants, including the antibiotics, has negative effects on many functions of the fish immune system. Romero et al. (2012) in their review on the use of antibiotics in aquaculture noted that treatment with oxytetracycline and oxolinic acid could induce significant immunosuppression in many fish species, while a less pronounced effect was observed after a treatment with florfenicol. All these findings stress therefore the urgency to minimize the use of any chemotherapeutant in aquaculture and indeed many countries have already developed strict legislations concerning their uses.

This necessity to reduce the use of chemicals is an important issue not only in aquaculture but in the whole animal farming industry. According to a report by World Human Organization (WHO 2011), the implementation of effective biosecurity measures, the development of new vaccines, the use of prebiotics and probiotics, and good hygiene and management practices are quite important for the control of many infectious diseases in both terrestrial and aquatic animal farming and can lead to a significant reduction in the use of antibiotics in animal farming. Furthermore, new legislations that would regulate and monitor the use of antibiotics should be implemented, while the use of antibiotics as growth promoters should be banned worldwide. Only qualified people, preferably veterinarians, should be responsible for monitoring the use of all chemicals used in animal farming. Experience from the terrestrial animal husbandry indicates that indeed strict legislations that require reduced use of antibiotics do not necessary result in increased costs to the farmers, as for example a survey in swine farms in Denmark has demonstrated (Aarestrup et al. 2010).

There is however a significant variation between countries concerning the use of chemotherapeutants, which may reflect the diverse degree of awareness of each society for environmental issues. This results in heterogeneity between the legislations in effect, in aquaculture producing countries. For example, Burridge et al., (2010) reported that the amount of antibiotics used in salmon farming between 2007 and 2008 in Chile and Norway, the two main salmon producing countries, was a few hundred metric tons in Chile and less than a metric ton in Norway. Furthermore, in many countries, fish and shellfish farmers use increased amounts of various antimicrobial substances, even on a daily basis, as a preventive measure (Heuer et al. 2009).

As societies become more aware of the negative effects of the various treatments that are employed today in the control of the infectious diseases in aquaculture, various alternative approaches have been suggested. These include the use of probiotics to enhance the immune response of fish and shellfish, the use of bacteriophages against bacterial pathogens and the use of various natural products, such as essential oils, as antimicrobial agents (Romero et al. 2012). Among them, seaweeds have also been examined as potential sources of antimicrobial substances (Gupta and Abu-Ghannam 2011b).

Seaweeds versus fish and shrimp pathogens

The dietary value of seaweeds, as potential substitutes for fishmeal, or as binding agents, has been extensively studied and the findings indicate that seaweed-based diets can be used for the farming of many aquatic organisms, such as fish, shrimp, sea urchins and abalones (Bindu and Sobha 2004; Henry 2012). Seaweeds have relatively simple cultivation methods and can grow fast. It is also possible to control the production of some of their bioactive extracts through the manipulation of the cultivation conditions (Plaza et al. 2008). Recent studies have focused on culture systems integrating seaweed with fish or shrimp production. In these Integrated Multitrophic Aquaculture Systems (IMTA), the seaweeds play an important role first as biofilters and secondly as a source of biomass (Barrington et al. 2009). Seaweeds receive the nutrient-rich waste water from the fish or shellfish and use it for their growth. In this way, they can reduce the negative environmental impacts of fish farming through the removal of the waste materials (mainly N and P) that are released from the animals in the farms. The produced seaweed biomass adds market value to the production system, as they can later be used in food, or pharmaceutical industry (Al-Hafedh et al. 2012).

The antimicrobial properties of seaweed extracts against many human and terrestrial animal pathogens are known since the end of the nineteenth century (Genovese et al. 2012). These antimicrobial properties can be affected by many factors, such as the habitats, the cultivation method, the growth stage of seaweeds, the season and the method used for the extraction of the bioactive components (Karthikaidevi et al.

2009; Govindasamy et al. 2011). For example, Osman et al. (2012), after screening many seaweed species against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus* spp. and *Escherichia coli*, found that green seaweeds and particularly *Ulva fasciata*, tended to exhibit higher antimicrobial activity. This was more pronounced when the green seaweeds were collected in winter. On the other hand, Salvador et al. 2007 found that red seaweeds exhibited higher antimicrobial properties against many bacterial species, particularly the seaweeds which were collected in autumn. Regarding the method of extraction, organic solvents generally tend to be more efficient for the extraction of the active substances than water (Abu-Ghannam and Rajauria 2013) and fractioned seaweed extracts appear more effective compared to crude (Radhika et al. 2014). One important characteristic of seaweeds that may pose a health risk is that they are prone to absorb heavy metals from their surrounding environment, especially if they are located in particularly polluted areas (Bailey et al. 1999). Furthermore, they may contain substances, such as kainoids, aplysiatoxins and polycavernosides, which may be toxic to humans and animals (Smit 2004). For example, significant ichthyotoxic effects have also been reported by De Lara-Isassi et al. (2000), who used *Carassius auratus* to assess the toxicity of over 70 seaweed species. They concluded that Rhodophyta tended to be more toxic, while Chlorophyta appeared to be the least toxic. In some cases, the seaweed extracts can be toxic to certain fish and shellfish species, even at sub-antimicrobial concentrations (Mata et al. 2013).

In farmed fish, most studies on the antimicrobial properties of seaweeds have focused on various bacterial pathogens (14 out of the 17 presented in this review), while fewer studies exist on viral and parasitic pathogens (1 and 2, respectively, out of the 17 presented in this review). On the other hand, in farmed shrimp, the studies focused mainly on various pathogenic vibrios and the White Spot Syndrome Virus. Interestingly, although there are in vitro studies in the literature that demonstrate the antifungal activities of many seaweed extracts against human pathogenic fungi, such as *Aspergillus* spp. and *Candida albicans* (Plaza et al. 2010; Omar et al. 2012), there are no similar studies on the main pathogenic fish or shrimp fungi.

Despite the numerous studies on the antimicrobial effects of seaweed extracts against fish and shrimp pathogens, there is still limited information on the exact mechanism of action for most of these extracts. The reason is that although an assessment of any antimicrobial substance, as in the case of seaweed extracts, should include an initial in vitro screening followed by an in vitro study (Fig. 1), most studies on the antimicrobial effects of seaweeds in fish and shrimp are either only in vitro or only in vivo. For example, 8 out of the 39 studies on seaweeds versus fish and shrimp pathogens discussed in this review included both in vitro and in vivo assays (Tables 1 and 2). Furthermore, none of the eight studies on the White Spot Syndrome Virus included any preliminary in vitro study. Thus, it is not always clear if the observed protective result is either due to the direct antimicrobial effect, or due to immunostimulation, or the synergic effect.

Bacterial pathogens

The main identified active antibacterial compounds found in seaweeds are as follows: fatty acids, lipophilic and phenolic compounds, lectins, acetogenins, terpenes, alkaloids, polyphenolics, isoprenoid metabolites and hydrogen peroxide (Mohamed et al. 2012). In general, these substances can (a) attack the bacterial cell walls and the cell membranes, which results in an extensive release of intracellular substances or/and disruption of the uptake and transportation of substances, as for example various phlorotannins (Hierholtzer et al. 2014); (b) reduce the protein and nucleic acid synthesis in the bacterial cells (Cai et al. 2014) and (c) inhibit respiration (Cai et al. 2014). Phlorotannins, as many other terrestrial tannins do, may also form complexes with some extracellular bacterial enzymes (Stern et al. 1996), thus reducing their effects. In most cases, the effects are dose dependent.

An area that has received a lot of attention is the effect of seaweeds and particularly some of their metabolites, on the quorum sensing mechanism, by which bacterial cells communicate between each other. This process, which depends on the population density, involves the production of certain substances, such as peptides, or lactones, which are then released into the extracellular environment. When the concentration of these substances increases beyond a certain level, they are then detected by specific receptors, located in the bacterial cell membranes, or cytoplasms. This in turn regulates the expression of certain genes. Many Gram positive and negative bacteria use this process to collectively regulate many processes, such as bioluminescence, formation of biofilms and the production of various virulence factors (Manefield et al. 2001; Rutherford and Bassler 2014). Active substances released from seaweeds, such as furanones, can disrupt this process, thus affecting the virulence of many pathogenic bacteria, as for example the virulence of many pathogenic *Vibrio* species (Defoirdt et al. 2006) (Fig. 2). Because of these properties and particularly the effect on the biofilm formation, seaweed extracts have also been studied as antifouling agents in aquaculture (Jha et al. 2013). It is worth mentioning that an important advantage of such quorum sensing inhibitors is that they do not induce strong selection pressure on the bacteria, as antibiotics do (Dobretsov et al. 2009).

Numerous studies have focused on the study of the direct antibacterial (either bactericidal or bacteriostatic) properties of seaweed extracts against human bacterial pathogens, such as: *B. subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Clostridium* spp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus* spp., *Salmonella typhimurium*, *Shigella*

sonnei, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes* and *Vibrio cholerae* (Vairappan and Suzuki 2000; Vairappan et al. 2001; Xu et al. 2003; Christobel et al. 2011; Vijayabaskar and Shiyamala 2011; Ganeshamurthy et al. 2012; Marudhupandi and Kumar 2013; Saritha et al. 2013). In most cases, only in vitro assays were used to establish the antibacterial activities, such as dick diffusion or tube dilution methods.

Most of the bacterial species that can cause diseases in fish and shrimp are quite ubiquitous in the aquatic environment, as for example many members of the genus *Aeromonas* and the various pathogenic *Vibrio* species, such as *Vibrio anguillarum* (also known as *Listonella anguillarum*), *Vibrio alginolyticus* and *Vibrio harveyi* (Genovese et al. 2012; Cavalo et al. 2013). Some of these bacteria, such as some pathogenic *Vibrio* species, can affect both fish and shrimp and in many cases the manifestation and the progress of the associated diseases are affected by the presence of various stressful conditions. In comparison to human bacterial pathogens, fewer studies have been conducted to identify the antibacterial potential of seaweed metabolites against these pathogens.

Comparisons between the different studies on the antibacterial properties of seaweeds against fish and shrimp are difficult, as different experimental protocols were used and particularly in relation to the extraction methods. However, it is worth noticing that in only 5 out of the 28 studies on fish and shrimp bacterial pathogens, water was used for the extraction (Table 1). Although none of the three groups of seaweeds appears to be significantly more effective, as different species belonging to all groups are effective against many bacterial pathogens, *Asparagopsis* spp. (red seaweed) and *Sargassum* spp. (brown seaweed) appear to exhibit a broader

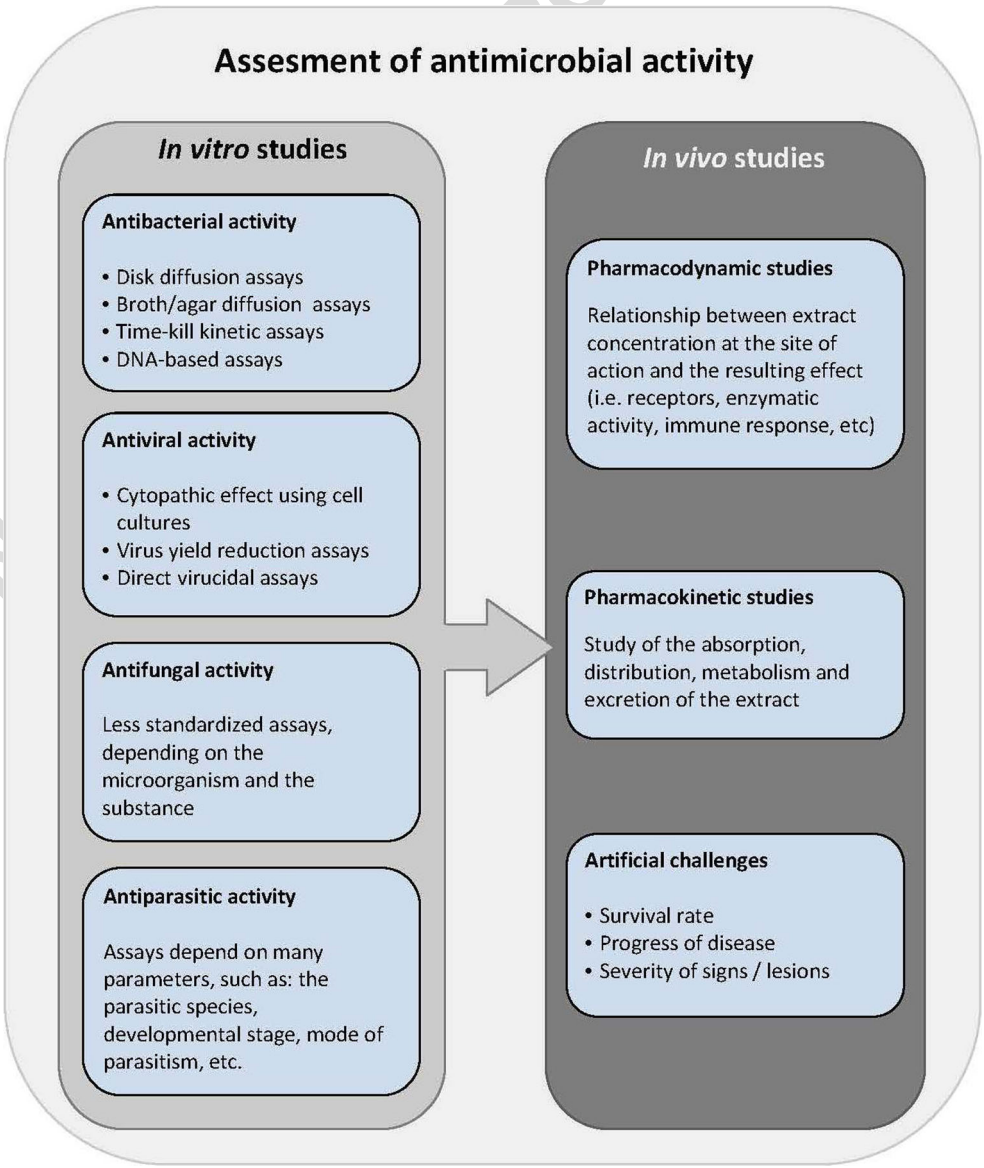

Fig. 1 A general scheme used in the assessment of antimicrobial activity of seaweed extracts or metabolites. The initial in vitro screening indicates the best candidates for the in vivo studies. This stage can include many assays, depending on the bioactive component and its potential application. The in vivo studies are designed in such a way so that the important information is collected by using the minimum number of animals. Based on all available information, the best method of administration of the tested extract is then proposed

t1.1 **Table 1** Assessment of the antimicrobial properties of seaweed extracts against fish pathogens

t1.2		Seaweed genus/species	Extraction method	Fish species	In vitro assays	In vivo assays	Pathogen	Results
t1.3	Bacterial	*Asparagopsis armata* (a) (red)	Organic solvents	–	Agar diffusion assay	–	*Vibrio anguillarum* *Pseudomonas anguilliseptica* *Aeromonas salmonicida* *Aeromonas hydrophila* *Yersinia ruckeri*	In vitro antibacterial activity
t1.4		*Laurencia chondrioides* (b) (red)	Organic solvents	–	Agar diffusion assay	–	*Vibrio anguillarum* *Pseudomonas anguilliseptica* *Aeromonas salmonicida* *Aeromonas hydrophila* *Yersinia ruckeri* *Photobacterium damselae* sbsp *piscicida*	In vitro antibacterial activity
t1.5		*Mastocarpus stellatus* (c) (red) *Ceramium rubrum* (c) (red) *Laminaria digitata* (c) (brown)	Organic solvents	–	Bacterial growth inhibition assay	–	*Aeromonas salmonicida* *Vibrio anguillarum* *Photobacterium damselae* subsp. *damselae* *Vibrio alginolyticus* *Yersinia ruckeri*	In vitro antibacterial activity
t1.6		*Halimeda micronesia* (d) (green)	Organic solvents	–	Agar well diffusion assay	–	*Aeromonas hydrophila* *Vibrio alginoticus* *V. parahaemolyticus* *Edwarsiella tarda*	In vitro antibacterial activity
t1.7		*Asparagopsis taxiformis* (e) (red)	Organic solvents	–	Agar diffusion assay	–	*Aeromonas salmonicida* *Photobacterium damselae* subsp *damselae* *Photobacterium damselae* subsp *piscicida* *Vibrio alginolyticus* *Vibrio harveyi* *Vibrio parahaemolyticus* *Vibrio vulnificus*	In vitro antibacterial activity
t1.8		*Ulva* spp. (f) (green)	Organic solvents	–	Agar well diffusion assay	–	*Aeromonas hydrophila* *Edwarsiella tarda*	In vitro antibacterial activity
t1.9		*Padina gymnospora* (g) (brown) *Padina tetrastomatica* (g) (brown) *Sargassum wightii* (g) (brown) *Turbinaria ornata* (g) (brown)	Organic solvents	–	Disc diffusion assay Minimum inhibitory concentrations	–	*Edwardsiella tarda* *Vibrio alginolyticus* *Aeromonas hydrophila* *Renibacterium salmoninarum*	In vitro antibacterial activity
t1.10		*Gracilaria dura* (h) (red) *Gracilaria gracilis* (h) (red) *Gracilariopsis longissima* (h) (red)	Organic solvents	–	Disc diffusion assay	–	*Vibrio ordalii* *Vibrio salmonicida* *Vibrio alginolyticus* *Vibrio vulnificus*	In vitro antibacterial activity

JmlID 10811_ArtID 506_Proof# 1 - 20/12/2014

Q2 t1.11 **Table 1** (continued)

	Seaweed genus/species	Extraction method	Fish species	In vitro assays	In vivo assays	Pathogen	Results
	Chaetomorpha linum (h) (green) *Cladophora rupestris* (h) (green) *Ulva prolifera* (h) (green)						
t1.11	*Gracilaria corticata* (i) (red) *Caulerpa racemosa* (i) (green) *Caulerpa sertularioides* (i) (green) *Chaetomorpha antennina* (i) (green) *Padina gymnospora* (i) (brown) *Sargassum wightii* (i) (green)	Organic solvents	–	Agar well diffusion assay	–	*Vibrio parahaemolyticus* *Aeromonas hydrophila*	In vitro antibacterial activity
t1.12	*Hypnea musciformis* (j) (red) *Gracilaria corticata* (j) (red) *Ulva fasciata* (j) (green) *Codium tomentosum* (j) (green) *Sargassum wightii* (j) (brown) *Dictyota dichotoma* (j) (brown) *Padina tetrastromatica* (j) (brown)	Water	–	Disc diffusion assay	–	*Vibrio alginolyticus* *Vibrio fischeri* *Vibrio harveyi*	In vitro antibacterial activity
t1.13	*Ulva clathrata* (k) (green)	Water	–	Addition of bacterial suspension in seaweed cultures	–	*Vibrio anguillarum*	Inhibition of bacterial growth in the water
t1.14	*Ulva reticulata* (l) (green)	Organic solvents	–	Minimum inhibitory concentrations Enumeration of bacteria on the surface of seaweed	–	*Aeromonas hydrophila* *Vibrio alginolyticus* *Vibrio parahaemolyticus*	In vitro antibacterial activity Decrease in number of bacterial colonies
t1.15	*Padina tetrastomatica* (m) (brown) *Stoechospermum marginatum* (m) (brown) *Ulva fasciata* (m) (green)	Organic solvents	–	Agar well diffusion method	–	*Aeromonas hydrophila*	In vitro antibacterial activity
t1.16	*Asparagopsis taxiformis* (n) (red)	Water	*Lates calcarifer*	Solid media antagonism assay Broth dilution assay	Immersion challenge followed by	*Streptococcus iniae*	Delay of the growth of the bacterium in the water

JrnlID 10811_ArtID 506_Proof# 1 - 20/12/2014

t1.17 **Table 1** (continued)

		Seaweed genus/species	Extraction method	Fish species	In vitro assays	In vivo assays	Pathogen	Results
						administration of the extract through the water		Not significant reduction in the mortality rate
t1.17	Viral	*Polysiphonia morrowii* (o) (red)	Organic solvents	–	Cytotoxicity assay Cytopathic effect reduction assay Plaque reduction assay Cytotoxicity assay.	–	Infectious Hematopoietic Necrosis Virus Infectious Pancreatic Necrosis Virus	In vitro antiviral activity
t1.18	Parasitic	*Fucus vesiculosus* (p) (brown)	–	*Oreochromis niloticus*	–	Feeding trial using naturally infected fish	*Ichthyophonus hoferi*	Reduced mortality
t1.19		*Ulva* spp. (q) (green) *Asparagopsis taxiformis* (q) (red)	Water	*Lates calcarifer*	Immersion treatment of various developmental stages of the parasites	Immersion treatment of infected fish	*Neobenedenia* spp.	Inhibition of the embryonic development, increase in the time of first and last hatch and reduced hatching success of the parasite

References: (a) Bansemir et al. (2006); (b) Bansemir et al. (2004); (c) Dubber and Harder (2008); (d) Ganeshamurthy et al. (2012); (e) Genovese et al. (2012); (f) Rebecca et al. (2012); (g) Singh et al. (2012); (h) Cavallo et al. (2013); (i) Maheswaran et al. (2013); (j) Christobel et al. (2011); (k) Lu et al. (2008); (l) Vairappan and Suzuki (2000); (m) Radhika et al. (2014); (n) Mata et al. (2013); (o) Kim et al. (2011); (p) El Ghany and Alla (2008); (q) Hutson et al. (2013)

range of antibacterial properties (Table 3). Interestingly, most studies were conducted in Asia (mainly India), while considerably fewer in other parts of the world, which can be associated with the extensive use of seaweed in the human diet in this area.

Fish bacterial pathogens

Antibacterial activities of seaweed extracts have been found against many Gram positive and Gram negative fish pathogenic bacteria, as many in vitro screenings have indicated (Table 3): many pathogenic *Vibrio* species, *Aeromonas hydrophila* and *Aeromonas salmonicida*, *Edwarsiella tarda*, *Renibacterium salmoninarum*, *Photobacterium damselae* sbsp *piscicida*, *Pseudomonas anguilliseptica*, *Streptococcus iniae* and *Yersinia ruckeri* (Vairappan and Suzuki 2000; Bansemir et al. 2004; 2006; Dubber and Harder 2008; Ganeshamurthy et al. 2012; Genovese et al. 2012; Rebecca et al. 2012; Singh et al. 2012; Cavallo et al. 2013; Maheswaran et al. 2013; Mata et al. 2013; Radhika et al. 2014).

Few of these studies investigated the potential of using seaweeds to control bacterial pathogens in the aquatic environment (Fig. 2). Lu et al. (2008) demonstrated the antimicrobial properties of *Ulva clathrata* in a series of experiments. In one experiment in particular, they added *V. anguillarum* in tanks containing cultures of the seaweed (10 g fresh algae L^{-1}). The seaweed significantly reduced the growth of the bacterium in the water. However, the study did not include any experiment with fish and thus the applicability of these findings was not assessed. Mata et al. (2013) examined both in vitro and in vivo the antibacterial effect of the aqueous extracts bromoform and dibromoacetic acid from the red seaweed *Asparagopsis taxiformis* against the fish pathogen *Streptococcus iniae*. In that study, the extracts were added into the water containing barramundi (*Lates calcarifer*) fingerlings already infected with *Streptococcus iniae*. The findings indicated that addition of approximately 28 μg L^{-1} bromoform and 5 μg L^{-1} dibromoacetic acid could delay the growth of the bacterium in the water, but did not affect significantly the mortalities caused by *Streptococcus iniae*. This study however examined the activity of the extracts after the infection, while the possible prophylactic effect prior to infection was not investigated. Addition of higher concentration of the extracts was more effective against the pathogen, but also induced mortality in the fish.

Shrimp bacterial pathogens

Almost all studies related to the antibacterial effects of seaweed extracts against shrimp pathogenic bacteria have focused on the bacterial genus *Vibrio* spp., as this represents the main bacterial group that can induce significant mortalities in shrimp farming (Defoirdt et al. 2006; Baleta et al. 2011;

Selvin et al. 2011; Dashtiannasab et al. 2012; Manilal et al. 2012; Cavalo et al. 2013; Silva et al. 2013; Sivakumar et al. 2014; Thanigaivel et al. 2014). When in vivo studies were carried out, the extracts were delivered to the shrimp mainly through enriched *Artemia* or medicated dry feeds. In one study, the extracts were added into the water that contained infected shrimp (Thanigaivel et al. 2014).

Traifalgar et al. (2009) examined and demonstrated the overall protective effect of fucoidan extracted from *Undaria pinnatifida* against *V. harveyi* in post-larvae black tiger shrimp (*Penaeus monodon*). In that study, the shrimp that were fed with 500–2,000 mg kg^{-1} body weight for 1 month exhibited significantly lower mortality when infected artificially with the bacterial pathogen. Interestingly, the shrimp that were fed with the medicated feeds also exhibited improved growth performance. Selvin et al. (2011) confirmed the protective effect of *U. fasciata* extracts after feeding black tiger shrimp post-larvae with medicated feed for 2 weeks. Subsequently, they challenged the shrimp with four pathogens, namely *Vibrio fischeri*, *V. harveyi*, *V. alginolyticus* and *Aeromonas* spp. The group of shrimp fed with 1 g kg^{-1} seaweed extract exhibited significantly lower mortality. Similarly, Manilal et al. (2012) examined the protective and therapeutic effect of ethyl acetate partitioned fraction of *Asparagopsis* spp. in black tiger shrimp post-larvae. For this, they fed the shrimp for 3 weeks and then challenged them with lethal doses of *V. harveyi*, *V. alginolyticus*, *Vibrio parahaemolyticus* and *Photobacterium damselae*. In this study, the authors examined the therapeutic effect as the shrimp were also fed with the medicated feed after the infection. Shrimp fed with 850 and 1, 150 mg kg^{-1} exhibited significantly increased survival rate. In all the above studies, the exact mode of action of the extracts was not determined.

In some studies, the authors attempted to explain the protective effect of the extracts only through their immunostimulatory properties. For example, Sirirustananun et al. (2011) studied the immunostimulatory effect of hot-water extract of *Gracilaria tenuistipitata* by feeding white shrimp (*Litopenaeus vannamei*) with 0.5, 1.0, and 2.0 g kg^{-1} dry diet for 14 days, before challenging them with *V. alginolyticus* and White Spot Syndrome Virus. The extracts induced a significant immunostimmulatory effect and increased survival rates. However, the study did not include any in vitro antibacterial assays, to indicate any possible direct antibacterial effect, which could also play an important role.

Kanjana et al. (2011) studied both in vitro and in vivo the protective role of some solvent extracts of the red seaweed *Gracilaria fisheri* against *V. harveyi*. After an initial screening using a disc diffusion assay, the authors used only the ethanol extracts for further in vivo studies. For the in vivo study, the authors fed the shrimp with enriched *Artemia salina* instars II (either with 0.5 or 1.0 mg mL^{-1}) for 2 weeks and then they artificially infected shrimp post-larvae with the bacterial pathogens. The results indicated both an antibacterial as well as an immunostimulatory effect (i.e. increased total haemocyte and granulocyte counts, increased phenoloxidase (PO) and superoxide dismutase (SOD) activities and increased super oxide anion production). Immanuel et al. (2004) also studied in vitro and in vivo the protective role of some seaweeds extracts against the shrimp pathogen *V. parahaemolyticus* by feeding *Penaeus indicus* post-larvae with *Artemia franciscana* preadults enriched with 400 mg L^{-1} of butanolic extracts from *Ulva lactuca* and *Sargassum wightii*. In this study, the authors maintained the shrimp in water containing the pathogen for 30 days, while fed them with the seaweed extract-enriched *Artemia*. Interestingly, they found that the extract that exhibited the highest inhibition zone in the initial in vitro screening also induced reduced bacterial load in the internal organs of the infected shrimp and increased the survival rate.

Thanigaivel et al. (2014) conducted a study which has demonstrated the potential of using seaweed extracts as alternatives to antibiotics. The authors examined the antioxidant and antibacterial properties of an ethanol extract from the green seaweed *Chaetomorpha antennina*. Regarding the antibacterial properties, the authors first infected *Penaeus monodon* (mean weight 12 g) with *V. parahaemolyticus* and then treated the diseased shrimp by immersing them into water containing 250 mg L^{-1} of the seaweed extract for 12–48 h. This treatment resulted in 98 % of survival of the treated shrimp. In addition, i.m. injection of 25 μL of the extract per shrimp protected the animals when they were subsequently infected by the bacterial pathogen. This is the first report that shows the therapeutic effect of a short-term administration of seaweed extracts.

A recent study by Sivakumar et al. (2014) demonstrated possible mechanisms that could explain the antimicrobial properties of *U. fasciata* against the pathogen *V. harveyi*. Thus, they demonstrated that solvent seaweed extracts reduced the phospholipase, proteolysis, lipolysis and thermonuclease activities of treated bacteria. The study included also an immersion challenge trial, in which *Penaeus monodon* post-larvae were maintained in water containing *V. harveyi* for 30 days. Addition of 200 μg mL^{-1} of extracts into the water resulted in significantly reduced mortality.

Defoirdt et al. (2006) examined the antibacterial effect of halogenated furanone extracted from the red seaweed *Delisea pulchra* against the shrimp bacterial pathogens *Vibrio campbellii*, *V. harveyi* and *V. parahaemolyticus*. They reported that this natural product at the concentration of 20 mg L^{-1} could protect in vivo the brine shrimp *Artemia franciscana* against these bacterial pathogens, although the substance did not have any effect on the growth rate of the pathogens in the water. Higher concentrations were toxic to *Artemia*. The authors concluded that the protective effect was probably due to the disruption of the quorum sensing mechanism, as assessed

t2.1 **Table 2** Assessment of the antimicrobial properties of seaweed extracts against shrimp pathogens

		Seaweed genus/species	Extraction method	Shrimp species	In vitro assays	In vivo assays	Pathogen	Results
t2.3	Bacterial	*Undaria pinnatifida* (a) (brown)	Organic solvents	*Penaeus monodon*	–	Feeding trial and immersion challenge	*Vibrio harveyi*	Reduced mortality
t2.4		*Ulva fasciata* (b) (green)	Organic solvents	*Penaeus monodon*	–	Feeding trial and injection challenge	*Vibrio alginolyticus V. harveyi Aeromonas spp.*	Reduced mortality
t2.5		*Asparagopsis* spp. (c) (red)	Organic solvents	*Penaeus monodon*	–	Feeding trial and injection challenge	*Vibrio harveyi Vibrio alginolyticus Vibrio parahaemolyticus Photobacterium damsela*	Reduced mortality
t2.6		*Gracilaria tenuistipitata* (d) (red)	Water	*Litopenaeus vannamei*	–	Feeding trial and injection challenge	*Vibrio alginolyticus*	Reduced mortality
t2.7		*Gracilaria fisheri* (e) (red)	Organic solvents	*Penaeus monodon*	Disc diffusion assay Minimum inhibitory concentrations	Safety test for the seaweed ethanol extract Enrichment of *Artemia salina* Immersion challenge of shrimp post-larvae and juveniles	*Vibrio harveyi*	In vitro antibacterial effect Reduced mortality
t2.8		*Ulva lactuca* (f) (green) *Sargassum wightii* (f) (brown)	Organic solvents	*Penaeus indicus*	Disc diffusion assay	Enrichment of *A. salina* Immersion challenge of shrimp juveniles	*Vibrio parahaemolyticus*	In vitro antibacterial effect Reduced bacterial load in the internal organs Reduced mortality
t2.9		*Delisea pulchra* (g) (red) Synthetic furanone (g)	Organic solvents	*Artemia franciscana*	Growth inhibition of furanone in liquid growth medium and water (plate counts) Disruption of AI-2 quorum sensing by synthetic furanone	Addition of the extract into the water and challenge tests	*Vibrio harveyi Vibrio campbellii Vibrio parahaemolyticus*	Disruption of the quorum sensing mechanism
t2.10		*Sargassum polycystum* (h) (brown)	Water	*Penaeus monodon*	Agar diffusion assay Minimum inhibitory concentrations	Feeding trial and incubation challenge	*Vibrio harveyi*	Reduced mortality
t2.11		*Ulva fasciata* (i) (green)	Organic solvents	*Penaeus monodon*	Agar well diffusion assay Minimum inhibitory concentrations Effect on virulence factors	Immersion challenge	*Vibrio harveyi*	In vitro antibacterial effect Reduced activity of many virulence factors Reduced mortality
t2.12		*Delisea pulchra* (j) (red)	Organic solvents	*Penaeus monodon*	Inhibition of luminescence T1 toxin production	Toxicity of supernatant extracts from furanone-treated *V. harveyi* cultures assess by i.m. injection	*Vibrio harveyi*	Inhibition of luminescence and T1 toxin production Reduced mortality
t2.13		*Chaetomorpha antennina* (k) (green)	Organic solvents	*Penaeus monodon*	Well diffusion method	Immersion treatment after i.m. and immersion challenge i.m. injection of extract followed by infection	*Vibrio parahaemolyticus*,	In vitro antibacterial effect Therapeutic effect after challenge

t2.14 **Table 2** (continued)

		Seaweed genus/species	Extraction method	Shrimp species	In vitro assays	In vivo assays	Pathogen	Results
								Improved histological picture after treatment with the extracts Protective effect of the i.m. injection of the extract
t2.14		*Padina gymnospora* (l) (brown)	Organic solvents	–	Disc diffusion assay	–	*Vibrio parahaemolyticus*, *Vibrio brasiliensis*, *Vibrio xuii*, *Vibrio navarrensis*	In vitro antibacterial effect
t2.15		*Sargassum oligocystum* (m) (brown)	Organic solvents	–	Disc diffusion method	–	*Vibrio alginolyticus*, *Vibrio parahaemolyticus* *Vibrio harveyi*	In vitro antibacterial effect
t2.16		*Sargassum latifoliumn* (brown)	Organic solvents	–	Disc diffusion method	–	*Vibrio alginolyticus*, *Vibrio parahaemolyticus* *Vibrio harveyi*	In vitro antibacterial activity
t2.17	Viral	*Sargassum wightii* (o) (brown)	Organic solvents		–	Enrichment of *Artemia* nauplii with fucoidan Immersion challenge	White Spot Syndrome Virus	Reduced mortality
t2.18		*Sargassum wightii* (p) (brown) *Sargassum duplicatum* (p) (brown)	Water	*Penaeus monodon*	–	Enrichment of *A. salina* Immersion challenge of shrimp post-larvae	White Spot Syndrome Virus	Reduced mortality
t2.19		*Sargassum wightii* (q) (brown)	Organic solvents	*Penaeus monodon*	–	Enrichment of *Artemia franciscana* nauplii Immersion challenge Viral load using nested PCR	White Spot Syndrome Virus	Reduced mortality
t2.20		*Sargassum* polycystum (h) (brown)	Water	*Penaeus monodon*	–	Feeding trial and immersion challenge	White Spot Syndrome Virus	Reduced mortality
t2.21		*Acrosiphonia orientalis* (r) (green)	Organic solvents	*Penaeus monodon*	–	Feeding trial and immersion challenge	White Spot Syndrome Virus	Reduced mortality
t2.22		*Cladosiphon okamuranus* (s) (brown)	–	*Penaeus japonicus*	–	Feeding trial and immersion challenge	White Spot Syndrome Virus	Reduced mortality
t2.23		*Sargassum wightii* (t) (brown)	Water	*Penaeus indicus* *Paratelphusa hydrodomous*	–	Determination of viral inactivation using i.m. injection of shrimp	White Spot Syndrome Virus	Reduced mortality
t2.24		*Gracilaria tenuistipitata* (d) (red)	Water	*Litopenaeus vannamei*	–	Feeding trial and injection challenge	White Spot Syndrome Virus	Reduced mortality

References: (a) Traifalgar et al. (2009); (b) Selvin et al. (2011); (c) Manilal et al. (2012); (d) Sirirustananun et al. (2011); (e) Kanjana et al. (2011); (f) Immanuel et al. (2004); (g) Defoirdt et al. (2006); (h) Chotigeat et al. (2004); (i) Sivakumar et al. (2014); (j) Manefield et al. (2000); (k) Thanigaivel et al. (2014); (l) Silva et al. (2013); (m) Baleta et al. (2011); (n) Dashtiannasab et al. (2012); (o) Sivagnanavelmurugan et al. (2012); (p) Immanuel et al. (2010); (q) Immanuel et al. (2012); (r) Manilal et al. (2009); (s) Takahashi et al. (1998); (t) Balasubramanian et al. (2006)

JrnlID 10811_ArtID 506_Proof# 1 - 20/12/2014

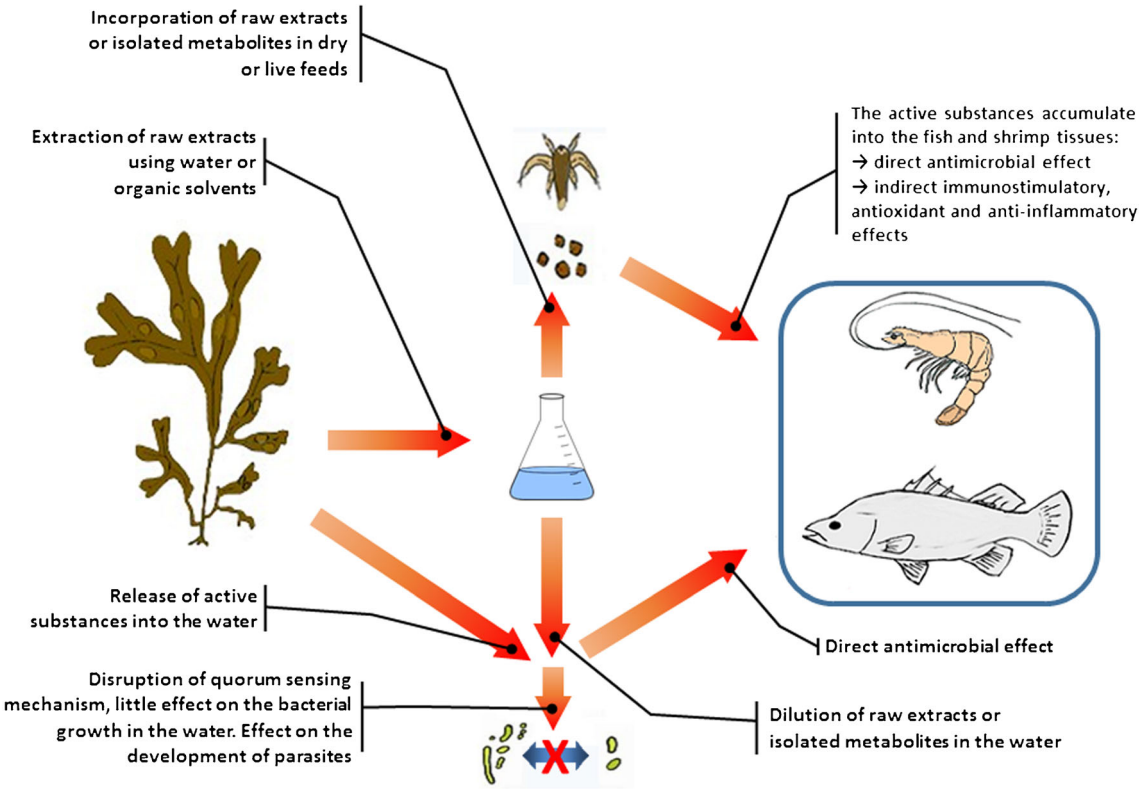

Fig. 2 Modes of administration of the seaweed extracts in fish and shrimp farming

by inhibition of bioluminescence, although a possible interaction between furanone and the shrimps was not excluded. Earlier, Manefield et al. (2000) had found that there is a link between bioluminescence and toxin production in *V. harveyi* and that the furanone that Defoirdt et al. (2006) also used could decrease the production of toxin by the bacterium. They also observed a protective effect in *P. monodon*, when they injected intramuscularly the animals with furanone-treated *V. harveyi* cultures. Rasch et al. (2004) examined the potential of using a synthetic halogenated furanone at significantly lower concentration (2.5 μg L^{-1}) to minimize the mortality caused by *V. anguillarum* in rainbow trout (*Oncorhynchus mykiss*). Although no natural seaweed extracts were used, the use of synthetic furanone decreased the mortality caused by the bacterial pathogen, probably through the disruption of the quorum sensing mechanism. As in the study by Defoirdt et al. (2006), no effect of the synthetic furanone were observed on the growth, the survival, the respiratory activity and the motility of the bacterium.

Viral pathogens

Currently, no antiviral drugs are used in aquaculture and thus the study of any substance with antiviral properties that can be used against fish or shellfish viruses is of great importance. The strategies that are currently used in aquaculture to control viral diseases rely on the use of effective vaccines (mostly in fish farming) and the development of lines of animals resistant to certain diseases through selective breeding (Kibenge et al. 2012). In shrimp farming, oral administration of immunostimulants has been suggested as a particularly promising method against viral pathogens (Sivagnanavelmurugan et al. 2012), as vaccination is a rather experimental control method (Sudheer et al. 2012).

The antiviral properties of seaweed extracts against human viruses are well reported. Various water-soluble extracts from red, brown and green seaweeds and particularly sulphated polysaccharides, exhibit antiviral properties against many viruses, such as the herpes simplex viruses (Saha et al. 2012; Son et al. 2013), the Japanese encephalitis virus (flavivirus) (Chiu et al. 2012) and the influenza virus (Jiao et al. 2012). The antiviral activities against human viruses have been assessed mainly by in vitro studies, on cell lines, but also by in vivo studies, using experimental animals (e.g. mice). These studies have shown that the extracts can suppress the replication of the viruses, and delay the manifestation of the disease symptoms, increasing the survival rates of the infected animals. The active substances found in seaweed extracts include among others: sulphoglycolipids, carrageenans and fucoidans (Mohamed et al. 2012). The mode of action depends on the substance but also on the virus. For instance, many sulphated polysaccharides may bind to the surface of the viruses (mainly enveloped viruses), or to virus receptors on the host cell surface, thus interfering with the attachment and the

adsorption of the viruses to the host cells (Wang et al. 2012). Some carrageenans can also exhibit post-binding inhibitory effects, affecting the intracellular stages of the infection (Buck et al. 2006), and particularly the virus transcription and replication (Wang et al. 2012). Factors that may affect the antiviral properties of the sulphated polysaccharides include the sugar composition, the main chain length, the sulphation level and the sulphate pattern (Jiao et al. 2012). Phlorotannins from the brown seaweed *Ecklonia cava* were also found to exhibit inhibitory effect on HIV-1 reverse transcriptase and proteases (Ahn et al. 2004).

Currently, there is only one study that indicates a possible protective effect of seaweed extracts against fish viruses (Infectious Hematopoietic Necrosis Virus and Infectious Pancreatic Necrosis Virus), while there are many studies on White Spot Syndrome Virus of shrimp. In contrast to bacterial pathogens, both water and organic solvents were used for the extraction (Table 2). The seaweed species that exhibited the antiviral activity were as follows: for WSSV: red seaweeds—*G. tenuistipitata*, brown seaweeds—*Sargassum* spp. and *Cladosiphon okamuranus*, green seaweeds—*Acrosiphonia orientalis*; and for IHNV and IPNV—the red seaweed *Polysiphonia morrowii* (Table 3). All studies discussed in the present review took place in Asia, probably because there is an increased interest to develop effective control strategies against WSSV, as no effective vaccines are yet available for the shrimp industry.

Fish viral pathogens

Kim et al. (2011) used cell-based assay to assess the antiviral properties of the red alga *Polysiphonia morrowii*. They found that the 80 % (*v*/*v*) methanolic extract had significant antiviral activity against two important fish viruses, the Infectious Hematopoietic Necrosis Virus (IHNV—family Rhabdoviridae) and the Infectious Pancreatic Necrosis Virus (IPNV—family Birnaviridae). Although, the study was in vitro and the authors did not provide any evidence on the mechanism of action of these extracts on the viruses, the results indicate the potential of using seaweed extracts against these viruses.

Shrimp viral pathogens

The White Spot Syndrome Virus (WSSV—family Nimaviridae) is the major pathogen affecting the shrimp production worldwide. WSSV can induce up to 100 % mortality within a few days, particularly at larval and juvenile stages. Various authors studied therefore the antiviral properties of the seaweed extracts in particular against the WSSV by administrating the extracts to shrimp either via enriched *Artemia* nauplii (Immanuel et al. 2010; Immanuel et al. 2012; Sivagnanavelmurugan et al. 2012) or through medicated feeds (Chotigeat et al. 2004; Manilal et al. 2009). Based on these studies, the effective concentration of extracts that can be used to enrich *Artemia* ranges from 400 to 750 mg L^{-1}, while the shrimp should be fed for about 20 days prior I order to acquire protection against the virus. On the other hand, medicated feeds were efficient when the seaweed extracts were added at a concentration of 250–500 mg kg^{-1} body weight. The active components were found to be polysaccharides, in particular fucoidans and sodium alginates (Takahashi et al. 1998; Chotigeat et al. 2004; Manilal et al. 2009; Immanuel et al. 2012; Sivagnanavelmurugan et al. 2012). Chotigeat et al. (2004) examined in particular the prophylactic and therapeutic effect of crude fucoidan extracted from *Sargassum polycystum* against WSSV. Black tiger shrimps of different sizes were fed with medicated feed 4 days prior to and 10 days after an experimental infection. The results showed that crude fucoidan at the concentration of 400 mg kg^{-1} of body weight day^{-1} increased significantly the survival rate, while at the same time increased the phagocytic activity of the shrimp haemocytes. Similar results were obtained in an earlier study by Takahashi et al. (1998) who fed kuruma shrimp (*Penaeus japonicus*) with fucoidan extracted from the brown seaweed *C. okamuranus*, at the concentration of 100 mg kg^{-1} of body weight day^{-1}.

In another study by Balasubramanian et al. (2006), the extracts, after their extraction by either water or organic solvents, were first mixed with suspensions of WSSV in order to de-activate the virus. Subsequently, the treated viral preparations were injected intramuscularly into marine shrimp (*Penaeus indicus*) and freshwater crab (*Paratelphusa hydrodomous*). Aqueous extracts of *Sargassum weightii* at a concentration of 3 mg per animal resulted in significantly less mortality in the infected animals.

In all the above studies on WSSV, the mechanisms explaining the antiviral action of these seaweed extracts were not determined. However, apart from the immunostimulatory effects, a direct antiviral effect of the extracts similar to that observed in other viruses cannot be excluded as a study by Rudtanatip et al. (2014) indicates. These authors reported that sulphated galactans isolated from the red seaweed *G. fisheri* attached to certain sites on the viral envelope and hence inhibited the attachment of the viruses to the host cells.

Parasitic pathogens

The antiparasitic properties of many seaweed extracts have been studied on a wide range of human parasites, such as protozoa (e.g. *Plasmodium* spp. and *Trichomonas* spp.) (Moo-Puc et al. 2008; Vonthron-Sénécheau et al. 2011), helminthes (e.g. *Ascaris* spp.) (Higa and Kuniyoshi 2000) and insects (e.g. mosquito larvae) (Bianco et al. 2013). The mechanism of action varies according to the extracts and the parasites. Thus, the extracts can either interfere with the binding of the

Table 3 Seaweed species tested against fish and shrimp pathogens. The table summarizes the findings presented in Tables 1 and 2 of this review

Seaweed genus/species	Geographical area	Pathogen
Red seaweeds		
Asparagopsis armata	Atlantic, France	Vang, Pang, Asal, Ahyd, Yruc
Asparagopsis taxiformis	Italy	Valg, Vpar, Vhar, Vvul, Asal, Pdad, Pdap,
Asparagopsis taxiformis	Australia	Sini, Neo
Ceramium rubrum	North Sea	Asal, Valg, Yruc
Delisea pulchra	India	Vhar, Vcam, Vpar
Delisea pulchra	Australia	Vhar
Gracilaria corticata	India	Vpar, Ahyd, Valg, Vhar, Vfis
Gracilaria dura	Italy	Vord, Valg
Gracilaria fisheri	Thailand	Vhar
Gracilaria gracilis	Italy	Vsal
Gracilaria tenuistipitata	Taiwan	Valg, WSSV
Gracilariopsis longissima	Southern Italy	Valg, Vvul
Hypnea musciformis	India	Vhar, Vfis
Laurencia chondrioides	Gran Canaria	Vang, Pang, Asal, Ahyd, Yruc, Pdapi
Mastocarpus stellatus	North Sea	Asal, Vang
Polysiphonia morrowii	South Korea	IHNV, IPNV
Green seaweeds		
Acrosiphonia orientalis	India	WSSV
Caulerpa racemosa	India	Vpar, Ahyd
Caulerpa sertulrioides	India	Vpar, Ahyd
Chaetomorpha antennina	India	Vpar, Ahyd
Chaetomorpha linum	Southern Italy	Vvul, Vord
Chladophora rupestris	Southern Italy	Vvul, Vsal, Vord
Codium tomentosum	India	Valg, Vhar, Vfis
Halimeda micronesia	India	Valg, Vpar, Ahyd, Etar
Ulva clathrata	China	Vang
Ulva fasciata	India	Valg, Vhar, Vfis, Aero
Ulva prolifera	Southern Italy	Vord
Ulva lactuca	India	Vpara
Ulva reticulata	Malaysia	Valg, Vpar, Ahyd
Ulva spp.	Australia	Neo
Brown seaweeds		
Cladosiphon okamuranus	Japan[a]	WSSV
Dictyota dichotoma	India	Valg
Fucus vesiculosus	Egypt[a]	Icth
Laminaria digitata	North Sea	Vang, Pdada, Yruc
Padina gymnospora	India	Vpar, Ahyd, Valg,
Padina gymnospora	Brazil	Vpar, Vbra, Vxui, Vnav
Padina tetrastomatica	India	Valg, Vhar, Etar, Ahyd
Sargassum duplicatum	India	WSSV
Sargassum latifolium	Persian Gulf	Vpar, Valg, Vhar
Sargassum oligocystum	Philippines	Vpar, Valg, Vhar
Sargassum polycystum	Thailand	Vhar, WSSV
Sargassum wightii	India	Vpar, Ahyd, Valg, Vhar, Vfis, Rsal, WSSV
Stoechospermum marginatum	India	Ahyd
Undaria pinnatifida	Japan	Vhar
Turbinaria ornata	India	Rsal

The relevant references are cited in Tables 1 and 2

Aero Aeromonas spp., *Ahyd Aeromonas hydrophila*, *Asal Aeromonas salmonicida*, *Etar Edwardsiella tarda*, *Icth I. hoferi*, *IHNV* Infectious Hematopoietic Necrosis Virus, *IPNV* Infectious Pancreatic Necrosis Virus, *Neo Neobenedenia* spp., *Pang Pseudomonas anguilliseptica*, *Pdad Photobacterium damselae* sbsp damselae, *Pdap Photobacterium damselae* sbsp *piscicida*, *Rsal R. salmoninarum*, *Sini Streptococcus iniae*, *Valg V. alginolyticus*, *Vang V. anguillarum*, *Vbra Vibrio brasiliensis*, *Vcam Vibrio campelii*, *Vfis V. fischeri*, *Vhar V. harveyi*, *Vord Vibrio ordalii*, *Vpar V. parahaemolyticus*, *Vsal Vibrio salmonicida*, *Vvul Vibrio vulnificus*, *Vxui Vibrio xuii*, *WSSV* White Spot Syndrome Virus, *Yruc Y. ruckeri*

[a] Area where the study took place

parasites to the target host cells and the subsequent invasion (Patel 2012) or have a direct toxic effect on the parasites. For example, Moo-Puc et al. (2008) demonstrated the direct antiprotozoan activity of organic extracts derived from many seaweed species against *Trichomonas vaginalis* trophozoites, while Bianco et al. (2013) reported significant larvicidal activity of the red seaweed *Laurencia dendroidea* organic extracts against the larval stages of the mosquito *Aedes aegypti*. Despite the many studies on human parasites, the information on the antiparasitic properties of seaweeds against fish parasites is limited, while there are no published studies on shrimp parasites.

Hutson et al. (2012) examined the effect of aqueous extracts from two seaweeds *Ulva* spp. and *Asparagopsis taxiformis* on the parasitism of barramundi (*L. calcarifer*) by the monogenean ectoparasite *Neobenedenia* spp. The extracts, at the concentration of 1/100 *v*/*v*, mainly affected the initial stages of the cycle of the parasites. In particular, they inhibited the embryonic development, delayed the time of first and last hatching, and reduced the hatching success rate of the parasite. The *Asparagopsis taxiformis* extracts appeared substantially more effective. Both extracts however had no significant effect on the survival of the attached adult parasites or the

infection success of oncomiracidia. The authors suggested that these extracts could be particularly effective in either closed or integrated farming systems, if these seaweed species are co-cultivated along with the fish. There was however no assessment of the applicability of this method under farming conditions.

Ghany and Alla (2008) reported that when Nile tilapias (*Oreochromis niloticus)* experimentally infected with the protozoan fish endoparasite *Ichthyophonus hoferi*, they exhibited reduced mortality when fed post-infection with extracts from the seaweed *Fucus vesiculosus* (2 g kg^{-1} body weight) for 3 months. It should be noted though that the study did not provide adequate information on the characteristics of the extracts, or how they were produced.

Conclusions and future priorities

Aquaculture is a growing industry and infectious diseases constitute one of the main limiting factors, affecting the production volume and cost. Assessment of the exact effects of the microbial diseases on the aquaculture production is very difficult, as there are direct and indirect effects. Stressful conditions can also compromise the immune system of fish and shellfish and subsequently reduce their response to any infectious agent (Huntingford et al. 2006).

Seaweeds represent a group of aquatic organisms which is an important part of the marine food chain, as well as the human diet. In addition to their nutritional value, they also exhibit antimicrobial, immunostimulatory and antioxidant properties. In the last 20 years, there is an increasing interest in using various seaweed extracts as prophylactic and therapeutic agents in aquaculture.

Although there are fewer published studies on fish and shrimp pathogens compared to human and husbandry animal pathogens, the findings indicate that seaweeds can play an important role in the upcoming aquaculture sustainable practices.

There are few published studies, which included both in vivo and in vitro assessment of the direct antimicrobial properties of seaweeds. Regarding the fish pathogens, almost all published information comes from in vitro screenings, where extracts of different seaweed species were tested against many bacterial pathogens, while there is only one published study on fish viruses (IHNV and IPNV) and two on fish parasites (*I. hoferi* and *Neobendenia* spp.). Interestingly, there are no published studies on salmons and carps, which are extensively farmed. The studies on shrimp have focused on the antimicrobial effects of seaweed extracts mainly against many *Vibrio* species and WSSV. Although all the studies indicate the overall positive effect of the extracts, they do not elucidate the exact mechanism of action and particularly within the animal tissues (Fig. 1). Furthermore, although it is known that many seaweed extracts also exhibit immunostimulatory properties, which can contribute to the protective effect, in most studies these effects were never examined in parallel to the antimicrobial effects.

In general terms, all three groups of seaweeds (red, green and brown) exhibit antimicrobial properties, but the genera that appear to exhibit a broader range of activity are *Asparagopsis* spp. (red) and *Sargassum* spp. (brown). It should be noted though that comparison between species is difficult, as there are many factors that can affect the antimicrobial properties, and the same seaweed species may exhibit different properties depending on the season or the geographical area.

The extraction method is also an important factor that can affect the efficacy of the produced extracts. In 27 out of 39 of the studies that are presented in this review, organic solvents were used for the extraction rather than water.

The modes of delivery of the active seaweed substances can either be through the water (released directly from the seaweeds or added into it after their extraction), or through medicated feed (again after their extraction), as outlined in Fig. 2. In the first case, mainly water-soluble substances of seaweeds can be released or added into the aquatic environment of the farmed fish and shrimp. These substances appear to affect the quorum sensing mechanism in bacteria with limited effects on the bacterial growth. When the extracts are added into the feeds (live or dry), they can act directly against the pathogens or by stimulating the immune system. In addition, there are no complete pharmacodynamic and pharmacokinetic studies, which can demonstrate the exact mode of action of any seaweed extract. This important issue should be included in future studies.

An important point that none of the published studies presented in our review has examined is the applicability of using any of these extracts on a commercial scale. The main issues related to this are the extraction cost and how the extracts can be delivered to fish or shrimp under the intensive farming conditions.

The production cost of seaweeds varies according to the country and it can be between € 160 and € 330 T^{-1} dry, in Asia and Europe, respectively, but new seaweed culture techniques are expected to reduce this cost (Bruton et al. 2009). For the extraction of the active substances, there are a few methods that are available on a commercial scale and at the moment the cost of these methods is relatively high (Takahashi et al. 1998; Ibañez et al. 2012). The yield of the active substances extracted from seaweed is between less than 1 % up to 40 % of the dry algal mass, depending on various factors, such the metabolite, seaweed species and season (Pereira and Costa-Lotufo 2012). Possible solutions to the high production cost can be the production of synthetic seaweed active compounds, as some of them exhibit properties similar to the natural

substance (Rasch et al. 2004; Defoirdt el. 2006), or the incorporation of the responsible seaweed genes into microorganism as Pereira et al. (2012) suggested. However, some of these techniques have many complex steps and can be applied only when the antimicrobial effect of the natural analogs is well demonstrated.

As discussed before, one mode of action is through the inhibition of the quorum sensing mechanism of the bacterial pathogens that exist in the water column, prior to infection. The active substances need to be constantly added into the water for long periods, as Rasch et al. (2004) did during their experimental challenges. Mata et al. (2013) examining the therapeutic effect of seaweed extracts also added the extracts to the water containing infected fish for a long period. In practice, this method can only be applied on land facilities, when fish are reared in small tanks and the water exchange rate is low (e.g. in hatcheries). In addition, the administration of therapeutics extracted from seaweed must be monitored continuously, as sudden increases of the concentration of the antimicrobial substance can be lethal (Rasch et al. 2004; Mata et al. 2013) and exposure periods must be as short as possible (Thanigaivel et al. 2014). More studies on short-term exposures are therefore required to confirm the efficacy of such treatments, particularly against parasitic pathogens.

The safest delivery method reported is through medicated feed, as the dose of the extract per animal treated can be calculated more accurately. This method applies to all farming systems and can decrease the bacterial load in the tissues (Immanuel et al. 2004). Thus, this method of delivery will probably be the most effective and applicable one. Nevertheless, more studies investigating the effect seaweed extracts on pathogens are necessary to support this hypothesis.

References

Aarestrup FM, Jensen VF, Emborg HD, Jacobsen E, Wegener HC (2010) Changes in the use of antimicrobials and the effects on productivity of swine farms in Denmark. Am J Vet Res 71:726–33

Abu-Ghannam N, Rajauria G (2013) Antimicrobial activity of compounds isolated from algae. In: Domínguez H (ed) Functional ingredients from algae for foods and nutraceuticals. Woodhead, Sawston, pp 287–306

Ahn MJ, Yoon KD, Min SY, Lee JS, Kim JH, Kim TG, Kim SH, Kim NG, Huh H, Kim J (2004) Inhibition of HIV-1 reverse transcriptase and protease by phlorotannins from the brown alga *Ecklonia cava*. Biol Pharm Bull 27:544–547

Al-Hafedh YS, Alam A, Buschmann AH, Fitzsimmons KM (2012) Experiments on an integrated aquaculture system (seaweeds and marine fish) on the Red Sea coast of Saudi Arabia: efficiency comparison of two local seaweed species for nutrient biofiltration and production. Rev Aquac 4:21–31

Bailey SE, Olin TJ, Bricka RM, Adrian DD (1999) A review of potentially low-cost sorbents for heavy metals. Water Res 33:2469–2479

Balasubramanian G, Sudhakaran R, Syed Musthaq S, Sarathi M, Sahul Hameed AS (2006) Studies on the inactivation of white spot syndrome virus of shrimp by physical and chemical treatments, and seaweed extracts tested in marine and freshwater animal models. J Fish Dis 29:569–572

Baleta FN, Laureta LV, Apines-Amar MJS, Padilla PIP, Quinitio GF (2011) Biological activity of extracts of *Sargassum oligocystum* (Magnaye) against aquaculture pathogenic bacteria. Isr J Aquac IIC 63(2011):667

Bansemir A, Just N, Michalik M, Lindequist U, Lalk M (2004) Extracts and sesquiterpene derivatives from the red alga *Laurencia chondrioides* with antibacterial activity against fish and human pathogenic bacteria. Chem Biodivers 1:463–467

Bansemir A, Blume M, Schröder S, Lindequist U (2006) Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. Aquaculture 252:79–84

Barrington K, Chopin T, Robinson S (2009) Integrated multi-trophic aquaculture (IMTA) in marine temperate waters. In: D. Soto (ed). Integrated mariculture: a global review. FAO Fisheries and Aquaculture Technical Paper. No. 529. Rome, FAO, pp 7–46

Bianco EM, Pires L, Santos GKN, Dutra KA, Reis TNV, Vasconcelos ERTPP, Cocentino ALM, Navarro DMAF (2013) Larvicidal activity of seaweeds from northeastern Brazil and of a halogenated sesquiterpene against the dengue mosquito (*Aedes aegypti*). Ind Crop Prod 43:270–275

Bindu MS, Sobha V (2004) Conversion efficiency and nutrient digestibility of certain seaweed diets by laboratory reared *Labeo rohita* (Hamilton). Indian J Exp Biol 42:1239–1244

Bruton T, Lyons H, Lerat Y, Stanley M, Rasmussen MB (2009) A review of the potential of marine algae as a source of biofuel in Ireland. Report prepared for Sustainable Energy Ireland. http://www.seai.ie/Publications/Renewables_Publications_/Bioenergy/Algaereport.pdf. Accessed 30 Oct 2014

Buchholz CM, Krause G, Buck BH (2012) Seaweed and man. In: Wiencke C, Bischof K (eds) Seaweed biology. Springer, Berlin, pp 471–493

Buck CB, Thompson CD, Roberts JN, Muller M, Lowy DR, Schiller JT (2006) Carrageenan is a potent inhibitor of papillomavirus infection. PLoS Pathog 2(7):e69

Burridge L, Weis J, Cabello F, Pizarro J, Bostick K (2010) Chemical use in salmon aquaculture: a review of current practices and possible environmental effects. Aquaculture 306:7–23

Cai J, Feng J, Xie S, Wang F, Xu Q (2014) *Laminaria japonica* extract, an inhibitor of *Clavibater michiganense* subsp. *sepedonicum*. PLoS One 9(4):e94329

Caipang CMA, Lazado CC, Berg I, Brinchmann MF, Viswanath K (2011) Influence of alginic acid and fucoidan on the immune responses of head kidney leukocytes in cod. Fish Physiol Biochem 37:603–612

Cavallo RA, Acquaviva M, Stabili L, Cecere E, Petrocelli A, Narracci M (2013) Antibacterial activity of marine macroalgae against fish pathogenic *Vibrio* species. Cent Eur J Biol 8:646–653

Chiu YH, Chan YL, Li TL, Wu CJ (2012) Inhibition of Japanese encephalitis virus infection by the sulfated polysaccharide extracts from *Ulva lactuca*. Mar Biotech 14:468–478

Chotigeat W, Tongsupa S, Supamataya K, Phongdara A (2004) Effect of fucoidan on disease resistance of black tiger shrimp. Aquaculture 233:23–30

Christobel JG, Lipton AP, Aishwarya MS, Sarika AR, Udayakumar A (2011) Antibacterial activity of aqueous extract from selected macroalgae of southwest coast of India. Seaweed Res Util 33:67–75

Costello MJ (2009) The global economic cost of sea lice to the salmonid farming industry. J Fish Dis 32:115–118

Dashtiannasab A, Kakoolaki S, Sharif Rohani M, Yeganeh V (2012) In vitro effects of *Sargassum latifolium* (Agardeh, 1948) against selected bacterial pathogens of shrimp. Iran J Fish Sci 11(4):765–775

Dawczynski C, Schubert R, Jahreis G (2007) Amino acids, fatty acids, and dietary fibre in edible seaweed products. Food Chem 103:891–899

de Lara-Isassi G, Álvarez-Hernández S, Collado-Vides L (2000) Ichtyotoxic activity of extracts from Mexican marine macroalgae. J Appl Phycol 12:45–52

Defoirdt T, Crab R, Wood TK, Sorgeloos P, Verstraete W, Bossier P (2006) Quorum sensing-disrupting brominated furanones protect the gnotobiotic brine shrimp *Artemia franciscana* from pathogenic *Vibrio harveyi*, *Vibrio campbellii*, and *Vibrio parahaemolyticus* isolates. Appl Environ Microbiol 72:6419–6423

Dillehay TD, Ramírez C, Pino M, Collins MB, Rossen J, Pino-Navarro JD (2008) Monte Verde: seaweed, food, medicine, and the peopling of South America. Science 320:784–786

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

Dubber D, Harder T (2008) Extracts of *Ceramium rubrum*, *Mastocarpus stellatus* and *Laminaria digitata* inhibit growth of marine and fish pathogenic bacteria at ecologically realistic concentrations. Aquaculture 274:196–200

El Gamal AA (2010) Biological importance of marine algae. Saudi Pharm J 18:1–25

El Ghany NAA, Alla HMLA (2008) A trial for treatment of ichthyophonosis in cultured *Oreochromis niloticus* using fucus and neem plants. 8th International Symposium on Tilapia in Aquaculture. Proceedings. Cairo, Egypt, 12–14 October, 2008. pp. 1329–1349

Food and Agriculture Organization of the United Nations (FAO) (2014) Global aquaculture production. http://www.fao.org/fishery/statistics/global-aquaculture-production/en. Accessed 14 May 2014

Ganeshamurthy R, Kumar TTA, Dhayanithi NB (2012) Effect of secondary metabolites of the seaweed (*Halimeda micronesia*) at Lakshadweep islands against aquatic pathogens. Int J Pharm Bio Sci 3:B213–B220

Genovese G, Faggio C, Gugliandolo C, Torre A, Spanò A, Morabito M, Maugeri TL (2012) In vitro evaluation of antibacterial activity of *Asparagopsis taxiformis* from the Straits of Messina against pathogens relevant in aquaculture. Mar Environ Res 73:1–6

Govindasamy C, Narayani S, Arulpriya M, Ruban P, Anantharaj K, Srinivasan R (2011) In vitro antimicrobial activities of seaweed extracts against human pathogens. J Pharm Res 4:2076–2077

Gupta S, Abu-Ghannam N (2011a) Bioactive potential and possible health effects of edible brown seaweeds. Trends Food Sci Tech 22: 315–326

Gupta S, Abu-Ghannam N (2011b) Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality of foods. Innov Food Sci Emerg 12:600–609

Henry EC (2012) The use of algae in fish feeds as alternatives to fish meals. http://users.auth.gr/kganias/Aquaculture/AQUAFEED_selection.pdf. Accessed May 14 2014

Heuer OE, Kruse H, Grave K, Collignon P, Karunasagar I, Angulo FJ (2009) Human health consequences of use of antimicrobial agents in aquaculture. Clin Infect Dis 49:1248–1253

Hierholtzer A, Chatellard L, Kierans M, Akunna JC, Collier PJ (2014) The impact and mode of action of phenolic compounds extracted from brown seaweed on mixed anaerobic microbial cultures. J Appl Microbiol 114:964–973

Higa T, Kuniyoshi M (2000) Toxins associated with medicinal and edible seaweeds. J Toxicol Toxin Rev 19:119–137

Holdt SL, Kraan S (2011) Bioactive compounds in seaweed: functional food applications and legislation. J Appl Phycol 23:543–597

Huntingford FA, Adams C, Braithwaite VA, Kadri S, Pottinger TG, Sandøe P, Turnbull JF (2006) Current issues in fish welfare. J Fish Biol 68:332–372

Hutson KS, Mata L, Paul NA, de Nys R (2012) Seaweed extracts as a natural control against the monogenean ectoparasite, *Neobenedenia* sp., infecting farmed barramundi (*Lates calcarifer*). Int J Parasitol 42:1135–1141

Ibañez E, Herrero M, Mendiola JA, Castro-Puyana M (2012) Extraction and characterization of bioactive compounds with health benefits from marine resources: macro and micro algae, cyanobacteria, and invertebrates. In: Hayes M (ed) Marine bioactive compounds: sources, characterization and applications. Springer, Berlin, pp 55–98

Immanuel G, Vincybai VC, Sivaram V, Palavesam A, Marian MP (2004) Effect of butanolic extracts from terrestrial herbs and seaweeds on the survival, growth and pathogen (*Vibrio parahaemolyticus*) load on shrimp *Penaeus indicus* juveniles. Aquaculture 236:53–65

Immanuel G, Sivagnanavelmurugan M, Balasubramanian V, Palavesam A (2010) Effect of hot water extracts of brown seaweeds *Sargassum* spp. on growth and resistance to white spot syndrome virus in shrimp *Penaeus monodon* postlarvae. Aquac Res 41:e545–e553

Immanuel G, Sivagnanavelmurugan M, Balasubramanian V, Palavesam A (2012) Sodium alginate from *Sargassum wightii* retards mortalities in *Penaeus monodon* postlarvae challenged with white spot syndrome virus. Dis Aquat Org 99:187–196

Jha B, Kavita K, Westphal J, Hartmann A, Schmitt-Kopplin P (2013) Quorum sensing inhibition by *Asparagopsis taxiformis*, a marine macro alga: separation of the compound that interrupts bacterial communication. Mar Drugs 11:253–265

Jiao G, Yu G, Wang W, Zhao X, Zhang J, Ewart SH (2012) Properties of polysaccharides in several seaweeds from Atlantic Canada and their potential anti-influenza viral activities. J Ocean Univ China 11:205–212

Johansen LH, Jensen I, Mikkelsen H, Bjørn PA, Jansen PA, Bergh Ø (2011) Disease interaction and pathogens exchange between wild and farmed fish populations with special reference to Norway. Aquaculture 315:167–186

Kang MC, Kim KN, Kang SM, Yang X, Kim EA, Song CB, Nah JW, Jang MK, Lee JS, Jung WK, Jeon YJ (2013) Protective effect of dieckol isolated from *Ecklonia cava* against ethanol caused damage in vitro and in zebra fish model. Environ Toxicol Pharmacol 36: 1217–1226

Kanjana K, Radtanatip T, Asuvapongpatana S, Withyachumnarnkul B, Wongprasert K (2011) Solvent extracts of the red seaweed *Gracilaria fisheri* prevent *Vibrio harveyi* infections in the black tiger shrimp *Penaeus monodon*. Fish Shellfish Immunol 30:389–396

Karthikaidevi G, Manivannan K, Thirumaran G, Anantharaman P, Balasubaramanian T (2009) Antibacterial properties of selected green seaweeds from Vedalai coastal waters; Gulf of Mannar marine biosphere reserve. Glob J Pharmacol 3:107–112

Kibenge FSB, Godoy MG, Fast M, Workenhe S, Kibenge MJT (2012) Countermeasures against viral diseases of farmed fish. Antivir Res 95:257–281

Kim SY, Kim SR, Oh MJ, Jung SJ, Kang SY (2011) In vitro antiviral activity of red alga, *Polysiphonia morrowii* extract and its bromophenols against fish pathogenic infectious hematopoietic necrosis virus and infectious pancreatic necrosis virus. J Microbiol 49: 102–106

Lu K, Lin W, Liu J (2008) The characteristics of nutrient removal and inhibitory effect of *Ulva clathrata* on *Vibrio anguillarum* 65. J Appl Phycol 20:1061–1068

Maheswaran ML, Padmavathy S, Gunalan B (2013) Screening and characterization of marine seaweeds and its antimicrobial potential against fish pathogens. Int J Fish Aquat Stud 1:1–13

Manefield M, Harris L, Rice SA, de Nys R, Kjelleberg S (2000) Inhibition of luminescence and virulence in the black tiger prawn (*Penaeus monodon*) pathogen *Vibrio harveyi* by intercellular signal antagonists. Appl Environ Microbiol 66:2079–2084

Manefield M, Welch M, Givskov M, Salmond GPC, Kjelleberg S (2001) Halogenated furanones from the red alga, *Delisea pulchra*, inhibit carbapenem antibiotic synthesis and exoenzyme virulence factor production in the phytopathogen *Erwinia carotovora*. FEMS Microbiol Lett 205:131–138

Manilal A, Sujith S, Selvin J, Seghal Kiran G, Shakir C (2009) In vivo antiviral activity of polysaccharide from the Indian green alga, *Acrosiphonia orientalis* (J. Agardh): potential implication in shrimp disease management. World J Fish Mar Sci 1:278–282

Manilal A, Selvin J, George S (2012) In vivo therapeutic potentiality of red seaweed, *Asparagopsis* (Bonnemaisoniales, Rhodophyta) in the treatment of vibriosis in *Penaeus monodon* Fabricius. Saudi J Biol Sci 19:165–175

Martinez JL (2009) The role of natural environments in the evolution of resistance traits in pathogenic bacteria. Proc R Soc B 276:2521–2530

Marudhupandi T, Kumar TTA (2013) Antibacterial effect of fucoidan from *Sargassum wightii* against the chosen human bacterial pathogens. Int Curr Pharm J 2:156–158

Mata L, Wright E, Owens L, Paul N, de Nys R (2013) Water-soluble natural products from seaweed have limited potential in controlling bacterial pathogens in fish aquaculture. J Appl Phycol 25:1963–1973

McLoughlin M (2006) Fish vaccination—a brief overview. http://www.imb.ie/images/uploaded/documents/Fish%20Vaccine%20Overview.pdf. Accessed 14 May 2014

Miranda CD, Zemelman R (2002) Bacterial resistance to oxytetracycline in Chilean salmon farming. Aquaculture 212:31–47

Mohamed S, Hashim SN, Rahman HA (2012) Seaweeds: a sustainable functional food for complementary and alternative therapy. Trends Food Sci Technol 23:83–96

Moo-Puc R, Robledo D, Freile-Pelegrin Y (2008) Evaluation of selected tropical seaweeds for in vitro anti-trichomonal activity. J Ethnopharmacol 120:92–97

Omar HH, Gumgumji NM, Shiek HM, El Kazan MM, El Gendy AM (2012) Inhibition of the development of pathogenic fungi by extracts of some marine algae from the Red Sea of Jeddah, Saudi Arabia. Afr J Biotechnol 11:13697–13704

Osman MEH, Abu-Shady AM, Elshobary ME (2012) The seasonal fluctuation of the antimicrobial activity of some macroalgae collected from Alexandria Coast, Egypt. In: Annous BA, Gurtler JB (eds) *Salmonella*: distribution, adaptation, control measures and molecular technologies, InTech, pp 173–186

Patel S (2012) Therapeutic importance of sulfated polysaccharides from seaweeds: updating the recent findings. 3 Biotechnol 2:171–185

Pereira RC, Costa-Lotufo LV (2012) Bioprospecting for bioactives from seaweeds: potential, obstacles and alternatives. Rev Bras Farmacogn Braz J Pharmacogn 22:894–905

Petersen A, Andersen JS, Kaewmak T, Somsiri T, Dalsgaard A (2002) Impact of integrated fish farming on antimicrobial resistance in a pond environment. Appl Environ Microbiol 68:6036–6042

Plaza M, Cifuentes A, Ibáñez E (2008) In the search of new functional food ingredients from algae. Trends Food Sci Technol 19:31–39

Plaza M, Santoyo S, Jaime L, García-Blairsy Reina G, Herrero M, Señoráns FJ, Ibáñez E (2010) Screening for bioactive compounds from algae. J Pharm Biomed Anal 51:450–455

Radhika D, Veerabahu C, Priya R, Mohaideen A (2014) A comparative study of biopotential of crude and fractionated extracts of some sea weeds from Tuticorin coast. Int J Phytopharmacol 5:27–30

Rasch M, Buch C, Austin B, Slierendrecht WJ, Ekmann KS, Larsen JL, Johansen C, Riedel K, Eberl L, Givskov M, Gram L (2004) An inhibitor of bacterial quorum sensing reduces mortalities caused by vibriosis in rainbow trout (*Oncorhynchus mykiss*, Walbaum). Syst Appl Microbiol 27:350–359

Rebecca LJ, Dhanalakshmi V, Sharmila S (2012) Effect of the extract of *Ulva* sp on pathogenic microorganisms. J Chem Pharm Res 4:4875–4878

Romero J, Feijoó CG, Navarrete P (2012) Antibiotics in Aquaculture – Use, Abuse and Alternatives. In: Carvalho ED, David GS, Silva RJ (eds) Health and environment in aquaculture. InTech, Croatia, pp 159–198

Rudtanatip T, Asuvapongpatana S, Withyachumnarnkul B, Wongprasert K (2014) Sulfated galactans isolated from the red seaweed *Gracilaria fisheri* targeted the envelope proteins of white spot syndrome virus and protected against viral infection in shrimp haemocytes. J Gen Virol. doi:10.1099/vir. 0.062919-0

Rutherford ST, Bassler BL (2014) Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harb Perspect Med 2:a012427

Saha S, Navid MH, Bandyopadhyay SS, Schnitzler P, Ray B (2012) Sulfated polysaccharides from *Laminaria angustata*: structural features and in vitro antiviral activities. Carbohydr Polym 87:123–130

Salvador N, Garreta AG, Lavelli L, Ribera MA (2007) Antimicrobial activity of Iberian macroalgae. Sci Mar 71:101–113

Saritha K, Mani AE, Priyalaxmi M, Patterson J (2013) Antibacterial activity and biochemical constituents of seaweed *Ulva lactuca*. Glob J Pharmacol 7:276–282

Selvin J, Manilal A, Sujith S, Kiran GS, Lipton AP (2011) Efficacy of marine green alga *Ulva fasciata* extract on the management of shrimp bacterial diseases. Lat Am J Aquat Res 39:197–204

Silva GC, Albuquerque-Costa R, Oliveira-Peixoto JR, Pessoa-Nascimento FE, de Macedo-Carneiro PB, dos Fernandes-Vieira RHS (2013) Tropical Atlantic marine macroalgae with bioactivity against virulent and antibiotic resistant *Vibrio*. Lat Am J Aquat Res 41:183–188

Singh M, Manikandan S, Kumaraguru AK (2012) In vitro antibacterial activity of selected brown marine macroalgae extracts collected from the Pudumadam Coast of "Gulf of Mannar" region against fish pathogens. Int J Human Genet Med Biotechnol Microbiol Stud. ISSN (Online) 2319–1732

Sirirustananun N, Chen JC, Lin YC, Yeh ST, Liou CH, Chen LL, Sim SS, Chiew SL (2011) Dietary administration of a *Gracilaria tenuistipitata* extract enhances the immune response and resistance against *Vibrio alginolyticus* and white spot syndrome virus in the white shrimp *Litopenaeus vannamei*. Fish Shellfish Immunol 31:848–855

Sivagnanavelmurugan M, Marudhupandi T, Palavesam A, Immanuel G (2012) Antiviral effect of fucoidan extracted from the brown seaweed, *Sargassum wightii*, on shrimp *Penaeus monodon* postlarvae against White Spot Syndrome Virus. J World Aquacult Soc 43:697–706

Sivakumar K, Kannappan S, Dineshkumar M, Patil PK (2014) Evaluation of marine macro alga, *Ulva fasciata* against bioluminescent causing *Vibrio harveyi* during *Penaeus monodon* larviculture. Afr J Microbiol Res 8:803–813

Smit AJ (2004) Medicinal and pharmaceutical uses of seaweed natural products: a review. J Appl Phycol 16:245–262

Son M, Lee M, Sung GH, Lee T, Shin YS, Cho H, Lieberman PM, Kang H (2013) Bioactive activities of natural products against Herpesvirus infection. J Microbiol 51:545–551

Stern J, Hagerman A, Steinberg P, Magon P (1996) Phlorotannin–protein interactions. J Chem Ecol 22:1877–1899

Sudheer NS, Philip R, Singh ISB (2012) Anti-white spot syndrome virus activity of *Ceriops tagal* aqueous extract in giant tiger shrimp *Penaeus monodon*. Arch Virol 157:1665–1675

Takahashi Y, Uehara K, Watanabe R, Okumura T, Yamashita T, Omura H, Yomo T, Kawano T, Kanemitsu A, Narasaka H, Suzuki N, Itami T (1998) Efficacy of oral administration of fucoidan, a sulfated polysaccharide, in controlling white spot syndrome in kuruma shrimp in Japan. In: Flegel TW (ed) Advances in shrimp biotechnology.

National Center for Genetic Engineering and Biotechnology, Bangkok, pp 171–173

Thanigaivel S, Vijayakumar S, Mukherjee A, Chandrasekaran N, Thomas J (2014) Antioxidant and antibacterial activity of *Chaetomorpha antennina* against shrimp pathogen *Vibrio parahaemolyticus*. Aquaculture 433:467–475

The Fish Site (2010) Aquatic animal diseases and their economic impact. http://www.thefishsite.com/articles/896/aquatic-animal-diseases-and-their-economic-impact#sthash.CrjIsPk9.dpuf. Accessed 11 March 2014

Traifalgar RF, Serrano AE, Corre V, Kira H, Tung HT, Michael FR, Kader MA, Laining A (2009) Evaluation of dietary fucoidan supplementation effects on growth performance and vibriosis resistance of *Penaeus monodon* postlarvae. Aquac Sci 57:167–174

Vairappan CS, Suzuki M (2000) Dynamics of total surface bacteria and bacterial species counts during desiccation in the Malaysian sea lettuce, *Ulva reticulata* (Ulvales, Chlorophyta). Phycol Res 48:55–61

Vairappan CS, Daitoh M, Suzuki M, Abe T, Masuda M (2001) Antibacterial halogenated metabolites from the Malaysian *Laurencia* species. Phytochemistry 58:291–297

Vijayabaskar P, Shiyamala V (2011) Antibacterial activities of brown marine algae (*Sargassum wightii* and *Turbinaria ornata*) from the Gulf of Mannar biosphere reserve. Advan Biol Res 5:99–102

Vonthron-Sénécheau C, Kaiser M, Devambez I, Vastel A, Mussio I, Rusig AM (2011) Antiprotozoal activities of organic extracts from French marine seaweeds. Mar Drugs 9:922–933

Wang W, Wang SX, Guan HS (2012) The antiviral activities and mechanisms of marine polysaccharides: an overview. Mar Drugs 10: 2795–2816

Wijesinghe WAJP, Kim EA, Kang MC, Lee WW, Lee HS, Vairappan CS, Jeon YJ (2014) Assessment of anti-inflammatory effect of 5-hydroxypalisadin B isolated from red seaweed *Laurencia snackeyi* in zebrafish embryo in vivo model. Environ Toxicol Pharmacol 37: 110–117

World Health Organization (2011) Tackling antibiotic resistance from a food safety perspective in Europe. http://www.euro.who.int/__data/assets/pdf_file/0005/136454/e94889.pdf Accessed 16 Oct 2014

Xu N, Fan X, Yan X, Li X, Niu R, Tseng CK (2003) Antibacterial bromophenols from the marine red alga *Rhodomela confervoides*. Phytochemistry 62:1221–1224

AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES.

Q1. Please check if the affiliations are presented correctly.
Q2. Kindly check the modifications made in Tables 1 and 2 if appropriate.
Q3. "Hutson et al. (2013)" is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.
Q4. The citation "Hierholtzer et al., 2012" (original) has been changed to "Hierholtzer et al. 2014". Please check if appropriate.
Q5. The citation "Rutherford and Bussler 2012" (original) has been changed to "Rutherford and Bassler 2014". Please check if appropriate.
Q6. "Pereira et al. (2012)" is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.