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# Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases

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## Abstract

Many classical therapeutants are going to be banned in Europe, and an urgent need for alternatives is emerging. This issue can be exemplified by one major parasitic disease in aquaculture and ornamental fish breeding: velvet disease. This disease, caused by dinoflagellates of the genera *Amyloodinium* and *Piscinoodinium*, is an important infection affecting cultured freshwater and marine ornamental and food fish, and consistently causes great financial loss to the associated industries. Therapeutants available contain copper, malachite green, or methylene blue, and which can be toxic to non-target organisms in the surrounding environment. As a result, these chemicals are banned for use by the aquaculture industry in several countries, and a prohibition for commercial ornamental fishkeeping is likely to follow in most countries. Increasing development of resistance to therapeutants, and growing public awareness for animal welfare and environmental protection, have prompted research in the areas of alternative treatment options and immunostimulants. Hydrogen peroxide and peracetic acid are possible 'green' therapeutants which do not contribute residues to the environment. Natural feed supplements such as pre- and probiotics can increase animal welfare and prevent stress and/or infections. Humic substances are another promising, natural immunostimulants which will be considered in depth. The aim of this review is to provide an overview of risks and benefits of current treatment options and new approaches to replace harmful therapeutants and minimize the number of toxic residues discharged into the environment. Treatments will be discussed on various parasitic infections and focus, where available, on *Amyloodinium* and *Piscinoodinium*.

**Key words:** biotics, copper sulphate, disease prevention, humic substances, immunostimulants, *Oodinium*.

## Introduction

Finfish are hosts to a plethora of both ecto- and endoparasites. These parasites are part of every ecosystem and they generally have limited effects on the fitness of healthy fish. However, parasites can become a problem under stressful conditions which can occur when fish are reared in captivity (e.g., public aquariums, the ornamental fish trade and

aquaculture). In aquaculture, up to 50% of production loss is caused by diseases (Assefa & Abunna 2018). High stocking densities and poor water quality present optimal conditions for the infestation and reproduction of parasites, leading quickly to pathogenic levels. Furthermore, spreading of infectious pathogens is supported by the inevitable transport of fish and equipment (Subasinghe *et al.* 2001). Parasitic infections can spread not only inside a system but

also to adjacent culture units via aerosol transfer (Paperna 1980). These anthropogenic stressors can lead to acute and chronic stress, and the need to cope with the allostatic load requires (energetic) metabolic reorganization, subsequently decreasing growth performance, depressing immune system functions and leaving fish even more vulnerable to infections (Bly *et al.* 1997; Tort 2011). For example, catfish exposed to 30-min low-water stress had about 20% mortality when challenged with *Edwardsiella ictaluri* compared to control fish (Small & Bilodeau 2005). This review focuses on velvet disease, a major disease threat in warm water culture, as a foundation to review the transition to emerging treatment approaches. Information on this disease is often distributed to different databases, paper, and books of research experts. In the first part we summarize information on the different parasites, life cycles, and misinterpretations due to outdated taxonomical terms.

Current treatment options such as triphenylmethane dyes, copper, acriflavine and formalin, as well as their effects on different life stages, are considered. Risks associated with many of these ‘traditional’ chemical therapeutics to fish, to the environment and to consumers have led to bans for their use in the aquaculture industry of many countries. The search for alternative environmentally friendly approaches for diseases has increased during the last decade and shall be considered in detail with emphasis on velvet disease where available.

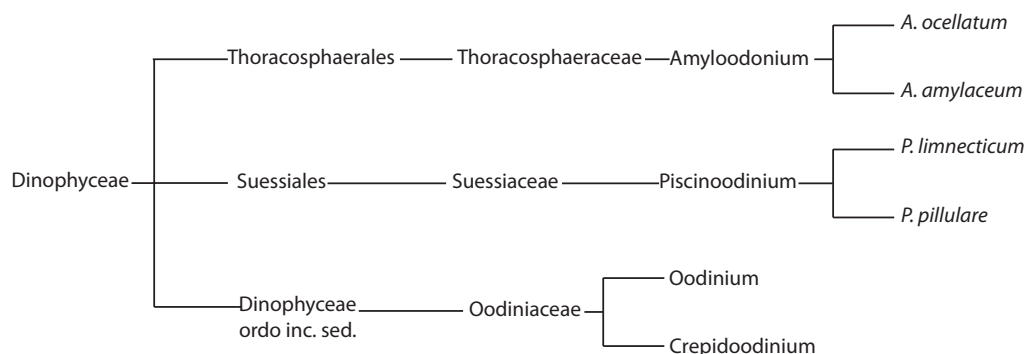
Furthermore, we reviewed recent international literature, as well as ‘grey literature’ and included results from our own research. Application of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and peracetic acid (PAA) as disinfectants to reduce the pathogenic load will be discussed. Supplementing food with live cultures of microorganisms and their immunostimulatory effects have been the subject of many studies in human and fish. We will discuss the different types of “biotics” including their interaction and different modes of action. Finally, supplementation of food and culture water with different plant extracts and humic substances is presented.

These supplements are used to increase of overall and immune-related health of fish and thereby stimulation of host-specific defense mechanisms against pathogens. We discuss the idea of whether or not the stimulation of the defense system increases overall protection and helps to prevent velvet disease as well as other pathogen-induced diseases.

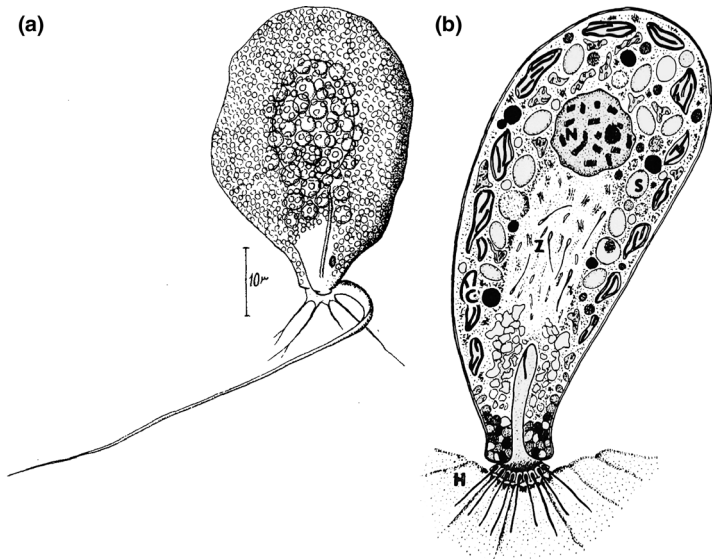
### Velvet disease is a risk to both freshwater and marine finfish

Velvet disease (Noga 2011), also known as Rust or Gold Dust disease, is caused by dinoflagellates of the genera *Amyloodinium* (Brown 1931; Brown & Hovasse 1946) and *Piscinoodinium* (Schäperclaus 1954; Lom 1981) and is a major threat in warm water fish culture facilities. It also poses a threat in coolwater facilities, although white spot disease, caused by *Ichthyophthirius multifiliis* in freshwater and *Cryptocaryon irritans* in marine environments salmon lice (*Lepeophtheirus salmonis*), and species of the genus *Myxobolus* are more of a threat. The name ‘Oodinium’, which can be found in old publications, is still used by ichthyopathologists and aquarium hobbyists but does not distinguish between the two genera (Lom & Dyková 1992). Taxonomically, the genus *Oodinium* Chatton, 1912, still exists and contains six species; however, their exact systematic position is unclear (Guiry & Guiry 2018). These species are ectoparasitic dinoflagellates of marine invertebrates. Species of the genus *Crepidoodinium* are also ectoparasites to marine fish but do not cause velvet disease. Figure 1 shows the systematic positions and relations of the different genera.

*Piscinoodinium pillulare* (Figure 2a) and *P. limneticum* are important pathogens of both, tropical and temperate freshwater fishes, while *Amyloodinium ocellatum* (Figure 2b) is the marine analogue and is one of the most important parasites infesting warm water marine or brackish water fishes (Noga 2012). These two dinoflagellate



**Figure 1** Systematic position and relation of Dinophyceae historically termed as “Oodinium”. Created, based on information from Guiry and Guiry (2018) and Guillou and Not (2018).

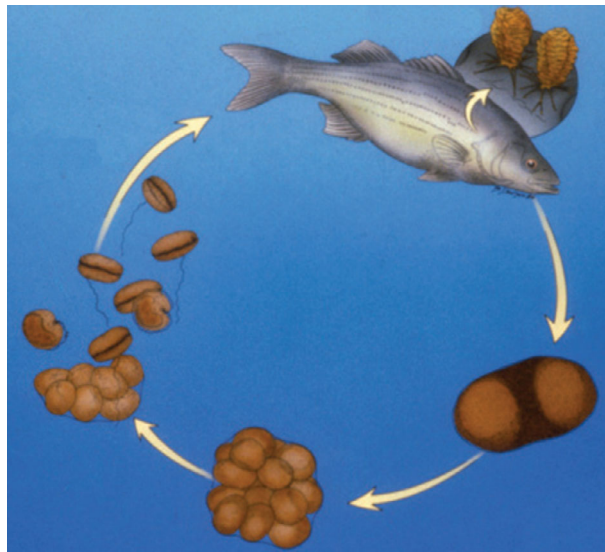


**Figure 2** Trophonts of a: *Amyloodinium ocellatum* from Brown and Hovasse (1946) with permission from John Wiley and Sons and b: *Piscinoodinium pillulare* from Lom and Schubert (1983) with permission from Folia Parasitologica.

genera are morphologically very similar and range in size from 80 to 150  $\mu\text{m}$ . However, *Piscinoodinium* obtains essential parts of its nutrition from photosynthesis, although light is not crucial for survival, while *Amyloodinium* lacks chloroplasts (Hoffman 1999; Abowei *et al.* 2011; Noga 2012). The life cycle of both genera is controlled by temperature and consists of three stages. First, free-swimming dinospores (also called gymnospires; Lom and Dyková (1992) infect the host and penetrate the epithelium (typically skin or gills) with an extension called a 'rhizoid'. As Figure 2 indicates, the extension of *Piscinoodinium* is nail-shaped (Abowei *et al.* 2011), while that of *Amyloodinium* is root-shaped (Lom & Lawler 1973; Cheung *et al.* 1981). Furthermore, *Amyloodinium* has a special tentacle-like movable organ called a stomopode, which *Piscinoodinium* lacks. The function may be food ingestion and injection of lytic bodies into the host cells (Guillou & Not 2018).

After attaching to the host, dinospores transform into trophonts which feed on the host's cells. Finally, the parasites detach and transform into tomonts. This encapsulated form is the reproductive stage, and a single tomont produces as many as 256 new dinospores that are ready to infect a new host. Tomont division occurs between 16 and 30°C (Saraiva *et al.* 2011). At 25°C and under ideal reproduction conditions (e.g. salinity, host density), the entire life cycle (Figure 3) takes about 4 to 5 days.

The high pathogenicity of these organisms is caused not only by extensive structural damage to the epithelium by the rhizoid itself but also by histopathological changes such as degeneration and necrosis in cells adjacent to the attachment site. These changes are possibly induced by toxic substances or irritants produced by the parasites (Lom & Lawler 1973; Paperna 1980). Under such conditions, the



**Figure 3** Life cycle of *Amyloodinium ocellatum*. Free swimming dinospores embed in the host skin using rhizoids and ultimately turn into trophonts. Detaching trophonts encapsulate and divide into tomonts, the reproductive stage. After several cell divisions, dinospores are released from the capsule and infest new hosts. From Francis-Floyd and Floyd (2011) and Noga (1987) reprinted with permission from Science.

host is very vulnerable to osmoregulatory impairment and secondary infections by other pathogens such as bacteria or fungi (Woo & Buchmann 2012).

### Diagnosis, current treatments, and related risks

Velvet disease is highly contagious and considering its rapid lifecycle, it is imperative to diagnose and treat as early as possible. Visible symptoms of an infestation in fish are

flashing, rapid gill movement, loss of appetite (and consequently loss of weight), clamped fins and lethargy. The key symptom is a velvet film or 'dusting' on the skin ranging in colour from gold to brown to green. In an early stage of infestation, symptoms may be weakly pronounced, if they occur at all. Analyzing smears of mucus and skin or gills under the microscope can help to detect dinospores at an early stage of infestation. Since dinospores detach after host death, fish have to be diagnosed while still living or directly after death (Woo & Buchmann 2012). Levy *et al.* (2007) developed a sensitive and specific PCR assay to detect *Amyloodinium* using ribosomal DNA. They were able to detect a single cell from each of the life cycle stages. An even more sensitive and specific approach is a loop-mediated isothermal amplification (LAMP) assay developed by Picón-Camacho *et al.* (2013) to detect all life stages of *Amyloodinium*. Using an enzyme-linked immunosorbent assay, Smith *et al.* (1992) showed the production of antibodies against *Amyloodinium* after infestation; this might also be used to monitor pathogens. All these methods, however, require specific laboratory equipment as well as qualified personnel to perform the assays. Therefore, while they may have great importance for research purposes, microscopy is still the standard diagnostic procedure for practical application in aquaculture facilities.

The infection is usually far advanced before being diagnosed, and by then, fish are already in a weakened condition. At this point, treatment of the disease is often difficult. Prolonged immersion in common, non-iodized table salt at about 5 g L<sup>-1</sup> (5 ppt or parts per thousand) is an effective treatment against *Piscinoodinium* infestations as the trophonts dislodge (Puello-Cruz *et al.* 2010). Conversely, short periods of hyposalinity (0 g L<sup>-1</sup>) dislodge *Amyloodinium* trophonts from the skin and gills (Kingsford 1975; Lawler 1977). After either treatment, fish need to be transferred to a clean environment to prevent a new infestation, and the treatment has to be repeated several times with periods of a few days recovery between treatments to exclude recurrence. Multiple treatments are essential for an effective regimen because the life cycles of individual organisms are not synchronized; therefore, the parasite may be present in several stages concurrently. Immersion treatments may be employed on a small-scale, but are not logistically practical for public aquaria, large professional aquaculture operations or wholesale of ornamental finfish. In addition, not all fish can tolerate the osmotic changes equally well and immersion treatment, as well as the necessary transfer of fish, adds to increased stress levels.

Commercially available therapeutants for velvet disease contain metals (mostly copper), triphenylmethane dyes, acriflavine, or formalin. These chemicals are effective at killing parasites, but are also harmful to non-target organisms, in particular plants and invertebrates such as

crustaceans and gastropods (Hodson *et al.* 1979; Flemming & Trevors 1989). Not only the basic substance, but residues and derivatives of these compounds can be unpredictably toxic to the environment and pose a high risk to fish, particularly when already weakened by infection (Alderman & Clifton-Hadley 1993; Jung *et al.* 2001; Srivastava *et al.* 2004).

Copper sulphate (CuSO<sub>4</sub>) is the most commonly used therapeutant to treat *Piscinoodinium* and *Amyloodinium* infestations. Concentrations of 0.5 to 10 mg L<sup>-1</sup> CuSO<sub>4</sub> were lethal to sporulating tomonts and dinospores of *Amyloodinium* but did not interrupt the division process of the encapsulated tomont (Paperna 1984). Exposure to 1 mg L<sup>-1</sup> CuSO<sub>4</sub> for 12 to 24 h reduced the reproductive success of tomonts, but dinospores, which could cause reinfestation, were still produced. Ideal treatment strategies would either use concentrations high enough to ensure complete inhibition of the encapsulated tomont or continuous exposure with low concentrations to kill dinospores; this could be harmful or cause stress to fish. Continuous treatment with a desired therapeutic concentration of 0.15 to 0.2 mg L<sup>-1</sup> free copper ion for 2 to 3 weeks helped to eliminate infection with *Amyloodinium* in marine systems (Francis-Floyd & Floyd 2011). Concentrations need to be monitored at least once a day as the free copper ion does not stay in solution for long and multiple treatments are often necessary. Problems occur, however, as copper is highly toxic to invertebrates and algae, even at low concentrations (Francis-Floyd & Floyd 2011; Noga 2012). For extremely sensitive fish, copper is toxic at concentrations close to those required for treatment (Cardeilhac & Whitaker 1988). A concentration of 10 µM CuSO<sub>4</sub> (1.6 mg L<sup>-1</sup> CuSO<sub>4</sub>) is used to induce leucocyte recruitment in zebrafish larvae (d'Alençon *et al.* 2010).

Depending on different factors such as alkalinity, organic matter content, as well as adsorption to surfaces, copper can quickly precipitate which reduces the amount of bioavailable copper (Smith *et al.* 2015). Furthermore, the concentration of specific ions (e.g., Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, H<sup>+</sup>) influence the active uptake of copper by Na<sup>+</sup> transporters as they compete for the binding sites (Niyogi & Wood 2004). In freshwater ponds, risk of acute toxicity can be reduced if the concentration does not exceed 1% of the total alkalinity (Boyd & Massaut 1999). McNevin and Boyd (2004) reported that 99% of the administered copper is bound to pond sediments. To decrease the likelihood of copper entrance into the effluents, water should not be discharged for at least 72 hours. When copper is used in the marine environment, the concentration must be monitored frequently because the free copper ion is unstable (Francis-Floyd & Floyd 2011; Noga 2012). Different ion configurations of copper might have different specific toxicities; however, the evidence is contradictory whether Cu<sup>2+</sup> or the



reduced form  $\text{Cu}^+$  is more toxic (Fargašová 1998; Mathews *et al.* 2015). Chelated, or complexed, forms of copper are sometimes recommended as they reportedly reduce the toxicity to fish. However, the bioavailable concentration is also reduced, which limits the effectiveness against pathogens (Keith 1981). While efforts are being made to obtain United States (U.S.) Food and Drug Administration (FDA)-approval for the use of copper sulphate as a therapeutic to treat freshwater fish diseases in ponds and hatcheries (Straus *et al.* 2016), it is not approved. Likewise, environmental concerns in the European Union (EU) prohibit the use of copper compounds.

The triphenylmethane dyes include malachite green, methylene blue and crystal (gentian) violet. These have been used since the mid-1930s against a plethora of pathogens in fish culture. They are fungicides (especially effective against *Saprolegnia* spp.), antiseptics and are effective treatments against internal and external parasites (Sudova *et al.* 2007; Andersen *et al.* 2009). None of the tested concentrations of malachite green (0.1–100  $\mu\text{g L}^{-1}$ ) had a lethal effect on *Amyloodinium*, although concentrations of 0.5  $\mu\text{g L}^{-1}$  suppressed sporulation (Paperna 1984). However, the compounds themselves, as well as their metabolites are toxic and carcinogenic. Malachite green and methylene blue are toxic to channel catfish (*Ictalurus punctatus*) and rainbow trout (*Oncorhynchus mykiss*) at concentrations of 0.2 and 16  $\mu\text{g L}^{-1}$ , respectively (Willford 1966). Furthermore, current studies showed malachite green and its reduced form, leucomalachite green, to be *in vivo* mutagens (Mitrowska *et al.* 2005, 2008; Culp *et al.* 2006). The same applies to crystal violet and brilliant green (Thomas & MacPhee 1984; Andersen *et al.* 2009), and highly concentrated methylene blue has been linked to neurotoxicity and encephalopathy (Sweet & Standiford 2007; Patel *et al.* 2012). These substances persist in tissue for an extended period of time and therefore pose a risk not only to the fish but also to human health when fish are consumed. These chemicals are labeled as harmful to human health and the environment, and also as toxic by EU regulation (EG1272/2008) 1272/2008 (CLP); they are prohibited for use in aquaculture within the EU. Malachite green and crystal violet are also banned to use on fish intended for human consumption in the US and Canada (Culp & Beland 1996; Srivastava *et al.* 2004; Andersen *et al.* 2009).

Acridine (acridinium chloride) is used to treat and prevent external infections in ornamental fish and has similar antimicrobial and antifungal properties as the triphenylmethane dyes (Plakas *et al.* 1999). Paperna (1984) reported that total sporulation of *Amyloodinium* was only suppressed on incubation with 6  $\mu\text{g L}^{-1}$  acridine. This concentration is in the range of the 48 h-LC<sub>50</sub> value (6.8  $\mu\text{g L}^{-1}$ ) determined for channel catfish by Clemens and Sneed (1958), while Willford (1966) determined the LC<sub>50</sub> value as

33.2  $\mu\text{g L}^{-1}$ . This discrepancy can be caused by different strains of a given fish species as well as differences in the condition of fish, water chemistry or compound quality used for testing. It shows, however, that toxicity can vary massively even in laboratory studies, and application in tanks or rearing facilities can be challenging. Furthermore, Van Duijn (1967) reported on fish sterility after application of acriflavine. This compound binds to DNA and inhibits protein biosynthesis; the mutagenic potential was reported in different species such as *Drosophila melanogaster* and *Chlamydomonas reinhardtii* (Tubbs *et al.* 1964; Xamena *et al.* 1984; Dorthu *et al.* 1992). Obstoy *et al.* (2015) reported DNA damage in cultured human lymphocytes and lung cancer cells after exposure to 0.025% acriflavine. In summary, acriflavine is genotoxic and is banned for use in aquaculture in the EU (Penninks *et al.* 2017).

As mentioned previously, formalin is also used as a treatment against ectoparasitic infections in cultured fish, including velvet disease. *Amyloodinium* infestation on the skin and gills of juvenile bullseye puffers (*Sphoeroides annulatus*) was significantly decreased after treatment with 51 mg L<sup>-1</sup> formalin for 1 h or 4 mg L<sup>-1</sup> formalin for 7 h (Fajer-Ávila *et al.* 2003). Effectiveness however, is affected by ambient temperature, water quality (i.e., salinity and content of dissolved organic carbon [DOC]), and storage conditions (Abowei *et al.* 2011). During *in vitro* studies, trophonts exposed to 25 to 200 mg L<sup>-1</sup> formalin retracted their rhizoid and transformed into tomonths within 1 to 3 h (Paperna 1984), explaining the reduced infection load in the studies mentioned above. Complete inhibition of tomont division was observed only after 24 h exposure to 200 mg L<sup>-1</sup> at 27–30°C. After 12 h exposure to 200 mg L<sup>-1</sup> formalin, cell division was only reduced by about 40% leaving enough motile dinospores for recurrence. Formalin (50 to 150 mg L<sup>-1</sup>) has been used successfully to reduce infections with *Saprolegnia parasitica* in rainbow trout (Giesecker *et al.* 2006). However, the 96-h-LC<sub>50</sub> values of formalin at pH 7 on silver barb (*Barbonymus gonionotus* Bleeker), common carp (*Cyprinus carpio* L) and snakehead (*Channa striatus* Flower) were 75, 107 and 150 mg L<sup>-1</sup>, respectively (Chinabut *et al.* 1988), which is in the range of therapeutic concentration. The parent compound of formalin, formaldehyde, is classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (Group 1) (Cogliano *et al.* 2005; Bianchi *et al.* 2007). It is currently approved for specific uses in aquaculture in the US, but not in the EU.

Noga (2011) cites a personal communication mentioning chloroquine diphosphate as a treatment against *Amyloodinium*. Treating false percula clownfish (*Amphiprion ocellaris*) with 5 to 10 mg L<sup>-1</sup> chloroquine diphosphate for 10 days killed dinospores when they were exiting the cyst. Oral administration of chloroquine was used in cultured

red drum (*Sciaenops ocellata*) as a chemotherapeutic agent against amyloodinosis (Lewis *et al.* 1988). As chloroquine is used as an anti-malaria drug for humans, it is expensive. Furthermore, it is highly toxic to invertebrates, especially echinoderms and corals, and not approved for fish intended for human consumption. *In vitro* studies using the fish cell line PLHC-1 derived from topminnow (*Poeciliopsis lucida*) showed reduced lysosomal function, increased metallothionein and glucose-6P-dehydrogenase levels as well as increased activity of succinate dehydrogenase (Zurita *et al.* 2005).

Another major problem of many therapeutants used against pathogens in fish is the development of resistance. Resistance can often be attributed to misuse or over use of precautionary treatments to prevent infections, or application of low doses for treatments that only kill the weak pathogens. Since therapeutants are applied to the water body, residues are being discharged into the natural environment. This dilution exerts a selection pressure and increases development of resistance even further, which not only endangers fish and environment but also leads to human safety concerns, including human pathogen resistance and foodborne transmission of antimicrobial- and multi-drug resistant microorganisms (White *et al.* 2002; Hur *et al.* 2012). Multi-drug resistance (resistance to more than two classes of antimicrobial agents) has been found for example in *Yersinia ruckeri*, *Aeromonas salmonicida* and *Piscirickettsia salmonis* (Balta *et al.* 2010; Kim *et al.* 2011).

Furthermore, resistance genes of important fish pathogen species have been found to be commonly located on mobile genetic elements such as the transferable plasmid IncA/C and Class 1 integrons, allowing easy transmission of resistances by horizontal gene transfer, even between different bacterial species (Miller & Harbottle 2018). In contrast to the resistance of bacteria to antimicrobial substances, comparably little is known about the resistance of parasites against chemical treatments. Most occurrences of the resistance of parasites usually originate from human or veterinary medicine. Ectoparasites of fish can be treated with antiparasitic compounds which are routinely used in other higher vertebrates like birds and mammals. Livestock intensification has led to an increased reliance on the use of antiparasitic compounds to control parasites.

In Norway, Scotland and other countries in recent years, the patterns of sea lice (*Lepeophtheirus salmonis*) treatment have changed as the resistance to traditional treatments has increased (Sevatdal *et al.* 2005; Costello 2006; Lees *et al.* 2008; Jones *et al.* 2013). The emergence of resistant parasites has limited the choice of effective compounds with a resulting adverse effect on animal welfare and loss in animal productivity (Taylor 1999). Resistance of parasites was further described by Roth *et al.* (1993), Burka *et al.* (1997) and Athanassopoulou *et al.* (2009) against

organophosphates, commonly used against parasitic crustaceans and monogeneans. The use of drugs like trichlorfon or praziquantel in bath treatments for ectoparasites also has the disadvantage of resistance development (Umeda *et al.* 2006). Reverter *et al.* (2014) report the resistance of platyhelminthes to praziquantel. There is limited available knowledge about any resistances of *Oodinium* against antiparasitic compounds, but the development of resistance must be expected.

### Alternative treatment strategies

The downside of many 'traditional' chemical therapeutants as well as the lack of substances allowed in aquaculture, require new strategies that have led to research focusing on alternative therapeutants. In general, there are two different approaches: 1) treatment of the culture water and the pathogen to reduce the overall load, and 2) treatment of the fish to make them less susceptible against infections by increasing the general stress resistance or by activating defense systems (stimulation of immune response) that protect against the pathogens.

### Hydrogen peroxide and peracetic acid as water disinfectants

Research on alternative therapeutants for the treatment of velvet disease is rare and the few existing studies evaluate the effects on *Amyloodinium*. Montgomery-Brock *et al.* (2001) studied the effect of H<sub>2</sub>O<sub>2</sub> on trophonts of *Amyloodinium* in cultured Pacific threadfin (*Polydactylus sexfilis*). Application of 75 and 150 mg L<sup>-1</sup> H<sub>2</sub>O<sub>2</sub> for 30 min resulted in a reduced number of parasites. However, treatment with 300 mg L<sup>-1</sup> H<sub>2</sub>O<sub>2</sub> resulted in mortality of exposed fish. Treating European seabass (*Dicentrarchus labrax*) with 100 and 200 mg L<sup>-1</sup> H<sub>2</sub>O<sub>2</sub> for 30 min significantly decreased the *Amyloodinium* load on gills (Seoud *et al.* 2017).

An alternative to using H<sub>2</sub>O<sub>2</sub> is the application of PAA (also known as peroxyacetic acid or acetylhydrogen peroxide), which is a stabilized mixture of acetic acid, H<sub>2</sub>O<sub>2</sub>, and water. It is used as a disinfectant for agriculture, food processing, and medical and veterinary facilities (Malchesky 1993; EPA 2005). More recently, PAA has been used in wastewater treatment, commercial laundries and in aquaculture in several countries (Straus *et al.* 2017). Peracetic acid has proven to be an effective treatment against various aquatic pathogens including fungi and bacteria (Jussila *et al.* 2011; Marchand *et al.* 2012). Straus *et al.* (2012) found that 2.5 mg L<sup>-1</sup> PAA was effective at controlling saprolegniasis on channel catfish eggs, whereas Mitchell *et al.* (2009) reported that 250 mg L<sup>-1</sup> H<sub>2</sub>O<sub>2</sub> was the optimal treatment in an earlier study with *Saprolegnia* in the

same system. Furthermore, PAA can be used as a treatment against the fish parasite *Ichthyophthirius multifiliis*, which causes Ich, or white spot disease in freshwater fish. This ciliated protozoan has a triphasic life cycle similar to *Piscinoodinium* and *Amyloodinium*. Meinelt *et al.* (2009) reported that different developmental stages of *I. multifiliis* have varying degrees of sensitivity to PAA. Application of 0.6 to 0.9 mg L<sup>-1</sup> PAA killed up to 82% of newly released tomonts, but none of the tested concentrations (0.5 to 3.0 mg L<sup>-1</sup>) halted theront production of encysted tomonts. Picón Camacho (2010) reported that treatment with 8 to 15 mg L<sup>-1</sup> PAA for 1 h was effective at killing all developmental stages, including cysts.

Similar to H<sub>2</sub>O<sub>2</sub>, PAA produces hydroxyl radicals that oxidize enzymes and proteins, and increase the permeability of cell walls by destruction of sulfhydryl and sulfur compounds. But is more potent than H<sub>2</sub>O<sub>2</sub> because of its fat solubility; in contrast to the microbial breakdown of H<sub>2</sub>O<sub>2</sub> by catalase activity, PAA is primarily degraded by chemical oxidation (Block 1991, 2001; Kitis 2004). This non-specific mode of action prevents adaptation of microorganisms, therefore preventing the development of resistance. The advantages of H<sub>2</sub>O<sub>2</sub> and PAA over conventional therapeutants become even more apparent when considering environmental impact. While H<sub>2</sub>O<sub>2</sub> degrades to water and oxygen, PAA degrades to water, oxygen and acetic acid, which is quickly metabolized by microorganisms. In contrast to conventional therapeutants, no toxic residues are discharged into the environment. Thus, H<sub>2</sub>O<sub>2</sub> and PAA have great potential to be effective against *Piscinoodinium* and to replace conventional therapeutants. Future research determining effective concentrations to treat the different lifecycle stages and the effects of both substances on the hosts would be extremely valuable.

### Vaccination to enhance the immune system

As in terrestrial vertebrates, vaccination is a method to protect against infections using the adaptive immune system. Exposition to an immunogen primes the immune system by forming T- and B-cells. If challenged again later, these primed cells recognize specific antigens, starting defense mechanisms and antibody production.

Oral vaccination with antigens was tested, but gave controversial results as antigens are often degraded by the gut biota (Hart *et al.* 1988; Irie *et al.* 2005). Most commonly, fish are vaccinated by dip or bath immersion, oral vaccination or by intramuscular or intraperitoneal injection (Somerset *et al.* 2005). Immersion is an easy way to vaccinate and can be enhanced by low frequency sonophoresis to increase skin permeability during the bath (Fernandez-Alonso *et al.* 2001; Zhou *et al.* 2002). As gills are very sensitive to sonication, intensities to be used have to be

evaluated carefully (Cobo *et al.* 2013; Cobo Labarca *et al.* 2015). A major problem with oral vaccination is that doses are not distributed equally among all fish but differ based on feeding behavior. Furthermore, the harsh gastric environment often breaks down the antigen, making it ineffective (Embregts & Forlenza 2016). Encapsulation with microalgae, alginate particles, nanoparticles or biofilms for example could help reduce this breakdown (Mutoloki *et al.* 2015; Embregts *et al.* 2019). Injecting the fish is the most effective way of protection. Viji *et al.* (2013) treated ornamental goldfish with whole cell, extracellular products, outer membrane proteins and biofilm vaccines from *Aeromonas hydrophila* which decreased mortality when the fish were challenged. Furthermore, they supplemented the vaccination with extracts from *Asparagus racemosus* which increased phagocytosis, bactericidal activity of the serum and albumin:globulin ratio compared to the group without immunoadjuvant.

In order to produce vaccination against parasites, those parasites have to be cultivated. In some cases, rearing can be done *in vitro*; *A. ocellatum* can be propagated using G1B cells (Noga 1987; Cobb *et al.* 1998). Often a cultivation of parasites requires a fish host population making the vaccination expensive and laborious. Recent advances include the development of multivalent vaccines, synthetic peptides, and subunit vaccines that use immunogenic parts (Plant & LaPatra 2011; Dadar *et al.* 2017). However, injection vaccination requires separate handling of each fish resulting in stress and subsequent feed intake reduction or injuries; fish also have to be of sufficient size (excluding young fingerling) to be eligible for injection vaccination. For more details about the present status of fish vaccination, we recommend the reviews by Muktar *et al.* (2016) and Assefa and Abunna (2018).

### Immunomodulatory feed supplements as an approach to increase animal welfare

Chronic stress and infection lead to immune system suppression of the host (Tort 2011; Zanzuzo *et al.* 2019). As a result, the defense systems are weakened and susceptibility to disease is increased. Subsequently, a treatment against an infestation is inevitable; however, as explained above, many conventional therapeutants can be toxic and cause additional stress which may result in loss of fish. Furthermore, inside the EU, many of the traditional chemical therapeutants are already banned<sup>1</sup>. An alternative approach is to prophylactically stimulate overall fish health and immune-related health to prevent diseases (Dawood *et al.* 2018).

<sup>1</sup>Chemicals banned in the European Union can be found in the 'List of chemicals: Annex I' (<https://echa.europa.eu/regulations/prior-informed-consent/list-chemicals>)



Stimulation of the fish's immune system and other defense mechanisms would have an effect primarily on the free-swimming dinospores. Increased mucus, for example, can prevent the dinospores from attaching to the skin and inhibiting the pathogen from completing its life cycle. Enzymes and other defense mechanisms in gill and skin epithelial cells could affect dinospores attached to the fish. This mucosal immunity will be discussed in later. Research on the effects of immune stimulatory substances against *Amyloodinium* or *Piscinoodinium* is scarce or even nonexistent. The following section will, therefore, focus on research with regard to increasing overall health and defense mechanisms with a focus on velvet disease where information is available. However, even studies about defense mechanisms against other pathogens give important information and may be worthwhile avenues to investigate for preventing outbreaks of velvet disease. We recommend the book entitled 'Aquatic Animal Nutrition' (Steinberg 2018) that explains how diets define the digestive tract and its microbiota, which in turn influences life history and behavioral traits of the host.

## Vitamins

A body of evidence exists illustrating the effects of dietary vitamin supplementation on health benefits. Vitamins are widely known to improve growth and survival and stimulate the immune response. Lim *et al.* (2002) reported that feeding 1,000 to 2,000 mg kg<sup>-1</sup> vitamin C increased resistance against osmotic stress in guppy (*Poecilia reticulata*). Furthermore, vitamin C enhanced resistance to infections in various fishes (Li & Lovell 1985; Navarre & Halver 1989; Hardie *et al.* 1991) and vitamin E supplements decreased stress caused by the fungicide copper oxychloride in Nile tilapia (*Oreochromis niloticus*) (Hassaan *et al.* 2014). As mentioned previously, reduction of stress will make fish less vulnerable to infections. Although supplementing fish feed with vitamins is beneficial, and lack of these substances leads to deficiency symptoms, there is also the risk of over-supplementation. Dietary oversupply (hypervitaminosis) of vitamin C and D leads to decreased growth, elevated levels of alkaline phosphatase and lethargy, and this can cause slow wound repair and depressed immunity (Merchie *et al.* 1996; Darias *et al.* 2011). In addition, skeletal abnormalities, jaw malformations, albinism and melanism caused by high concentrations of vitamin C have been reported in olive flounder (*Paralichthys olivaceus*) and in striped trumpeter (*Latris lineata*) (Negm *et al.* 2014; Takeuchi 2014). Overdose by supplementing with high concentrations of vitamins is particularly risky in the case of vitamin A. Dedi *et al.* (1995) demonstrated that feeding olive flounder with *Artemia* enriched with more than 40 mg vitamin A palmitate produced a negative effect on growth (body weight and total length) and a high

incidence of bone deformities. This shows that despite the beneficial effects on fish, similar to 'traditional' therapeutics, vitamin supplementation can have its downsides and needs to be used and monitored carefully.

## Prebiotics, probiotics, postbiotics, parabiotics, symbiotics

Vitamins are not the only group of dietary supplements that have been studied for their health benefit in aquatic animals. There are numerous available studies on the effect of food-supplementation with nutrients ('functional feeds') such as amino acids (Tesser *et al.* 2005; Peres & Oliva-Teles 2008), polyunsaturated fatty acids (Martinez-Rubio *et al.* 2012, 2013), short chain fatty acids (Hoseinifar *et al.* 2017b) and different kinds of 'biotics' have been evaluated in a plethora of studies (Hoseinifar *et al.* 2018; Van Doan *et al.* 2019). The following section will discuss the different kinds of biotics, their effects on the immune status and pathogen resistance of fish and effects against aquatic parasites.

Gibson and Roberfroid (1995) define prebiotics as in the following: '[Prebiotics] are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health'. Main targets are either the increase in potentially beneficial bacteria proliferation or counteracting the negative ones (Encarnação 2016). Beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium*, hydrolyse the prebiotics which results in production of various bio-active compounds such as short chain fatty acids (scFA), vitamins and peptides. These are capable of increasing fitness over less beneficial or pathogenic bacteria, thereby indirectly improving fish health (Hoseinifar *et al.* 2015a; Hoseinifar *et al.* 2017c; Jung-Schroers *et al.* 2016). Furthermore, prebiotics can directly improve fish health by increasing the immune response (Ringø *et al.* 2010; Song *et al.* 2014; Torrecillas *et al.* 2014). Acting as "risk" signals,  $\beta$ -glucans activate defense mechanisms that are also triggered after cell damage including antigen presenting cells and increased production of Th1 and Th2 cells (Dalmo & Bøgwald 2008). Information on combating challenges by parasites is scarce and often inconsistent. Feeding rainbow trout with 0.2%  $\beta$ -glucan Lauridsen and Buchmann (2010) decreased susceptibility to *I. multifiliis* while plasma lysozyme activity was increased. Feeding common carp with 3%  $\beta$ -1,3/1,6-glucan increased the relative gene expression of interleukin 1- $\beta$ ; however, susceptibility against *I. multifiliis* was not significantly altered (Herczeg *et al.* 2017). Protection against the marine parasitic flatworm *Neobenedenia girellae* was gained after feeding greater amberjack (*Seriola dumerili*) with different forms of mannan oligosaccharides (Fernández-Montero *et al.* 2019). A promising study for

the use of prebiotics against velvet disease was conducted by Buentello *et al.* (2010); in this research red drum feed was supplemented with several prebiotics at 10 g kg<sup>-1</sup> which resulted in increased serum lysozyme activity and survival after infection with *A. ocellatum*. Recently, Guerreiro *et al.* (2018) gave an overview about currently used prebiotics. They point out the importance of different factors that can influence effects such as fish age and size, environmental factors as temperature and give advises on timing and duration of administration as misuse of prebiotics can also cause adverse effects such as alterations in gut histomorphology.

The term postbiotics evolved in the last years of research of functional foods, referring to “soluble factors (products or metabolic byproducts) secreted by live bacteria or released after bacterial lysis” (Aguilar-Toalá *et al.* 2018). These include short-chain fatty acids (scFA), enzymes, peptides, cell surface proteins, and vitamins (Tsilingiri & Rescigno 2012; Konstantinov *et al.* 2013). Although immune response promotion was found, the exact mechanisms of postbiotic modes of action have not yet been fully elucidated (Kareem *et al.* 2016; Nawaz *et al.* 2018). Recent studies shed light on the mode of action of prebiotic on host immune response. It has been shown that dietary administration of prebiotics and their fermentation by beneficial communities of gut microbiota drastically increase the levels of scFAs, including acetate, propionate and butyrate (Hoseinifar *et al.* 2017a). These scFAs are capable of modulating immune response through binding to G protein coupled receptor, GPR43, which is expressed mainly on innate immune response and inflammatory cells (Maslowski & Mackay 2011). However, such scFAs-receptors have not been reported in fish.

The FAO/WHO defined probiotics as ‘live microorganisms which, when administered in adequate amounts, confer health benefits on hosts’ (FAO 2001). This includes host-associated microorganisms, such as *Lactobacillus*, *Bifidobacterium*, *Vibrio*, and *Aeromonas*, but also yeast (*Saccharomyces cerevisiae* and *Saccharomyces exiguus*) and microalgae (*Tetraselmis suecica*). Although immunostimulatory effects of probiotics have been analysed in several studies, there are limited studies available about effects of probiotics on resistance against parasites. Going into detail would go beyond the scope of this review; we therefore focus on a few selected examples where bacterial and parasitic challenges have been studied and recommend the reviews from Zorriehzakra *et al.* (2016) and Hoseinifar *et al.* (2018). *Bacillus* strains were screened for their anti-*Vibrio* properties and the effective mechanism was cell lysis by disruption of cell membranes (Gao *et al.* 2017a). Zebrafish (*Danio rerio*) treated with different strains of *Lactobacillus* and *Bacillus amyloliquefaciens* increased transcription of immune related genes, such as insulin-like growth factor-1 (*igf-1*), complement component

3a (*c3a*), and genes of interleukins, tumor necrosis factors, toll-like receptors and lysozyme. At the same time, relative expression of genes involved in oxidative stress such as superoxide dismutase (*SOD*), glutathione peroxidase 1a (*Gpx1a*), nitric oxide synthase 2a (*NOS2a*) and heat shock protein 70 (*Hsp70*) was decreased (Qin *et al.* 2018; Lin *et al.* 2019). In addition, mortality was decreased when challenged with *A. hydrophilia* and *S. agalactiae*. The few reported trials with dietary pre- and probiotics as therapeutic strategy against *Amyloodinium* are mixed. Reyes-Becerril *et al.* (2008) investigated the effects of the dietary intake of live yeast *Debaryomyces hansenii* on the immune response of juvenile leopard groupers (*Mycteroperca rosacea*) and on their resistance to infection by *A. ocellatum*; only fish with yeast-supplemented diet recovered from the parasitic challenge. The underlying stimulated immune response was based on a significant increase in immunoglobulin M content as well as the activity of superoxide dismutase (Reyes-Becerril *et al.* 2008). The second attempt we are aware of failed; Li *et al.* (2005) evaluated the effects of dietary supplementation with brewer’s yeast and nucleotides on juvenile red drum’s resistance to *A. ocellatum* infection and found that *in situ* challenge by this parasite caused 100% mortality regardless of dietary treatment within a 48 h period. Feeding rainbow trout for 14 days with 10<sup>8</sup>CFU g<sup>-1</sup> *Aeromonas sobria* (GC2) resulted in 100% survival after infection with *I. multifiliis* (0% in control) while, using 10<sup>10</sup> CFU g<sup>-1</sup> *Brochothrix thermosphacta* BA211 did not reduce the mortality (Pieters *et al.* 2008). As mentioned, *I. multifiliis* and *Amyloodinium/Piscinoodinium* have similar life cycles; therefore, *A. sobria* (GC2) might be a treatment option against velvet disease. However, BA211 significantly increased survival against *Aeromonas bestiarum* (Pieters *et al.* 2008). These results show that one probiotic strain can have different effects on different challenges, and that effects of timing, age and species of fish, as well as effect against specific pathogen or parasite has to be evaluated carefully prior to being recommended for application in aquaculture facilities.

Concerns have been raised about the functionality and applicability of live microbes including the viability in the supplementing product, persistence in the gut, horizontal gene transfer from pathogenic bacteria, and enhanced inflammatory responses (Kataria *et al.* 2009; van Reenen & Dicks 2011; Taverniti & Guglielmetti 2011). Inactivated (non-viable) preparations of probiotics (the so-called paraprobiotics) have also shown to be effective immune stimulants (Rampengan *et al.* 2010). Immunostimulatory effects (respiratory burst activity, increases of plasma lysozyme and myeloperoxidase activity) of heat-inactivated *Bacillus amyloliquefaciens* FPTB16 were found in the South Asian carp *Gibelion catla* (Hamilton, 1822) (Singh *et al.* 2017). In rainbow trout, *Enterobacter* sp. was used as effective supplementation against *Flavobacterium psychrophilum* and inactivated *Enterococcus faecalis* significantly decreased

cumulative mortality and frequency of infection with *A. salmonicida* (Rodriguez-Estrada *et al.* 2013; LaPatra *et al.* 2014). Application in aquaculture is still limited and so far no studies exist evaluating the potential protective effect against parasites. Choudhury and Kamilya () presented a good comprehensive review about current studies and the role of paraprobiotics in modifying responses in fish.

Often a combination of different treatment strategies is more successful. Over the past year, an updated idea was suggested regarding modulation of intestinal microbiota aimed at improved host health, the so-called 'Synbiotic' (Gibson & Roberfroid 1995). The idea refers to combined administration of a probiotic bacterium with an optimum prebiotic as substrate. Using mannan oligosaccharide combined with *E. faecalis* decreased the frequency of *A. salmonicida* even more than compared to application of each supplement alone (Rodriguez-Estrada *et al.* 2013). Feeding angelfish (*Pterophyllum scalare*) with symbiotic (*Pediococcus acidilactici* plus fructooligosaccharide) enriched *Artemia* was more effective in increasing skin mucus lysozyme, protease activity, and immunoglobulin concentration than singular enrichment with probiotics or prebiotics alone (Azimirad *et al.* 2016). Similar results were found after treating rainbow trout for 8 weeks with galactooligosaccharides (GOS), *P. acidilactici* and the combination (Hoseinifar *et al.* 2015b); activity of serum lysozyme, respiratory burst, and alternative complement was significantly increased compared to singular enrichment. The highest resistance against *Streptococcus iniae* was observed in the synbiotic group. Several combinations have been tested for their stimulatory potential (Cerezuela *et al.* 2011; Hoseinifar *et al.* 2016a; Huynh *et al.* 2017).

Dietary 'biotics' exhibit optima with regard to doses and frequency of application, and can exert adverse effects when applied above their optimum concentration or for too long. Feeding tiger shrimp (*Penaeus monodon*) with 1000 mg kg<sup>-1</sup> of the immunostimulant alginic acid for 35 days, Ojerio *et al.* (2018) found an enhanced resistance against white spot syndrome virus (WSSV), while groups fed higher concentrations had reduced survival rates. Apart from the beneficial effects of the feed supplementation on growth and immune parameters, few studies with real challenges against pathogens or parasites have been conducted yet. Molecular studies are also required to assess the mechanisms behind the stimulatory effects, and further understanding of the pathways of these compounds will be a crucial step towards their broad application in aquaculture and the replacement of antibiotics and traditional therapeutants.

### Plant secondary metabolites

A growing field of research is focusing on evaluating the effect of natural organic matter (NOM), including plant

secondary metabolites (PSM), on health benefits. Often inspired by traditional Chinese medicine, a plethora of fish and aquatic invertebrates have been treated with extracts from medicinal plants (i.e., herbal drugs). In addition to the direct promotion of health, such as benefits in growth and food conversion ratio, many PSM isolates increase specific and non-specific immunity, and thereby resistance against stress and various pathogens. One example is the change in the internal cell oxidative status (mitohormesis). Increasing evidence indicates that oxidative burst-released ROS not only cause oxidative stress, but rather may function as signaling molecules that promote health by preventing or delaying a number of chronic diseases, and ultimately extend lifespan (Ristow & Schmeisser 2014).

Apart from increasing the specific growth rate, very low concentrations of anthraquinone extract from rhubarb (*Rheum officinale*) increased the activities of blood lysozyme and resistance against crowding stress or artificial *A. hydrophila* infection in common carp and prevented high temperature stress in giant river prawn (*Macrobrachium rosenbergii*) (Xie *et al.* 2008; Liu *et al.* 2010). After treatment with an extract of Siberian ginseng (*Eleutherococcus senticosus*), olive flounder demonstrated increased non-specific immunity and resistance against infection with *Edwardsiella tarda* and *Vibrio anguillarum* (Won *et al.* 2008). Anusha *et al.* (2014) reported that extracts from jungle geranium (*Ixora coccinea*) protected goldfish (*Carassius auratus*) against *A. hydrophila* infection. Resveratrol, a phytoalexin found in grapes and several berries can extend lifespan, protect the nervous system, increase cognitive abilities and retard ageing-related histological markers in redbtail notho (*Nothobranchius guentheri*) (Yu & Li 2012; Genade & Lang 2013). Although, many studies focus on anti-bacterial effects of PSMs, there is evidence of anti-parasitic and anti-fungal effects as well. Applying extracts of garlic (*Allium sativum*) and Indian almond (*Terminalia catappa*) to rearing water of tilapia fingerlings, Chitmanat *et al.* (2005) were able to completely remove a *Trichodina* sp. infection After a 2-day treatment. Garlic was also effective at killing theronts (63.5 mg L<sup>-1</sup>) and tomocysts (570 mg L<sup>-1</sup>) of *I. multifiliis* (Buchmann *et al.* 2003). Magnolia (*Magnolia officinalis*), Euphorbiaceae (*Euphorbia fischeriana* Steud.), black tyme (*Thymbra spicata* L.), oregano (*Origanum onites* L.), and savory (*Satureja tymbra* L.) were used to control *Saprolegnia* sp. (Gomez & Diler 2014; Huang *et al.* 2015). Extracts from different medicinal herbs, e.g. cinnamon (*Cinnamomum cassia*), golden larch (*Pseudolarix amabilis*), juanilama (*Lippia alba*) applied either as bath or orally, were effective anthelmintics (Ji *et al.* 2012; Reverter *et al.* 2014; Soares *et al.* 2016). These are only a few examples of studies analyzing the effects of medicinal plants and secondary metabolites. Stratev *et al.* (2018) presented a

comprehensive overview about current treatments using medicinal plants as well as the mechanisms of action.

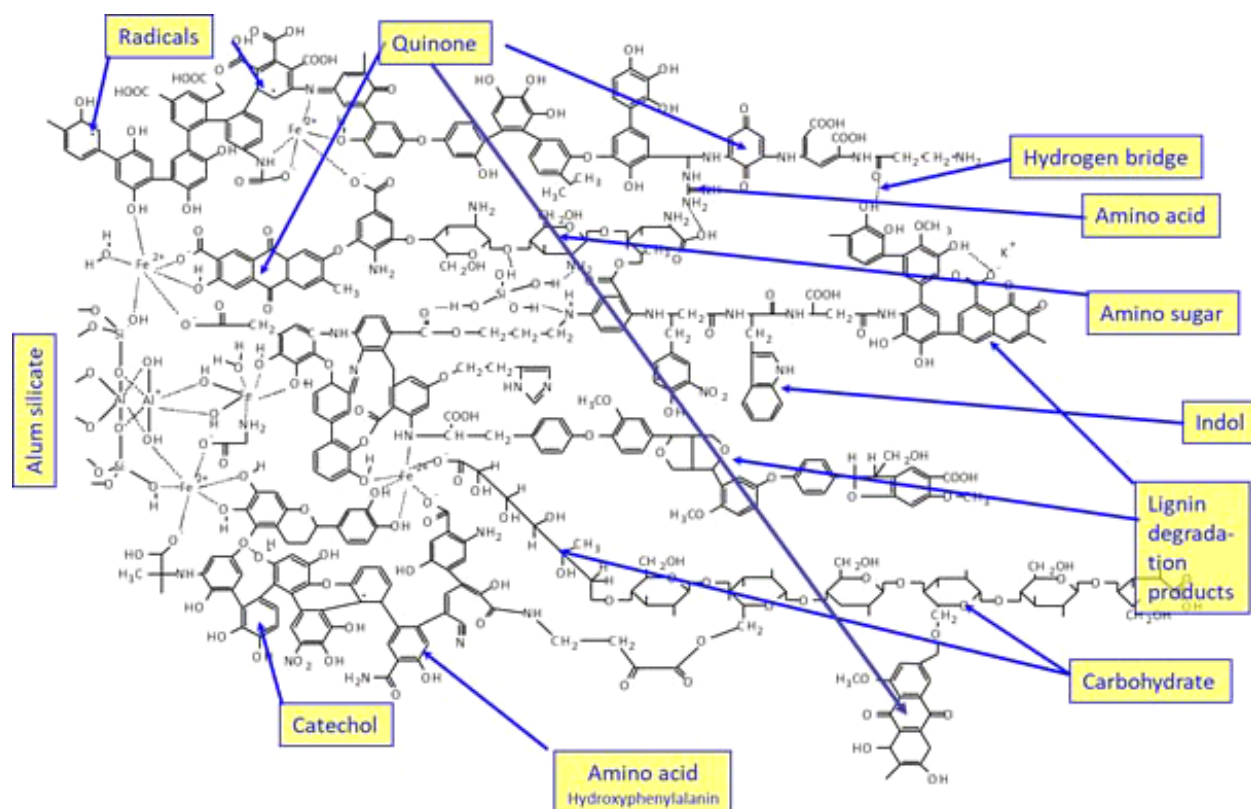
### Humic substances: rediscovered remedy

Major components of Natural Organic Matter (NOM) are humic substances. These ubiquitous, organic compounds arise from the physical, chemical and microbiological transformation (humification) of biomolecules and occur in decaying debris and soil. Up to 95% of dissolved organic matter (DOM) found in aquatic ecosystems are humic substances (Thurman 1985; Haitzer *et al.* 1998; Steinberg 2003). Humic substances can be divided into three main fractions: humic acids, fulvic acids and humins (Stevenson 1982). These subdivisions are arbitrarily based on the solubility of each fraction in water at different pH levels as well as their molecular weight (Pettit 2004).

Figure 4 shows a schematic structure of a humic acid with various functional groups which are important for interactions with exposed organisms. Most effects of humic substances have been evaluated in terrestrial animals so far. Administration of humic substances as food supplements has been shown to increase vitality traits such as growth

and immunity, and reduce diseases in poultry and rats (Yasar *et al.* 2002; TeraVita 2004, Islam *et al.* 2005). Vetrivicka *et al.* (2010) tested two different types of humic acids on humoral and cellular immune reactions of mice; evidence of immunomodulating activity was demonstrated through increased phagocytosis by neutrophils and macrophages, as well as stimulated secretion of IL-2 by spleen cells.

Increased vigor, growth, lifespan and increased stress resistance have also been reported after application of 5 to 180 mg L<sup>-1</sup> DOC of an artificial humic substance (HS 1500) for 21 weeks to culture water of swordtail (*Xiphophorus helleri*) (Meinelt *et al.* 2004). In the cladoceran *Moina macrocopa*, osmotic stress was reduced in the presence of 10 mg L<sup>-1</sup> DOC of a natural humic substance from a Brazilian polyhumic coastal lagoon (Suhett *et al.* 2011); stress resistance was inherited by the succeeding generation, most likely via DNA methylation (Menzel *et al.* 2011). Supplementing feed with 2 g kg<sup>-1</sup> of a fulvic acid significantly increased survival of white shrimp (*Litopenaeus vannamei*) challenged with *Vibrio parahaemolyticus* (Fierro-Coronado *et al.* 2018). In this research, TCTP (translationally controlled tumor protein), which is involved in the regulation



**Figure 4** Structure of a soil humic substance. Major functional groups are indicated. Redrawn after Kleinhempel (1970) with permission from Taylor & Francis.



of the host defense response against bacterial and viral infections, was significantly increased, while Hsp70 was significantly decreased 72 h after feeding; this indicates a modulatory effect of the fulvic acid on the immune and stress response. Adding 1% of humus extract to the feed of ayu fish (*Plecoglossus altivelis*) decreased the development of skin lesions during infection with *F. psychrophilum* (Nakagawa *et al.* 2009). A direct effect on the pathogen was excluded, as the pathogenic load in the water did not decrease due to the treatment. Supplementing feed of juvenile loach (*Paramisgurnus dabryanus* (Sauvage)) with 1.5% fulvic acid for 60 days resulted not only in elevated growth performance but in an increase in intestinal protease activity, antioxidant activity, lysozyme activity (LZM), complement 3 (C3) content, immune globulin M (IgM) content, acid phosphatase activity (ACP), and alkaline phosphatase activity (AKP) (Gao *et al.* 2017b). Intestinal health also increased as shown by elevated abundance of *Lactobacillus* and decreased amounts of *Serratia*, *Acinetobacter*, *Aeromonas* and *Edwardsiella*.

Studies using fish eggs and larvae demonstrated that humic substances increased defense against pathogenic fungi (*Saprolegnia* spp.) and different microorganisms (*Aeromonas* spp.) (Schreckenbach *et al.* 1994; Meinelt *et al.* 2008). Common carp had significantly reduced infection rates when challenged with *A. salmonicida* subsp. *salmonicida* after oral application of humic-rich sludge from a recirculating aquaculture system, a synthetic humic acid, and an extract derived from Leonardite (Yamin *et al.* 2017). Similar results were found by Kodama *et al.* (2007) after oral administration of humus extracts to carp infected with *A. salmonicida*. However, research of whether humic substances can be a suitable supplement to reduce stress in finfish caused by treatment against, or the infection itself, is still new and only few current studies have examined this topic yet.

Only a few studies have documented the rare phenomenon of bio-toxicity of humic substances (Gau *et al.* 2000; Chen *et al.* 2002; Hseu *et al.* 2008), yet the effect was found *in vitro* using different cell cultures. Bernacchi *et al.* (1996) examined the *in vivo* effect by feeding mice a single dose of an aqueous solution containing 4 g L<sup>-1</sup> of humic acids (100 mg kg<sup>-1</sup> body weight). Orally administered humic acids induced structural and numerical chromosomal abnormalities in intestinal cells. However, the concentration used during these experiments was 10 to 1000 times higher than those that are environmentally realistic.

As mentioned previously, the structure of humic substances is very diverse and different humic substances can therefore provoke contrasting effects. Combating the water mold *Saprolegnia parasitica* with 20 humic preparations, Meinelt *et al.* (2007) found that humic preparations with high aromaticity which contain a high number of organic

radicals were the most efficient in reducing fungal growth and caused sporangia formation. Steinberg *et al.* (2007) identified that those humic substances effective with *Saprolegnia* also triggered longevity in the nematode *Caenorhabditis elegans*. On the other hand, humic substances with high shares of aliphatic structures, such as carbohydrates, tended to support *Saprolegnia* growth and did not extend lifespan in *C. elegans* (Steinberg *et al.* 2007). These examples show that structure matters and structural aspects of humic substances have to be considered and evaluated when applying humic substances as treatments against velvet disease and similar pathogens.

Increased animal welfare, as well as enhanced immune-related and overall health, may help prevent diseases or help fish recover from infections easier. Whether an increase in mechanical and chemical defense mechanisms by application of humic substances helps to decrease susceptibility to *Piscinoodinium* and *Amyloodinium* should be evaluated in future research and may provide an alternative to conventional therapeutics. However, when considering humic substances as a treatment or preventative against fish pathogens, one must keep in mind that the very supplements that improve fish health may also promote the growth of dinoflagellates. Low concentrations (6 to 32 µg mL<sup>-1</sup>) of humic substances exerted a stimulatory effect that was reflected by increased yield, growth rate and <sup>14</sup>C uptake in marine dinoflagellates of the genus *Gonyaulax* (Prakash & Rashid 1968). At higher concentrations (over 35 µg mL<sup>-1</sup>), growth rate and yield significantly decreased. Effects of humic substances on hosts and parasites should be monitored and evaluated carefully to ensure proper treatment. Apart from that, humic substances have the potential to reduce susceptibility to infections and pathogens by increasing animal welfare and stimulating host-specific defense mechanisms.

## Defense mechanisms

The defense system of vertebrates is organized in two levels 1) the innate and 2) the adaptive defense system. Innate protection has a general character and protects against unspecific pathogens without recognition and is a fast acting defense system. The adaptive protection in contrast depends on recognition of distinct molecular structures of the invading organisms and memory development. Response is slow (weeks to month) (Van Muiswinkel 1995). Innate defense mechanisms of fish include epithelial barriers, lysozyme, phagocytic cells, and interferons and are similar to that of mammals and birds. However, due to the intimate contact with their environment, fish have established some special defense mechanisms which are less pronounced in other vertebrates.



## Mucosal immunity

Beside skin, there are other mucosal surfaces in fish which have important barrier function (Salinas 2015; Hoseinifar *et al.* 2019). These barriers are composed of the epithelia and their mucus secretions, is called mucosa-associated lymphoid tissue (MALT). The MALT is presented in gut (gut-associated lymphoid tissue or GALT), gills (gill-associated lymphoid tissue or GIALT), skin (skin-associated lymphoid tissue or SALT) and as recently discovered in nose (nasopharynx-associated lymphoid tissue (NALT)) (Lazado & Caipang 2014). The skin mucus can be considered a first line of defense against attacks of pathogens and parasites. Consequently, fish have developed means to improve the mucosal immunity. Feeding Persian hogweed (*Heracleum persicum*) powder to common carp, Hoseinifar *et al.* (2016b) found significantly elevated immunological factors such as immunoglobulins, lysozyme, protease and alternative complement activities in carp mucus but not in serum. Feeding Caspian white fish (*Rutilus frisii kutum*) and Caspian brown trout (*Salmo trutta caspius*) with dietary peppermint extracts increased skin mucus defense (protein concentration, alkaline phosphatase and antimicrobial activity) (Adel *et al.* 2015). Furthermore, immune genes in mucosal tissues were upregulated and resistance against parasites was increased after feeding Greater amberjack (*Seriola dumerili*) with cMOS supplemented diets (Fernández-Montero *et al.* 2019). These studies indicate that nutrition plays one central role in this defense process. Addition of a humic substance to culture water reduced attachment and penetration of the fish skin by pathogens (Schreckenbach *et al.* 1996). Furthermore, increased mucosal lysozyme activity was found when fish were exposed to a fulvic acid (which is also a humic substance) while serum lysozyme was unaffected (Lieke, *et al.*, in preparation). This indicates that apart from nutrition, other pathways seem to be involved in regulation of mucosal immunity and the mode of action has not been determined and merit further research.

## Antimicrobial peptides are natural defense mechanisms against pathogens

Antimicrobial peptides (AMPs), such as histone-like proteins (HLPs) and piscidines, are a diverse group of molecules that are widespread in the animal and plant kingdoms and are important parts of the innate immune system (Schröder 2013). They were discovered when an extract from a soil *Bacillus* strain was used to protect mice from pneumococci infection (Dubos 1939a, b). More than 5,500 AMPs have been discovered or synthesized to date (Zhao *et al.* 2013). In animals, AMPs are located in different tissues; especially those exposed to the environment

(i.e., epidermis and gills) and are a first line of defense against pathogens. The AMPs consist of up to 100 amino acids and most demonstrate an amphipathic design with clusters of hydrophobic and cationic amino acids (Schulze 2010). The basis of specificity of AMPs arises from different physicochemical properties of the outer membranes. The outer leaflet of the bilayer of bacteria consists of phospholipids with negatively charged headgroups, while that of plants and animals has no net charge (Op den Kamp 1979; Zasloff 2002). The mode of action of AMPs is not completely understood. According to the Shai–Matsuzaki–Huang–model (Matsuzaki 1999; Shai 1999; Yang *et al.* 2000), accumulation of AMPs at the outer layer is aided by electrostatic interactions, followed by formation of  $\alpha$ -helices and insertion of the hydrophobic clusters leading to membrane permeation. Inhibition of target organisms can be different, including the disruption of membrane integrity and arising plasmolysis, inhibition of proteins, DNA and RNA synthesis and interaction with intracellular targets (Bahar & Ren 2013). Because of the physical disruption of the cell membrane, resistance against AMPs will not develop. Due to the lack of net charge and the high content of cholesterol, AMPs do not accumulate at membranes of multicellular organisms (Matsuzaki *et al.* 1995; Toke 2005), preventing impairment of non-target organisms. Therefore, they have been analysed as a replacement for traditional therapeutants (Hancock & Patrzykat 2002; Finlay & Hancock 2004) and could be a useful tool to prevent velvet disease.

Noga *et al.* (2002, 2003) reported negative effects of HLP-1, which has high sequence homology to histone H2B, on the infection intensity and propagation of *A. occe-latum*. Addition of 100  $\mu\text{g mL}^{-1}$  HLP-1, isolated from rainbow trout (*Oncorhynchus mykiss*) and hybrid striped bass (female *Morone chrysops* x male *M. saxatilis*), inhibited young and mature trophonts *in vitro*. HLP-1 caused delayed or irregular development of the parasites and led to less aggressive feeding behavior and slower monolayer destruction (Noga *et al.* 2002, 2003). *In vitro* upregulation of the AMPs results in expression concentrations, especially in skin and gill epithelia, which are in the range of the anti-parasitic concentrations, measured *in vitro* (Ullal *et al.* 2008).

Stimulation of these host-specific defensive mechanisms could help protect fish against velvet disease, as parasites are challenged at an early stage of infection and immune system functions attempt to destroy them. Consequently, these host-specific defense mechanisms decrease the chances of parasite propagation, prevent mass infections and prevent excessively weakened health conditions of fish. As humic substances are immunostimulatory, they may induce expression of AMPs, but there is presently no research about this.

## Conclusion

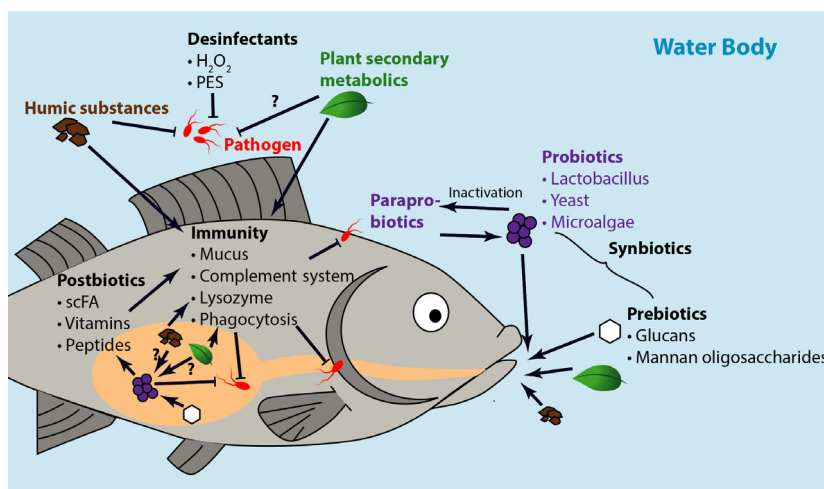
Diseases cause significant financial losses to public aquariums, ornamental enthusiasts and aquaculture operations. Few studies exist evaluating effects of environmental friendly therapeutants to diseases, such as velvet disease, maybe due to its lower infectivity at low temperatures. Household remedies such as saline baths are often used by hobby aquarists but are unsuitable for commercial usage. Commercially available therapeutants are toxic to non-target organisms and can increase stress levels, lower health conditions, and potentially cause death of treated fish. Discarded residues of these therapeutants can be harmful to the environment or lead to the development of resistance in microorganisms, which can pose a subsequent risk to human safety as well. Especially inside the EU regulations are strict and EU aquaculture is renowned for its high quality sustainability and consumer protection standards. These include regulations of environmental impact assessment, water use, food and safety hygiene, animal welfare, and animal health. The European Commission is helping and demanding to increase a sustainable aquaculture that is safe but also competitive with other countries. This shows the high demand of replacing chemical therapeutants not only by the government but by end consumers as well, and has fueled research approaches on alternative strategies such as disinfection of water, immunostimulatory feed ingredients, and water supplements. Figure 5 summarizes current knowledge on these three topics and how they can work to prevent disease outbreaks.

Two compounds,  $H_2O_2$  and PAA degrade into harmless residues and are therefore more environmental friendly than other compounds. They have been proven to be effective against a variety of aquatic pathogens and represent promising approaches to treat velvet disease. Nevertheless,

they could add to the stress level of the infected fish due to their high oxidative capacity and are toxic when applied at high concentrations. Conditioning the fish prior to treatment potentially helps decrease this stress.

Supplementing food with vitamins or biotics has been demonstrated to reduce stress-levels and susceptibility to infections; however, there is a risk of over-supplementation. Dose/response curve of immunostimulants does not follow a linear relationship (Bliznakov & Adler 1972). It is often a hormetic one, with beneficial effects increasing up to a certain concentration and then decreasing if that concentration is exceeded. In addition, life time, duration, and frequency of application can play a tremendous part. After continuous feeding for 40 days with glucan at a concentration of 2 g glucan  $kg^{-1}$  feed, which represents optimal concentration for Asian tiger shrimp (*Penaeus monodon*), immunity was reduced (Chang *et al.* 2000). Indian white shrimp (*Fenneropenaeus indicus*) had highest survival rate when challenged with white spot syndrome virus (WSSV) when 2 g glucan  $kg^{-1}$  was added once a week as feed supplement. Higher intervals decreased survival (Sajeewan *et al.* 2009). These results indicate that continuous use of immunostimulants even at an optimal dose may suppress the immunity and administration at definite intervals would give better performance resulting in enhanced survival. Clearly, more research is needed in order to identify optimal application strategies, particularly studies on fish are, to the best of our knowledge, not available.

Health benefits of natural organic matter have been proven in a plethora of different animals. Many plant extracts not only have general beneficial effects, such as enhanced growth and food conversion rate, but also increase specific and non-specific immunity. The effects of humic substances have recently been rediscovered and scientifically proven; used as feed additives, they can increase vitality



**Figure 5** Scheme of environmental friendly treatments used to prevent or treat diseases. →: stimulation; ⊥: inhibition; ?: proposed pathway.

and lifespan, stimulate growth, accelerate wound healing and increase resistance to pathogenic bacterial loads. The immune system and host-specific defense mechanisms in land vertebrates were stimulated by humic substances and they reduced susceptibility to stress and microorganisms. Conditioning fish using natural organic matter is a promising approach to enhance overall and immune-related health, making fish less susceptible to velvet disease. Ongoing research in our laboratory suggests that humic substances can increase the MALT system and thereby help in preventing diseases. Humic substances are therefore another promising approach of immunostimulation. Natural organic matter can increase host defense systems in order to decrease or even replace use of chemical therapeutants. Furthermore, humic substances are part of the natural environment of fish. In contrast to plant extracts, fish defense systems are 'accustomed' to these natural xenobiotics, making them ideal candidates for use in culture water.

Stimulation of immune response does not necessarily reflect an increased protection against pathogens. Studies where fish are challenged with pathogens after administration of immunostimulants are scarce and most focus on bacterial infections due to easier implementation of the experiment. Also, the higher energy demand needed to activate the immune system might even be accompanied by adverse effects such as impairment of growth. It is therefore important to monitor parameters from different regulatory pathways (metabolism, stress response, growth) in addition to the immune parameters in order to ensure safe application of new immunostimulants. Nevertheless, they have been proven to be able to help protect from stress and disease, often with the beneficial side effect of increased growth performance and have the potential to replace harmful therapeutants in aquaculture facilities. When transferring laboratory results to facilities, we highly recommend monitoring the health status of fish closely at different time points in order to ensure best output.

Practical application of immunostimulatory substances in aquaculture has just begun to be explored. The next step should be to focus on duration, frequencies and life stages as well as optimizing concentrations for different fishes in order to provide recommendations for large scale application. Pilot studies have to be conducted to evaluate effects in commercial aquaculture facilities and cope with possible problems due to upscaling from laboratory to facility devices. No strategy is without constraints; this also applies for treatment of stress and diseases in aquaculture. As demonstrated with the synbiotics, a combination of different strategies can lead to mutual assistance resulting in additive or even synergistic effects. In our opinion, the best disease management strategy would thus be a holistic one, including the physicochemical environment, the animals

and the microorganisms. A favourable concept for environmentally friendly treatments against velvet disease and other fish diseases is to concentrate on two aspects: 1) treatment of the invading parasites using residue-free or naturally occurring substances, and 2) fortifying the fish to reduce susceptibility to parasites and to allow significantly reduced use of antiparasitic therapeutants. In this way, the most sustainable outcome for the environment as well as for the farmer can be achieved.

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## Compliances with ethical standards

The authors declare that there are no conflicts of interest. The founding sponsors had no role in the design, content or decision to publish the review.

## References

- Abowei JFN, Briyai OF, Bassey SE (2011) A Review of Some Basic Parasite Diseases in Culture Fisheries Flagellids, Dinoflagellides and Ichthyophthiriasis, Ichtyobodiasis, Coccidiosis Trichodiniasis, Heminthiasis, Hirudinea Infestation, Crustacean Parsite and Ciliates. *British Journal of Pharmacology and Toxicology* 2(5): 213–226.
- Adel M, Abedian Amiri A, Zorriehzahra J, Nematolahi A, Esteban MÁ (2015) Effects of dietary peppermint (*Mentha piperita*) on growth performance, chemical body composition and hematological and immune parameters of fry Caspian white fish (*Rutilus frisii kutum*). *Fish and Shellfish Immunology* 45 (2): 841–847.
- Aguilar-Toalá JE, Garcia-Varela R, Garcia HS, Mata-Haro V, González-Córdova AF, Vallejo-Cordoba B *et al.* (2018) Postbiotics: An evolving term within the functional foods field. *Trends in Food Science and Technology* 75: 105–114.
- Alderman DJ, Clifton-Hadley RS (1993) Malachite green: A pharmacokinetic study in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* 16(4): 297–311.
- d'Alençon CA, Peña OA, Wittmann C, Gallardo VE, Jones RA, Loosli F *et al.* (2010) A high-throughput chemically induced inflammation assay in zebrafish. *BMC Biology* 8(1): 151.

- Andersen WC, Turnipseed SB, Karbiwnyk CM, Lee RH, Clark SB, Rowe WD *et al.* (2009) Multiresidue method for the triphenylmethane dyes in fish: Malachite green, crystal (gentian) violet, and brilliant green. *Analytica Chimica Acta* **637** (1–2): 279–289.
- Anusha P, Thangaviji V, Velmurugan S, Michaelbabu M, Citarasu T (2014) Protection of ornamental gold fish *Carassius auratus* against *Aeromonas hydrophila* by treating *Ixora coccinea* active principles. *Fish and Shellfish Immunology* **36**(2): 485–493.
- Assefa A, Abunna F (2018) Maintenance of fish health in aquaculture: Review of epidemiological approaches for prevention and control of infectious disease of fish. *Veterinary Medicine International* **2018**: 1–10.
- Athanassopoulou F, Pappas I, Bitchava K (2009) An overview of the treatments for parasitic disease in Mediterranean aquaculture. *Options Méditerran Ser A* **86**: 65–83.
- Azimirad M, Meshkini S, Ahmadi N, Hoseinifar SH (2016) The effects of feeding with synbiotic (*Pediococcus acidilactici* and fructooligosaccharide) enriched adult *Artemia* on skin mucus immune responses, stress resistance, intestinal microbiota and performance of angelfish (*Pterophyllum scalare*). *Fish and Shellfish Immunology* **54**: 516–522.
- Bahar AA, Ren D (2013) Antimicrobial peptides. *Pharmaceuticals* **6**(12): 1543–1575.
- Balta F, Sandalli C, Kayis S, Ozgumus O (2010) Molecular analysis of antimicrobial resistance in *Yersinia ruckeri* strains isolated from rainbow trout (*Oncorhynchus mykiss*) grown in commercial fish farms in Turkey. *Bulletin of the European Association of Fish Pathologists* **30**(6): 211–219.
- Bernacchi F, Ponzanelli I, Minunni M, Falezza A, Loprieno N, Barale R (1996) *In vivo* cytogenetic effects of natural humic acid. *Mutagenesis* **11**(5): 467–469.
- Bianchi F, Careri M, Musci M, Mangia A (2007) Fish and food safety: Determination of formaldehyde in 12 fish species by SPME extraction and GC–MS analysis. *Food Chemistry* **100** (3): 1049–1053.
- Bliznakov E, Adler A (1972) Nonlinear response of the reticuloendothelial system upon stimulation. *Pathobiology* **38**(6): 393–410.
- Block SS (1991) *Disinfection, Sterilization, and Preservation*. Lea & Febiger Publications, Philadelphia.
- Block SS (2001) *Peroxygen Compounds*. Lippincott Williams & Wilkins, Philadelphia.
- Bly JE, Quiniou SM, Clem LW (1997) Environmental effects on fish immune mechanisms. *Developments in Biological Standardization* **90**: 33–43.
- Boyd CE, Massaut L (1999) Risks associated with the use of chemicals in pond aquaculture. *Aquacultural Engineering* **20** (2): 113–132.
- Brown EM (1931) 16. Note on a new Species of Dinoflagellate from the Gills and Epidermis of Marine Fishes. Proceedings of the Zoological Society of London, Wiley Online Library.
- Brown EM, Hovasse R (1946) Amyloodinium ocellatum (Brown), a Peridinin Parasitic on Marine Fishes. A Complementary Study. Proceedings of the Zoological Society of London, Wiley Online Library.
- Buchmann K, Jensen P, Kruse K (2003) Effects of sodium percarbonate and garlic extract on *Ichthyophthirius multifiliis* theronts and tomocysts: *in vitro* experiments. *North American Journal of Aquaculture* **65**(1): 21–24.
- Buentello JA, Neill WH, Gatlin I, Delbert M (2010) Effects of dietary prebiotics on the growth, feed efficiency and non-specific immunity of juvenile red drum *Sciaenops ocellatus* fed soybean-based diets. *Aquaculture Research* **41**(3): 411–418.
- Burka JF, Hammell KL, Horsberg T, Johnson GR, Rainnie D, Speare DJ (1997) Drugs in salmonid aquaculture – A review. *Journal of Veterinary Pharmacology and Therapeutics* **20**(5): 333–349.
- Cardeilhac PT, Whitaker BR (1988) Copper Treatments: Uses and Precautions. *Veterinary Clinics of North America: Small Animal Practice* **18**(2): 435–448.
- Cerezuela R, Meseguer J, Esteban MA (2011) Current Knowledge in Synbiotic Use for Fish Aquaculture: A Review. *Journal of Aquaculture Research and Development* **01**: 1–7.
- Chang C-F, Chen H-Y, Su M-S, Liao I-C (2000) Immunomodulation by dietary  $\beta$ -1, 3-glucan in the brooders of the black tiger shrimp *Penaeus monodon*. *Fish and Shellfish Immunology* **10**(6): 505–514.
- Chen C-H, Liu J-J, Lu F-J, Yang M-L, Lee Y, Huang T-S (2002) The effect of humic acid on the adhesibility of neutrophils. *Thrombosis Research* **108**(1): 67–76.
- Cheung PJ, Nigrelli RF, Ruggieri GD (1981) Development of *Oodinium ocellatum* (Dinoflagellida): A scanning electron microscopic study. *Transactions of the American Microscopical Society* **100** (4): 415–420.
- Chinabut S, Limsuwan C, Tonguthai K, Pungkachonboon T (1988) Toxic and sublethal effects of formalin of freshwater fishes. [Cited 10 Oct 2018.] Available from <http://www.fao.org/docrep/field/003/AC278E/AC278E00.htm>
- Chitmanat C, Tongdonmuan K, Nunsong W (2005) The use of crude extracts from traditional medicinal plants to eliminate *Trichodina* sp. in tilapia (*Oreochromis niloticus*) fingerlings. *Songklanakarinn Journal of Science and Technology* **27**(Suppl 1): 359–364.
- Choudhury TG, Kamilya D (in press) Paraprobiotics: an aquaculture perspective. *Reviews in Aquaculture*. <https://doi.org/10.1111/raq.12290>
- Clemens HP, Sneed KE (1958) The chemical control of some diseases and parasites of channel catfish. *The Progressive Fish Culturist* **20**(1): 8–15.
- Cobb CS, Levy MG, Noga EJ (1998) Acquired immunity to amyloodiniosis is associated with an antibody response. *Diseases of Aquatic Organisms* **34**(2): 125–133.
- Cobo Labarca C, Makhutu M, Lumsdon AE, Thompson KD, Jung R, Kloas W *et al.* (2015) The adjuvant effect of low frequency ultrasound when applied with an inactivated *Aeromonas salmonicida* vaccine to rainbow trout (*Oncorhynchus mykiss*). *Vaccine* **33**(11): 1369–1374.



- Cobo C, Makosch K, Jung R, Kohlmann K, Knopf K (2013) Enhanced bacterin permeability and side effects using low frequency sonophoresis at 37 kHz in rainbow trout. *Fish and Shellfish Immunology* **34**(6): 1648.
- Cogliano VJ, Grosse Y, Baan RA, Straif K, Secretan MB, El Ghis-sassi F, the Working Group for Volume 88 (2005) Meeting report: Summary of IARC monographs on formaldehyde, 2-butoxyethanol, and 1-tert-butoxy-2-propanol. *Environmental Health Perspectives* **113**: 1205.
- Costello MJ (2006) Ecology of sea lice parasitic on farmed and wild fish. *Trends in Parasitology* **22**(10): 475–483.
- Culp SJ, Beland FA (1996) Malachite green: A toxicological review. *International Journal of Toxicology* **15**(3): 219–238.
- Culp SJ, Mellick PW, Trotter RW, Greenlees KJ, Kodell RL, Beland FA (2006) Carcinogenicity of malachite green chloride and leucomalachite green in B6C3F1 mice and F344 rats. *Food and Chemical Toxicology* **44**(8): 1204–1212.
- Dadar M, Dhama K, Vakharia VN, Hoseinifar SH, Karthik K, Tiwari R *et al.* (2017) Advances in Aquaculture Vaccines Against Fish Pathogens: Global Status and Current Trends. *Reviews in Fisheries Science and Aquaculture* **25**(3): 184–217.
- Dalmo RA, Bøgwald J (2008)  $\beta$ -glucans as conductors of immune symphonies. *Fish and Shellfish Immunology* **25**(4): 384–396.
- Darias M, Mazurais D, Koumoundouros G, Cahu C, Zambonino-Infante J (2011) Overview of vitamin D and C requirements in fish and their influence on the skeletal system. *Aquaculture* **315**(1–2): 49–60.
- Dawood MA, Koshio S, Esteban MÁ (2018) Beneficial roles of feed additives as immunostimulants in aquaculture: a review. *Reviews in Aquaculture* **10**(4): 950–974.
- Dedi J, Takeuchi T, Seikai T, Watanabe T (1995) Hypervitaminosis and safe levels of vitamin A for larval flounder *Paralichthys olivaceus* fed *Artemia* nauplii. *Aquaculture* **133**(2): 135–146.
- Dorthu M, Remy S, Michel-Wolwertz M, Colleaux L, Breyer D, Beckers M *et al.* (1992) Biochemical, genetic and molecular characterization of new respiratory-deficient mutants in *Chlamydomonas reinhardtii*. *Plant Molecular Biology* **18**(4): 759–772.
- Dubos RJ (1939a) Studies on a bactericidal agent extracted from a soil *Bacillus*: I. Preparation of the agent. Its activity *in vitro*. *Journal of Experimental Medicine* **70**: 1.
- Dubos RJ (1939b) Studies on a bactericidal agent extracted from a soil *Bacillus*: II. Protective effect of the bactericidal agent against experimental *Pneumococcus* infections in mice. *Journal of Experimental Medicine* **70**: 11–17.
- EG1272 (2008) Verordnung (EG) Nr. 1272/2008 des Europäischen Parlaments und des Rates vom 16. Dezember 2008 über die Einstufung, Kennzeichnung und Verpackung von Stoffen und Gemischen, zur Änderung und Aufhebung der Richtlinien 67/548/EWG und 1999/45/EG und zur Änderung der Verordnung (EG) Nr. 1907/2006 (Text von Bedeutung für den EWR).
- Embregts CWE, Forlenza M (2016) Oral vaccination of fish: Lessons from humans and veterinary species. *Developmental and Comparative Immunology* **64**: 118–137.
- Embregts CWE, Rigau-deau D, Tacchi L, Pijlman GP, Kampers L, Veselý T *et al.* (2019) Vaccination of carp against SVCV with an oral DNA vaccine or an insect cells-based subunit vaccine. *Fish and Shellfish Immunology* **85**: 66–77.
- Encarnação P (2016) 5 - Functional feed additives in aquaculture feeds. In: Nates SF (ed) *Aquafeed Formulation*, pp. 217–237. Academic Press, San Diego.
- EPA (2005) Peraclean 15% (Peroxyacetic Acid Solution). [Cited 20 May 2018] Available from [https://www3.epa.gov/pesticide/s/chem\\_search/ppls/054289-00004-20000919.pdf](https://www3.epa.gov/pesticide/s/chem_search/ppls/054289-00004-20000919.pdf)
- FAO (2001) Probiotics in food - Health and nutritional properties and guidelines for evaluation. FAO Food and Nutrition Paper. **85**, 1–10. Available from <http://www.fao.org/3/a-a0512e.pdf>.
- Fajer-Ávila EJ, Abdo-de la Parra I, Aguilar-Zarate G, Contreras-Arce R, Zaldívar-Ramírez J, Betancourt-Lozano M (2003) Toxicity of formalin to bullseye puffer fish (*Sphoeroides annulatus* Jenyns, 1843) and its effectiveness to control ectoparasites. *Aquaculture* **223**(1): 41–50.
- Fargašová A (1998) Comparative acute toxicity of  $\text{Cu}^{2+}$ ,  $\text{Cu}^+$ ,  $\text{Mn}^{2+}$ ,  $\text{Mo}^{6+}$ ,  $\text{Ni}^{2+}$  and  $\text{V}^{5+}$  to *Chironomus plumosus* larvae and *Tubifex tubifex* worms. *Section Cellular and Molecular Biology* **53**(3): 315–319.
- Fernandez-Alonso M, Rocha A, Coll JM (2001) DNA vaccination by immersion and ultrasound to trout viral haemorrhagic septicaemia virus. *Vaccine* **19**(23): 3067–3075.
- Fernández-Montero Á, Torrecillas S, Izquierdo M, Caballero MJ, Milne DJ, Secombes CJ *et al.* (2019) Increased parasite resistance of greater amberjack (*Seriola dumerili* Risso 1810) juveniles fed a cMOS supplemented diet is associated with upregulation of a discrete set of immune genes in mucosal tissues. *Fish and Shellfish Immunology* **86**: 35–45.
- Fierro-Coronado JA, Angulo C, Rubio-Castro A, Luna-González A, Cáceres-Martínez CJ, Ruiz-Verdugo CA *et al.* (2018) Dietary fulvic acid effects on survival and expression of immune-related genes in *Litopenaeus vannamei* challenged with *Vibrio parahaemolyticus*. *Aquaculture Research* **49**(9): 3218–3227.
- Finlay BB, Hancock RE (2004) Can innate immunity be enhanced to treat microbial infections? *Nature Reviews Microbiology* **2**(6): 497–504.
- Flemming CA, Trevors JT (1989) Copper toxicity and chemistry in the environment: a review. *Water, Air, and Soil Pollution* **44**(1): 143–158.
- Francis-Floyd R, Floyd MR (2011) *Amyloodinium ocellatum*, an important parasite of cultured marine fish. Southern Regional Aquaculture Center. Available from <http://agris.fao.org/agris-search/search.do?recordID=US201300003502>
- Gao X-Y, Liu Y, Miao L-L, Li E-W, Hou T-T, Liu Z-P (2017a) Mechanism of anti-*Vibrio* activity of marine probiotic strain *Bacillus pumilus* H2, and characterization of the active substance. *AMB Express* **7**: 23.
- Gao Y, He J, He Z, Li Z, Zhao B, Mu Y *et al.* (2017b) Effects of fulvic acid on growth performance and intestinal health of juvenile loach *Paramisgurnus dabryanus* (Sauvage). *Fish and Shellfish Immunology* **62**: 47–56.



- Gau R-J, Yang H-L, Chow S-N, Suen J-L, Lu F-J (2000) Humic acid suppresses the LPS-induced expression of cell-surface adhesion proteins through the inhibition of NF- $\kappa$ B activation. *Toxicology and Applied Pharmacology* **166**(1): 59–67.
- Genade T, Lang DM (2013) Resveratrol extends lifespan and preserves glia but not neurons of the *Nothobranchius guentheri* optic tectum. *Experimental Gerontology* **48**(2): 202–212.
- Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *Journal of Nutrition* **125**: 1401–1412.
- Gieseker CM, Serfling SG, Reimschuessel R (2006) Formalin treatment to reduce mortality associated with *Saprolegnia parasitica* in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **253**(1–4): 120–129.
- Gomez O, Diler O (2014) *In vitro* antifungal activity of essential oils from *Tymbra*, *Origanum*, *Satureja* species and some pure compounds on the fish pathogenic fungus, *Saprolegnia parasitica*. *Aquaculture Research* **45**(7): 1196–1201.
- Guerreiro I, Oliva-Teles A, Enes P (2018) Prebiotics as functional ingredients: Focus on Mediterranean fish aquaculture. *Reviews in Aquaculture* **10**(4): 800–832.
- Guillou L, Not F (2018) *AquaSymbio: Parasites and Endosymbioses in Aquatic Ecosystems*, Biological Station of Roscoff. CNRS, France.
- Guiry MD, Guiry GM (2018) AlgaeBase. World-wide electronic publication, National University of Ireland, Galway.
- Haitzer M, Höss S, Traunspurger W, Steinberg CEW (1998) Effects of dissolved organic matter (DOM) on the bioconcentration of organic chemicals in aquatic organisms - A review. *Chemosphere* **37**(7): 1335–1362.
- Hancock REW, Patrzykat A (2002) Clinical development of cationic antimicrobial peptides: From natural to novel antibiotics. *Current Drug Targets-Infectious Disorders* **2**(1): 79–83.
- Hardie LJ, Fletcher TC, Secombes CJ (1991) The effect of dietary vitamin C on the immune response of the Atlantic salmon (*Salmo salar* L.). *Aquaculture* **95**(3): 201–214.
- Hart S, Wrathmell A, Harris J, Grayson T (1988) Gut immunology in fish: a review. *Developmental and Comparative Immunology* **12**(3): 453–480.
- Hassaan MS, Goda AMAS, Mahmoud SA, Tayel SI (2014) Protective effect of dietary vitamin E against fungicide copperoxychloride stress on Nile tilapia, *Oreochromis niloticus* (L.), fingerlings. *International Aquatic Research* **6**(1): 1–17.
- Herczeg D, Sipos D, Dán Á, Loy C, Kallert DM, Eszterbauer E (2017) The effect of dietary immunostimulants on the susceptibility of common carp (*Cyprinus carpio*) to the white spot parasite, *Ichthyophthirius multifiliis*. *Acta Veterinaria Hungarica* **65**(4): 517–530.
- Hodson PV, Borgmann U, Shear H (1979) Toxicity of Copper to Aquatic Biota. *Copper in the Environment Part 2*: 307–372.
- Hoffman GL (1999) *Parasites of North American Freshwater Fishes*. Cornell University Press, Ithaca.
- Hoseinifar SH, Esteban MÁ, Cuesta A, Sun Y-Z (2015a) Prebiotics and fish immune response: a review of current knowledge and future perspectives. *Reviews in Fisheries Science and Aquaculture* **23**(4): 315–328.
- Hoseinifar SH, Mirvaghefi A, Amoozegar MA, Sharifian M, Esteban MÁ (2015b) Modulation of innate immune response, mucosal parameters and disease resistance in rainbow trout (*Oncorhynchus mykiss*) upon synbiotic feeding. *Fish and Shellfish Immunology* **45**(1): 27–32.
- Hoseinifar SH, Ringø E, Shenavar Masouleh A, Esteban MÁ (2016a) Probiotic, prebiotic and synbiotic supplements in sturgeon aquaculture: a review. *Reviews in Aquaculture* **8**(1): 89–102.
- Hoseinifar SH, Zoheiri F, Lazado CC (2016b) Dietary phytoimmunostimulant Persian hogweed (*Heracleum persicum*) has more remarkable impacts on skin mucus than on serum in common carp (*Cyprinus carpio*). *Fish and Shellfish Immunology* **59**: 77–82.
- Hoseinifar SH, Ahmadi A, Khalili M, Raeisi M, Van Doan H, Caipang CM (2017a) The study of antioxidant enzymes and immune-related genes expression in common carp (*Cyprinus carpio*) fingerlings fed different prebiotics. *Aquaculture Research* **48**: 5447–5454.
- Hoseinifar SH, Sun Y-Z, Caipang CM (2017b) Short-chain fatty acids as feed supplements for sustainable aquaculture: an updated view. *Aquaculture Research* **48**: 1380–1391.
- Hoseinifar SH, Sun Y, Zhou Z (2017c) *Prebiotics and Synbiotics. Diagnosis and Control of Diseases of Fish and Shellfish*. In: Austin B, Newaj-Fyzul A. John Wiley & Sons Ltd, New Jersey.
- Hoseinifar SH, Sun Y, Wang A, Zhou Z (2018) Probiotics as means of diseases control in aquaculture, A Review of current knowledge and future perspectives. *Frontiers in Microbiology* **9**: 2429.
- Hoseinifar SH, Khodadadian Zou H, Paknejad H, Hajimoradloo A, Van Doan H (2019) Effects of dietary white-button mushroom powder on mucosal immunity, antioxidant defence, and growth of common carp (*Cyprinus carpio*). *Aquaculture* **501**: 448–454.
- Hsu Y-C, Chen S-C, Chen Y-L, Chen J-Y, Lee M-L, Lu F-J et al. (2008) Humic acid induced genotoxicity in human peripheral blood lymphocytes using comet and sister chromatid exchange assay. *Journal of Hazardous Materials* **153**: 784–791.
- Huang X, Liu R, Whyte S, Du Z, Chen D, Deng Y et al. (2015) The *in vitro* antifungal activity of 30 Chinese herb extracts to *Saprolegnia* sp. *Journal of Applied Ichthyology* **31**: 681–686.
- Hur J, Jawale C, Lee JH (2012) Antimicrobial resistance of *Salmonella* isolated from food animals: A review. *Food Research International* **45**: 819–830.
- Huynh T-G, Shiu Y-L, Nguyen T-P, Truong Q-P, Chen J-C, Liu C-H (2017) Current applications, selection, and possible mechanisms of actions of synbiotics in improving the growth and health status in aquaculture: A review. *Fish and Shellfish Immunology* **64**: 367–382.
- Irie T, Watarai S, Iwasaki T, Kodama H (2005) Protection against experimental *Aeromonas salmonicida* infection in carp by oral immunisation with bacterial antigen entrapped liposomes. *Fish and Shellfish Immunology* **18**(3): 235–242.

- Islam KMS, Schuhmacher A, Gropp JM (2005) Humic acid substances in animal agriculture. *Pakistan Journal of Nutrition* **4** (3): 126–134.
- Ji J, Lu C, Kang Y, Wang G-X, Chen P (2012) Screening of 42 medicinal plants for *in vivo* anthelmintic activity against *Dactylogyrus intermedius* (Monogenea) in goldfish (*Carassius auratus*). *Parasitology Research* **111**(1): 97–104.
- Jones P, Hammell K, Gettinby G, Revie CW (2013) Detection of emamectin benzoate tolerance emergence in different life stages of sea lice, *Lepeophtheirus salmonis*, on farmed Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **36**(3): 209–220.
- Jung SH, Kim JW, Jeon IG, Lee YH (2001) Formaldehyde residues in formalin-treated olive flounder (*Paralichthys olivaceus*), black rockfish (*Sebastes schlegeli*), and seawater. *Aquaculture* **194**(3–4): 253–262.
- Jung-Schroers V, Adamek M, Jung A, Harris S, Dóza ÖS, Baumer A *et al.* (2016) Feeding of  $\beta$ -1, 3/1, 6-glucan increases the diversity of the intestinal microflora of carp (*Cyprinus carpio*). *Aquaculture Nutrition* **22**(5): 1026–1039.
- Jussila J, Makkonen J, Kokko H (2011) Peracetic acid (PAA) treatment is an effective disinfectant against crayfish plague (*Aphanomyces astaci*) spores in aquaculture. *Aquaculture* **320** (1): 37–42.
- Kareem K, Loh T, Foo H, Asmara S, Akit H (2016) Influence of postbiotic RG14 and inulin combination on cecal microbiota, organic acid concentration, and cytokine expression in broiler chickens. *Poultry Science* **96**: 966–975.
- Kataria J, Li N, Wynn JL, Neu J (2009) Probiotic microbes: do they need to be alive to be beneficial? *Nutrition Reviews* **67**: 546–550.
- Keith RE (1981) Loss of therapeutic copper in closed marine systems. *Aquaculture* **24**: 355–362.
- Kim JH, Hwang SY, Son JS, Han JE, Jun JW, Shin SP *et al.* (2011) Molecular characterization of tetracycline- and quinolone-resistant *Aeromonas salmonicida* isolated in Korea. *Journal of Veterinary Science* **12**: 41–48.
- Kingsford E (1975) *Treatment of Exotic Marine Fish Diseases*. Palmetto Publishing Company, North Charleston.
- Kitis M (2004) Disinfection of wastewater with peracetic acid: a review. *Environment International* **30**: 47–55.
- Kleinhempel D (1970) Contribution to the theory of the condition of humic substances (in German). *Archives of Agronomy and Soil Science* **14**: 3–14.
- Kodama H, Denso, Nakagawa T (2007) Protection against atypical *Aeromonas salmonicida* infection in carp (*Cyprinus carpio* L.) by oral administration of humus extract. *Journal of Veterinary Medical Science* **69**: 405–408.
- Konstantinov SR, Kuipers EJ, Peppelenbosch MP (2013) Functional genomic analyses of the gut microbiota for CRC screening. *Nature Reviews Gastroenterology and Hepatology* **10**: 741.
- LaPatra SE, Fehring TR, Cain KD (2014) A probiotic *Enterobacter* sp. provides significant protection against *Flavobacterium psychrophilum* in rainbow trout (*Oncorhynchus mykiss*) after injection by two different routes. *Aquaculture* **433**: 361–366.
- Lauridsen JH, Buchmann K (2010) Effects of short-and long-term glucan feeding of rainbow trout (*Salmonidae*) on the susceptibility to *Ichthyophthirius multifiliis* infections. *Acta Ichthyologica Et Piscatoria* **40**:61–66.
- Lawler AR (1977) The parasitic dinoflagellate *Amyloodinium ocellatum* in marine aquaria. *Drum and Croaker* **17**: 17–20.
- Lazado CC, Caipang CMA (2014) Mucosal immunity and probiotics in fish. *Fish and Shellfish Immunology* **39**: 78–89.
- Lees F, Baillie M, Gettinby G, Revie CW (2008) The efficacy of emamectin benzoate against infestations of *Lepeophtheirus salmonis* on farmed Atlantic salmon (*Salmo salar* L) in Scotland, 2002–2006. *PLoS ONE* **3**: e1549.
- Levy MG, Poore MF, Colorni A, Noga EJ, Vandersea MW, Litaker RW (2007) A highly specific PCR assay for detecting the fish ectoparasite *Amyloodinium ocellatum*. *Diseases of Aquatic Organisms* **73**: 219–226.
- Lewis DH, Wenxing W, Ayers A, Arnold CR (1988) Preliminary studies on the use of chloroquine as a systemic chemotherapeutic agent for amyloodinosis in red drum (*Sciaenops ocellatus*). *Contributions in Marine Science* **30**: 183–189.
- Li Y, Lovell RT (1985) Elevated levels of dietary ascorbic acid increase immune responses in channel catfish. *The Journal of Nutrition* **115**: 123–131.
- Li P, Burr GS, Goff J, Whiteman KW, Davis KB, Vega RR *et al.* (2005) A preliminary study on the effects of dietary supplementation of brewers yeast and nucleotides, singularly or in combination, on juvenile red drum (*Sciaenops ocellatus*). *Aquaculture Research* **36**: 1120–1127.
- Lim LC, Dhert P, Chew WY, Dermaux V, Nelis H, Sorgeloos P (2002) Enhancement of stress resistance of the guppy *Poecilia reticulata* through feeding with vitamin C supplement. *Journal of the World Aquaculture Society* **33**: 32–40.
- Lin Y-S, Saputra F, Chen Y-C, Hu S-Y (2019) Dietary administration of *Bacillus amyloliquefaciens* R8 reduces hepatic oxidative stress and enhances nutrient metabolism and immunity against *Aeromonas hydrophila* and *Streptococcus agalactiae* in zebrafish (*Danio rerio*). *Fish and Shellfish Immunology* **86**: 410–419.
- Liu B, Xie J, Ge X, Xu P, Wang A, He Y *et al.* (2010) Effects of anthraquinone extract from *Rheum officinale* Bail on the growth performance and physiological responses of *Macrobrachium rosenbergii* under high temperature stress. *Fish and Shellfish Immunology* **29**: 49–57.
- Lom J (1981) Fish invading dinoflagellates: a synopsis of existing and newly proposed genera. *Folia Parasitologica* **28**(1): 3–11.
- Lom J, Dyková I (1992) *Protozoan Parasites of Fishes*. Elsevier Science Publishers, Amsterdam.
- Lom J, Lawler AR (1973) An ultrastructural study on the mode of attachment in dinoflagellates invading gills of *Cyprinodontidae*. *Protistologica* **9**(2): 293–309.
- Lom J, Schubert G (1983) Ultrastructural study of *Piscinoodinium pillulare* (Schäperclaus, 1954) Lom, 1981 with special emphasis on its attachment to the fish host. *Journal of Fish Diseases* **6**(5): 411–428.

- Malchesky PS (1993) Peracetic acid and its application to medical instrument sterilization. *Artificial Organs* **17**(3): 147–152.
- Marchand P-A, Phan T-M, Straus DL, Farmer BD, Stüber A, Meinelt T (2012) Reduction of *in vitro* growth in *Flavobacterium columnare* and *Saprolegnia parasiticaby* products containing peracetic acid. *Aquaculture Research* **43**(12): 1861–1866.
- Martinez-Rubio L, Morais S, Evensen Ø, Wadsworth S, Ruohonen K, Vecino JLG et al. (2012) Functional feeds reduce heart inflammation and pathology in Atlantic salmon (*Salmo salar* L.) following experimental challenge with Atlantic salmon reovirus (ASRV). *PLoS One* **7**(11): e40266.
- Martinez-Rubio L, Morais S, Evensen O, Wadsworth S, Vecino JG, Ruohonen K et al. (2013) Effect of functional feeds on fatty acid and eicosanoid metabolism in liver and head kidney of Atlantic salmon (*Salmo salar* L.) with experimentally induced heart and skeletal muscle inflammation. *Fish and Shellfish Immunology* **34**(6): 1533–1545.
- Maslowski KM, Mackay CR (2011) Diet, gut microbiota and immune responses. *Nature Immunology* **12**(1): 5–9.
- Mathews S, Kumar R, Solioz M (2015) Copper Reduction and Contact Killing of Bacteria by Iron Surfaces. *Applied and Environmental Microbiology* **81**(18): 6399–6403.
- Matsuzaki K (1999) Why and how are peptide–lipid interactions utilized for self-defense? Magainins and tachyplesins as archetypes. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1462**(1–2): 1–10.
- Matsuzaki K, Sugishita K, Fujii N, Miyajima K (1995) Molecular basis for membrane selectivity of an antimicrobial peptide, magainin 2. *Biochemistry* **34**(10): 3423–3429.
- McNevin AA, Boyd CE (2004) Copper Concentrations in Channel Catfish *Ictalurus punctatus* Ponds Treated with Copper Sulfate. *Journal of the World Aquaculture Society* **35**(1): 16–24.
- Meinelt T, Schreckenbach K, Knopf K, Wienke A, Stüber A, Steinberg CEW (2004) Humic substances affect physiological condition and sex ratio of swordtail (*Xiphophorus helleri* Heckel). *Aquatic Sciences* **66**(2): 239–245.
- Meinelt T, Paul A, Phan TM, Zwirnmann E, Krüger A, Wienke A et al. (2007) Reduction in vegetative growth of the water mold *Saprolegnia parasitica* (Coker) by humic substance of different qualities. *Aquatic Toxicology* **83**(2): 93–103.
- Meinelt T, Schreckenbach K, Pietrock M, Heidrich S, Steinberg CEW (2008) Humic Substances. Part 1 - Dissolved humic substances (HS) in aquaculture and ornamental fish breeding. *Environmental Science and Pollution Research* **15**(1): 17–22.
- Meinelt T, Matzke S, Stüber A, Pietrock M, Wienke A, Mitchell AJ et al. (2009) Toxicity of peracetic acid (PAA) to tomonts of *Ichthyophthirius multifiliis*. *Diseases of Aquatic Organisms* **86**(1): 51–56.
- Menzel S, Bouchnak R, Menzel R, Steinberg CEW (2011) Dissolved humic substances initiate DNA-methylation in cladocerans. *Aquatic Toxicology* **105**(3–4): 640–642.
- Merchie G, Lavens P, Storch V, Übel U, Nelis H, De Leenheer A et al. (1996) Influence of dietary vitamin C dosage on turbot (*Scophthalmus maximus*) and European sea bass (*Dicentrarchus labrax*) nursery stages. *Comparative Biochemistry and Physiology Part A: Physiology* **114**(2): 123–133.
- Miller RA, Harbottle H (2018) Antimicrobial drug resistance in fish pathogens. *Microbiology Spectrum* **6**(1)1–20.
- Mitchell AJ, Radomski AA, Straus DL, Carter R (2009) The effect of hydrogen peroxide on the hatch rate and *Saprolegnia* spp. infestation of channel catfish eggs. *North American Journal of Aquaculture* **71**(3): 276–280.
- Mitrowska K, Posyniak A, Zmudzki J (2005) Determination of malachite green and leucomalachite green in carp muscle by liquid chromatography with visible and fluorescence detection. *Journal of Chromatography A* **1089**(1–2): 187–192.
- Mitrowska K, Posyniak A, Zmudzki J (2008) Determination of malachite green and leucomalachite green residues in water using liquid chromatography with visible and fluorescence detection and confirmation by tandem mass spectrometry. *Journal of Chromatography A* **1207**(1–2): 94–100.
- Montgomery-Brock D, Sato VT, Brock JA, Tamaru CS (2001) The Application of Hydrogen Peroxide as a Treatment for the Ectoparasite *Amyloodinium ocellatum* on the Pacific Threadfin *Polydactylus sexfilis*. *Journal of the World Aquaculture Society* **32**(2): 250–254.
- Mukhtar Y, Tesfaye S, Tesfaye B (2016) Present status and future prospects of fish vaccination: a review. *Journal of Veterinary Science and Technology* **2**: 299.
- Mutuloki S, Munang'andu HM, Evensen Ø (2015) Oral vaccination of fish – antigen preparations, uptake, and immune induction. *Frontiers in Immunology* **6**: 519. <https://doi.org/10.3389/fimmu.2015.00519>
- Nakagawa J, Iwasaki T, Kodama H (2009) Protection against *Flavobacterium psychrophilum* infection (cold water disease) in ayu fish (*Plecoglossus altivelis*) by oral administration of humus extract. *Journal of Veterinary Medical Science* **71**(11): 1487–1491.
- Navarre O, Halver JE (1989) Disease resistance and humoral antibody production in rainbow trout fed high levels of vitamin C. *Aquaculture* **79**(1–4): 207–221.
- Nawaz A, Bakhsh Javaid A, Irshad S, Hoseinifar SH, Xiong H (2018) The functionality of prebiotics as immunostimulant: Evidences from trials on terrestrial and aquatic animals. *Fish and Shellfish Immunology* **76**: 272–278.
- Negm RK, Cobcroft JM, Brown MR, Nowak BF, Battaglione SC (2014) Performance and skeletal abnormality of striped trumpeter *Latris lineata* larvae and post larvae fed vitamin A enriched *Artemia*. *Aquaculture* **422**: 115–123.
- Niyogi S, Wood CM (2004) Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals. *Environmental Science and Technology* **38**(23): 6177–6192.
- Noga EJ (1987) Propagation in cell culture of the dinoflagellate *Amyloodinium*, an ectoparasite of marine fishes. *Science* **236**(4806): 1302–1304.
- Noga EJ (2011) *Fish Disease: Diagnosis and Treatment*. John Wiley & Sons, Hoboken.
- Noga EJ, Fan Z, Silphaduang U (2002) Host site of activity and cytological effects of histone-like proteins on the parasitic

- dinoflagellate *Amyloodinium ocellatum*. *Diseases of Aquatic Organisms* **52**: 207–215.
- Noga EJ, Silphaduang U (2003) Piscidins: a novel family of peptide antibiotics from fish. *Drug News Perspect* **16**: 87–92.
- Noga EJ (2012) *Amyloodinium ocellatum*. Fish Parasites: Pathobiology and Protection Woo, P. T. K. Buchmann, K.: 19–29.
- Obstoy B, Salaun M, Veresezan L, Sesboüé R, Bohn P, Boland F-X *et al.* (2015) Safety and performance analysis of acriflavine and methylene blue for *in vivo* imaging of precancerous lesions using fibered confocal fluorescence microscopy (FCFM): An experimental study. *BMC Pulmonary Medicine* **15**(1): 30.
- Ojerio VT, Corre VL, Toledo NA, Andrino-Felarca KGS, Nievales LM, Traifalgar RFM (2018) Alginate acid as immunostimulant: effects of dose and frequency on growth performance, immune responses, and white spot syndrome virus resistance in tiger shrimp *Penaeus monodon* (Fabricius, 1798). *Aquaculture International* **26**(1): 267–278.
- Op den Kamp JAF (1979) Lipid asymmetry in membranes. *Annual Review of Biochemistry* **48**(1): 47–71.
- Paperna I (1980) *Amyloodinium ocellatum* (Brown, 1931) (Dinoflagellida) infestations in cultured marine fish at Eilat, Red Sea: Epizootiology and pathology. *Journal of Fish Diseases* **3**(5): 363–372.
- Paperna I (1984) Chemical control of *Amyloodinium ocellatum* (Brown 1931) (Dinoflagellida) infections: *In vitro* tests and treatment trials with infected fishes. *Aquaculture* **38**(1): 1–18.
- Patel HP, Chadwick DR, Harrison BJ, Balasubramanian SP (2012) Systematic review of intravenous methylene blue in parathyroid surgery. *British Journal of Surgery* **99**(10): 1345–1351.
- Penninks A, Baert K, Levorato S, Binaglia M (2017) Scientific report on the dyes in aquaculture and reference points for action. *European Food Safety Authority Journal* **15**(7): 4920. <https://doi.org/10.2903/j.efsa.2017.4920>
- Peres H, Oliva-Teles A (2008) Lysine requirement and efficiency of lysine utilization in turbot (*Scophthalmus maximus*) juveniles. *Aquaculture* **275**(1): 283–290.
- Pettit RE (2004) Organic matter, humus, humate, humic acid, fulvic acid and humin: Their importance in soil fertility and plant health. Available from <https://humates.com/pdf/ORGANICMATTERPettit.pdf>
- Picón Camacho SM (2010) Developing strategies for the control of Ichthyophthirius multifiliis Fouquet, 1876 (Ciliophora), University of Stirling.
- Picón-Camacho SM, Thompson WP, Blaylock RB, Lotz JM (2013) Development of a rapid assay to detect the dinoflagellate *Amyloodinium ocellatum* using loop-mediated isothermal amplification (LAMP). *Veterinary Parasitology* **196**(3): 265–271.
- Pieters N, Brunt J, Austin B, Lyndon AR (2008) Efficacy of in-feed probiotics against *Aeromonas bestiarum* and *Ichthyophthirius multifiliis* skin infections in rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Journal of Applied Microbiology* **105**(3): 723–732.
- Plakas SM, Doerge DR, Turnipseed SB (1999) Disposition and metabolism of malachite green and other therapeutic dyes in fish. In: Plakas SM, Doerge DR, Turnipseed SB (eds) *Xenobiotics in Fish*, pp. 149–166. Kluwer Academic/Plenum Publishers, New York.
- Plant KP, LaPatra SE (2011) Advances in fish vaccine delivery. *Developmental and Comparative Immunology* **35**(12): 1256–1262.
- Prakash A, Rashid MA (1968) Influence of humic substances on the growth of marine phytoplankton: Dinoflagellates. *Limnology and Oceanography* **13**(4): 598–606.
- Puello-Cruz AC, Velasco-Blanco G, Martínez-Rodríguez IE, Felix-Ramos E, Voltolina D (2010) Growth and survival of siamese fighting fish, *Betta Splendens*, larvae at low salinity and with different diets. *Journal of the World Aquaculture Society* **41**(5): 823–828.
- Qin C, Xie Y, Wang Y, Li S, Ran C, He S *et al.* (2018) Impact of *Lactobacillus casei* BL23 on the host transcriptome, growth and disease resistance in larval zebrafish. *Frontiers in Physiology* **9**: 1245.
- Rampengan NH, Manoppo J, Warouw SM (2010) Comparison of efficacies between live and killed probiotics in children with lactose malabsorption. *Southeast Asian Journal of Tropical Medicine and Public Health* **41**(2): 474–481.
- van Reenen CA, Dicks LM (2011) Horizontal gene transfer amongst probiotic lactic acid bacteria and other intestinal microbiota: what are the possibilities? A review. *Archives of Microbiology* **193**(3): 157–168.
- Reverter M, Bontemps N, Lecchini D, Banaigs B, Sasal P (2014) Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. *Aquaculture* **433**: 50–61.
- Reyes-Becerril M, Tovar-Ramírez D, Ascencio-Valle F, Civera-Cerecedo R, Gracia-López V, Barbosa-Solomieu V (2008) Effects of dietary live yeast *Debaryomyces hansenii* on the immune and antioxidant system in juvenile leopard grouper *Mycteroperca rosacea* exposed to stress. *Aquaculture* **280**(1): 39–44.
- Ringø E, Olsen R, Gifstad T, Dalmo R, Amlund H, Hemre GI *et al.* (2010) Prebiotics in aquaculture: A review. *Aquaculture Nutrition* **16**(2): 117–136.
- Ristow M, Schmeisser K (2014) Mitohormesis: Promoting health and lifespan by increased levels of reactive oxygen species (ROS). *Dose-Response* **12**(2): 288–341.
- Rodríguez-Estrada U, Satoh S, Haga Y, Fushimi H, Sweetman J (2013) Effects of inactivated *Enterococcus faecalis* and mannan oligosaccharide and their combination on growth, immunity, and disease protection in rainbow trout. *North American Journal of Aquaculture* **75**(3): 416–428.
- Roth M, Richards RH, Sommerville C (1993) Current practices in the chemotherapeutic control of sea lice infestations in aquaculture: A review. *Journal of Fish Diseases* **16**(1): 1–26.
- Sajeevan T, Philip R, Singh IB (2009) Dose/frequency: a critical factor in the administration of glucan as immunostimulant to Indian white shrimp *Fenneropenaeus indicus*. *Aquaculture* **287** (3–4): 248–252.



- Salinas I (2015) The Mucosal Immune System of Teleost Fish. *Biology* **4**(3): 525–539.
- Saraiva A, Jerónimo D, Cruz C (2011) *Amyloodinium ocellatum* (Chromalveolata: Dinoflagellata) in farmed turbot. *Aquaculture* **320**(1–2): 34–36.
- Schäperclaus W (1954) *Handbuch der Fischkrankheiten*. Akademie Verlag, Berlin.
- Schreckenbach K, Knösche R, Seubert B, Höke H (1994) *Klinische Prüfung des Synthesehuminstoffes HS 1500 bei Eiern und Larven von Regenbogenforellen (Oncorhynchus mykiss)*. Bericht, Institute of Inland Fisheries e.V. Potsdam Sacrow.
- Schreckenbach K, Kühnert M, Haase A, Höke H (1996) *Gutachten über die Wirkung des Arzneimittelgrundstoffes HS 1500 bei Nutz- und Zierfischen in der Aquakultur und Aquaristik*. Bericht, Institute of Inland Fisheries e.V. Potsdam Sacrow.
- Schröder J-M (2013) Antimikrobielle Peptide und Proteine: Effektoren des angeborenen Abwehrsystems der Grenzflächen. *Allergo Journal* **22**(6): 362–364.
- Schulze M (2010) *Die Wirkung Antimikrobieller Peptide (AMP) bei der Konservierung von Ebersperma*. Freie Universität Berlin, Berlin.
- Seoud SSM, Zaki VH, Ahmed GE, El-Khalek NKA (2017) Studies on *Amyloodinium* Infestation in European Seabass (*Dicentrarchus labrax*) Fishes with Special Reference for Treatment. *International Journal of Marine Science* **7**(24): 229–246.
- Sevatdal S, Copley L, Wallace C, Jackson D, Horsberg TE (2005) Monitoring of the sensitivity of sea lice (*Lepeophtheirus salmonis*) to pyrethroids in Norway, Ireland and Scotland using bioassays and probit modelling. *Aquaculture* **244**(1–4): 19–27.
- Shai Y (1999) “Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by  $\alpha$ -helical antimicrobial and cell non-selective membrane-lytic peptides”. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1462**(1–2): 55–70.
- Singh ST, Kamilya D, Kheti B, Bordoloi B, Parhi J (2017) Paraprobiotic preparation from *Bacillus amyloliquefaciens* FPTB16 modulates immune response and immune relevant gene expression in *Catla catla* (Hamilton, 1822). *Fish and Shellfish Immunology* **66**: 35–42.
- Small BC, Bilodeau AL (2005) Effects of cortisol and stress on channel catfish (*Ictalurus punctatus*) pathogen susceptibility and lysozyme activity following exposure to *Edwardsiella ictaluri*. *General and Comparative Endocrinology* **142**(1): 256–262.
- Smith SA, Levy MG, Noga EJ (1992) Development of an enzyme-linked immunosorbent assay (ELISA) for the detection of antibody to the parasitic dinoflagellate *Amyloodinium ocellatum* in *Oreochromis aureus*. *Veterinary Parasitology* **42**(1–2): 145–155.
- Smith KS, Balistrieri LS, Todd AS (2015) Using biotic ligand models to predict metal toxicity in mineralized systems. *Applied Geochemistry* **57**: 55–72.
- Soares BV, Neves LR, Oliveira MSB, Chaves FCM, Dias MKR, Chagas EC et al. (2016) Antiparasitic activity of the essential oil of *Lippia alba* on ectoparasites of *Colossoma macropomum* (tambaqui) and its physiological and histopathological effects. *Aquaculture* **452**: 107–114.
- Sommerset I, Krossøy B, Biering E, Frost P (2005) Vaccines for fish in aquaculture. *Expert Review of Vaccines* **4**(1): 89–101.
- Song SK, Beck BR, Kim D, Park J, Kim J, Kim HD et al. (2014) Prebiotics as immunostimulants in aquaculture: A review. *Fish and Shellfish Immunology* **40**(1): 40–48.
- Srivastava S, Sinha R, Roy D (2004) Toxicological effects of malachite green. *Aquatic Toxicology* **66**(3): 319–329.
- Steinberg CEW (2003) *Ecology of Humic Substances in Freshwaters: Determinants from Geochemistry to Ecological Niches*. Springer Science & Business Media, Heidelberg.
- Steinberg CEW (2018) *Aquatic Animal Nutrition - A Mechanistic Perspective from Individuals to Generations*. Cham, Switzerland, Springer Nature Switzerland AG.
- Steinberg CEW, Saul N, Pietsch K, Meinelt T, Rienau S, Menzel R (2007) Dissolved humic substances facilitate fish life in extreme aquatic environments and have the potential to extend lifespan of *Caenorhabditis elegans*. *Annals of Environmental Science* **1**: 81–90.
- Stevenson FJ (1982) *Humus Chemistry: Genesis, Composition, Reactions*. Wiley, New York.
- Stratev D, Zhelyazkov G, Noundou XS, Krause RWM (2018) Beneficial effects of medicinal plants in fish diseases. *Aquaculture International* **26**(1): 289–308.
- Straus DL, Meinelt T, Farmer BD, Mitchell AJ (2012) Peracetic acid is effective for controlling fungus on channel catfish eggs. *Journal of Fish Diseases* **35**(7): 505–511.
- Straus DL, Farmer BD, Ledbetter CK, Beck BH, Williams RS, Clark ML et al. (2016) Use of copper sulfate to control egg saprolegniasis at a commercial sunshine bass hatchery. *North American Journal of Aquaculture* **78**(3): 243–250.
- Straus DL, Meinelt T, Liu D, Pedersen L-F (2017) Toxicity of peracetic acid to fish: Variation among species and impact of water chemistry. *Journal of the World Aquaculture Society* **4**: 715–724.
- Subasinghe RP, Bondad-Reantaso MG, McGladdery SE (2001) Aquaculture development, health and wealth. Food and Agriculture Organization of the United Nations.
- Sudova E, Machova J, Svobodova Z, Vesely T (2007) Negative effects of malachite green and possibilities of its replacement in the treatment of fish eggs and fish: A review. *Veterinary Medicine* **52**(12): 527–539.
- Suhett AL, Steinberg CEW, Santangelo JM, Bozelli RL, Farjalla VF (2011) Natural dissolved humic substances increase the lifespan and promote transgenerational resistance to salt stress in the cladoceran *Moina macrocopa*. *Environmental Science and Pollution Research* **18**(6): 1004–1014.
- Sweet G, Standiford SB (2007) Methylene-blue-associated encephalopathy. *Journal of the American College of Surgeons* **204**(3): 454–458.
- Takeuchi T (2014) Progress on larval and juvenile nutrition to improve the quality and health of seawater fish: A review. *Fisheries Science* **80**(3): 389–403.
- Taverniti V, Guglielmetti S (2011) The immunomodulatory properties of probiotic microorganisms beyond their viability



- (ghost probiotics: proposal of paraprobiotic concept). *Genes and Nutrition* **6**(3): 261.
- Taylor MA (1999) Parasites, resistance and control strategies. *The Journal of the Fish Veterinary Society* **3**: 43.
- TeraVita (2004) Chapter 9: the use of humates in poultry and stock farming. In: *Humates in Poultry and Stock Farming TeraVita*. Available from <http://www.teravita.cyaom/Humates/Chapter9.htm>
- Tesser MB, Terjesen BF, Zhang Y, Portella MC, Dabrowski K (2005) Free- and peptide-based dietary arginine supplementation for the South American fish pacu (*Piaractus mesopotamicus*). *Aquaculture Nutrition* **11**(6): 443–453.
- Thomas SM, MacPhee DG (1984) Crystal violet: A direct-acting frameshift mutagen whose mutagenicity is enhanced by mammalian metabolism. *Mutation Research Letters* **140**(4): 165–167.
- Thurman EM (1985) *Organic Geochemistry of Natural Waters*. Nijhoff, M./Junk, W. Publishers, Dordrecht.
- Toke O (2005) Antimicrobial peptides: New candidates in the fight against bacterial infections. *Peptide Science* **80**(6): 717–735.
- Torrecillas S, Montero D, Izquierdo M (2014) Improved health and growth of fish fed mannan oligosaccharides: Potential mode of action. *Fish and Shellfish Immunology* **36**(2): 525–544.
- Tort L (2011) Stress and immune modulation in fish. *Developmental and Comparative Immunology* **35**(12): 1366–1375.
- Tsilingiri K, Rescigno M (2012) Postbiotics: What else? *Beneficial Microbes* **4**(1): 101–107.
- Tubbs RK, Ditmars WE, Van Winkle Q (1964) Heterogeneity of the interaction of DNA with acriflavine. *Journal of Molecular Biology* **9**(2): 545–557.
- Ullal AJ, Wayne Litaker R, Noga EJ (2008) Antimicrobial peptides derived from hemoglobin are expressed in epithelium of channel catfish (*Ictalurus punctatus*, Rafinesque). *Developmental and Comparative Immunology* **32**(11): 1301–1312.
- Umeda N, Nibe H, Hara T, Hirazawa N (2006) Effects of various treatments on hatching of eggs and viability of oncomiracidia of the monogenean *Pseudodactylogyrus anguillae* and *Pseudodactylogyrus bini*. *Aquaculture* **253**(1–4): 148–153.
- Van Doan H, Hoseinifar SH, Esteban MÁ, Dadar M, Thu TTN (2019) Chapter 2 - Mushrooms, seaweed, and their derivatives as functional feed additives for aquaculture: an updated view. In: Atta ur R (ed) *Studies in Natural Products Chemistry*, pp. 41–90. Elsevier; Susan Dennis, Amsterdam.
- Van Duijn C Jr (1967) *Diseases of Fishes*, Thomas. Springfield, Illinois.
- Van Muiswinkel W (1995) The piscine immune system: innate and acquired immunity. *Fish Diseases and Disorders* **1**: 729–750.
- Vetvicka V, Baigorri R, Zamarreño AM, Garcia-Mina JM, Yvin J-C (2010) Glucan and humic acid: Synergistic effects on the immune system. *Journal of Medicinal Food* **13**(4): 863–869.
- Viji VT, Deepa K, Velmurugan S, Donio MBS, Jenifer JA, Babu MM *et al.* (2013) Vaccination strategies to protect goldfish *Carassius auratus* against *Aeromonas hydrophila* infection. *Diseases of Aquatic Organisms* **104**(1): 45–57.
- White DG, Zhao S, Simjee S, Wagner DD, McDermott PF (2002) Antimicrobial resistance of foodborne pathogens. *Microbes and Infection* **4**(4): 405–412.
- Willford WA (1966) Toxicity of 22 therapeutic compounds to six fishes. Investigations in Fish Control.
- Won KM, Kim PK, Lee SH, Park SI (2008) Effect of the residue extract of Siberian ginseng *Eleutherococcus senticosus* on non-specific immunity in olive flounder *Paralichthys olivaceus*. *Fisheries Science* **74**(3): 635–641.
- Woo PTK, Buchmann K (2012) Fish parasites: Pathobiology and Protection.
- Xamena N, Creus A, Marcos R (1984) Mutagenic activity of some intercalating compounds in the *Drosophila zeste* somatic eye mutation test. *Mutation Research/Genetic Toxicology* **138** (2–3): 169–173.
- Xie J, Liu B, Zhou Q, Su Y, He Y, Pan L *et al.* (2008) Effects of anthraquinone extract from rhubarb *Rheum officinale* Bail on the crowding stress response and growth of common carp *Cyprinus carpio* var. Jian. *Aquaculture* **281**(1): 5–11.
- Yamin G, Falk R, Avtalion R, Shoshana N, Ofek T, Smirnov R *et al.* (2017) The protective effect of humic substances and water and sludge from a recirculating aquaculture system on *Aeromonas salmonicida* infection in common carp (*Cyprinus carpio*). *Journal of Fish Diseases* **40**(12): 1783–1790.
- Yang L, Weiss TM, Lehrer RI, Huang HW (2000) Crystallization of antimicrobial pores in membranes: Magainin and protegrin. *Biophysical Journal* **79**(4): 2002–2009.
- Yasar S, Gokcimen A, Altuntas I, Yonden Z, Petekkaya E (2002) Performance and ileal histomorphology of rats treated with humic acid preparations. *Journal of Animal Physiology and Animal Nutrition* **86**(7–8): 257–264.
- Yu X, Li G (2012) Effects of resveratrol on longevity, cognitive ability and aging-related histological markers in the annual fish *Nothobranchius guentheri*. *Experimental Gerontology* **47** (12): 940–949.
- Zanuzzo FS, Sabioni RE, Marzocchi-Machado CM, Urbinati EC (2019) Modulation of stress and innate immune response by corticosteroids in pacu (*Piaractus mesopotamicus*). *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology* **231**: 39–48.
- Zaslouff M (2002) Antimicrobial peptides of multicellular organisms. *Nature* **415**(6870): 389–395.
- Zhao X, Wu H, Lu H, Li G, Huang Q (2013) LAMP: A database linking antimicrobial peptides. *PLoS One* **8**(6): e66557.
- Zhou YC, Wang J, Zhang B, Su YQ (2002) Ultrasonic immunization of sea bream, *Pagrus major* (Temminck & Schlegel), with a mixed vaccine against *Vibrio alginolyticus* and *V. anguillarum*. *Journal of Fish Diseases* **25**(6): 325–331.
- Zorriehzahra MJ, Delshad ST, Adel M, Tiwari R, Karthik K, Dhama K *et al.* (2016) Probiotics as beneficial microbes in aquaculture: An update on their multiple modes of action: A review. *Veterinary Quarterly* **36**(4): 228–241.
- Zurita JL, Jos Á, del Peso A, Salguero M, López-Artíguez M, Repetto G (2005) Ecotoxicological evaluation of the anti-malarial drug chloroquine. *Aquatic Toxicology* **75**(2): 97–107.