












REVIEW

Microbial education for marine invertebrate disease prevention in aquaculture

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Abstract

The holobiont theory expands the notion of individual multicellular organisms as a community composed of a host and all its associated microorganisms. This concept has been extensively studied in the field of aquaculture, where increasing evidence has highlighted the importance of the host associated microorganisms in species fitness. Here, we focus our review on mollusc and crustacean species in which microbiota dysbiosis has recently been described in the context of various diseases, resulting in significant economic losses. Influencing the holobiont structure through the use of probiotics is a potential strategy that could improve the fitness or the robustness of cultivated species. We discuss here the possibility of developing microbiome targeted prophylactic approaches by promoting (1) methods to identify host microbial community that fosters good health status and (2) early life microbial education to favour long-term resistance to stress or disease. This review aims to inform the aquaculture industry about potential strategies in rearing practices to mitigate diseases and economic losses.

KEYWORDS

crustacean, holobiont, microbial education, mollusc

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1 | INTRODUCTION

Aquaculture production covers most of the increase in seafood needs since 1990 and has surpassed fisheries as a source of food for human consumption.¹ It has become the fastest growing food-producing sector in the world yielding 57.5 million tonnes of finfish, 17.7 million tonnes of molluscs and 11.2 million tonnes of crustaceans for the year 2020.² For the aquaculture industry, disease emergences pose a significant risk and can induce substantial economic losses. More concerning is the accumulation of evidence over the past decades that diseases affecting wild and cultivated animals have increased in frequency and severity in association with anthropogenic disturbances and global change.^{3–8} As mentioned below, marine invertebrates constitute an important contribution to aquaculture production in the world. In these species, evidence for recurrent diseases inducing mass mortalities have accumulated, demonstrating the devastating impact of epizootics.^{5,8} Diseases affecting farmed oysters, shrimp, abalone and various fish cost billions of dollars each year to this industry.⁹ In some cases, these diseases directly result from the emergence of viral, bacterial or parasitic pathogens.^{10,11} For example, the White Spot Syndrome Virus (WSSV) is the most prevalent viral pathogen in shrimp leading to massive mortalities (up to 100% within 3–10 days) that have impacted productions for decades.¹² This highly virulent virus quickly infects gills, stomach, haemocytes, haematopoietic tissues and antennal gland.¹³ Another concerning infection known as acute hepatopancreatic necrosis disease (AHPDN) or Early Mortality Syndrome (EMS) has been reported in different shrimp species in Asia. It causes rapid and drastic mortalities causing losses of up to 90% of the live-stock.¹⁴ This disease is caused by the bacterial agent *Vibrio parahaemolyticus* which damages the hepatopancreas. In molluscs, the abalone withering syndrome (WS) is another infectious disease caused by the bacteria *Xenohaliotis californiensis* responsible for moderate to mass mortality events depending on the species involved. Mortality rate of up to 99% have been observed in some species such as the black abalone *Haliotis cracherodii* in the United States.¹⁵ Several bacterial diseases caused by *Vibrio*, *Nocardia* and *Roseovarius* have also been reported in several bivalve species.¹¹

Other diseases that affect invertebrates of interest to aquaculture are multifactorial and polymicrobial: in this case, there is a destabilisation of the microbiota due to an initial harmful environmental pressure or to the presence of a pathogen. This dysbiosis eventually opens the door for the development or colonisation by opportunistic pathogens or may even lead to a shift from commensalism to opportunism/pathogenicity for some members of the microbiota. In shrimp species, the White Faeces Syndrome (WFS) is a gastrointestinal disorder affecting cultivated penaeid shrimp, leading to severe production losses worldwide. Shrimp with WFS usually show a reduced feed consumption and growth rate, hepatopancreatic discoloration, and loose shells. Diseased shrimp show significantly decreased microbial richness and diversity compared to healthy control.¹⁶ In molluscs, many epizootics affecting shellfish are polymicrobial. One of notable examples is the Pacific Oyster Mortality Syndrome (POMS) which emerged in 2008 and is still heavily impacting the Pacific oyster, *Crassostrea*

gigas, production worldwide.^{17,18} POMS is a multifactorial disease, influenced by biotic and abiotic factors.^{18,19} The central role of a herpes-like virus, OsHV-1- μ Var, in POMS has been demonstrated; viral infection triggers an immune-compromised state that leads to microbiota dysbiosis and subsequent bacteraemia caused by opportunistic bacteria, ultimately resulting in oyster death.²⁰ Additional studies have shown that OsHV-1 infection leads to variations in the microbiota characterised by changes in the bacterial groups which are normally the most abundant and an increase of rare OTU belonging to the *Vibrio* genus including known opportunistic pathogens such as *V. harveyi*²¹ and *Arcobacter*.²² Other studies have shown that environmental factors such as exposure to pollutants or warming temperatures are other potential causes of dysbiosis in molluscs leading to increased mortality rate in farms. Temperature stress in *C. gigas* (rapid increase from 20 to 25°C) alters the bacterial composition of the microbiota towards dominance of opportunistic pathogens such as *Vibrionaceae* correlating with increased in mortality.²³ Heat stress also leads to decreased stability of the haemolymph microbiota in the Pacific oyster *C. gigas*²⁴ and induces lower diversity in the mussel *M. coruscus*.²⁵ These changes in the haemolymph microbiota contributes to increased mortality in these two bivalves in response to infections. Other studies performed in the clams *Chamelea gallina*^{26,38–50} and *Tegillarca granosa*²⁷ have shown that chemical pollution leads to microbiota shift associated with the proliferation of opportunistic pathogens potentially contributing to the declining health of clams.

In order to fight these diseases threatening aquaculture activities, a series of approaches have been developed. Strategies such as the use of probiotics, mutualist symbionts and bacteriophages have been proposed.²⁸ These approaches are based on the identification of beneficial microorganisms that directly benefit by exerting antagonistic effects against potential pathogens. These effects include the production of antimicrobial compounds, interference with quorum sensing or predatory actions.^{29–33} Other strategies are based on changes in aquaculture practices through the application of methods that aim to manage the microbial community within rearing tanks. For instance, the implementation of biofloc system improves water quality^{34–36} and has been shown to promote a better host immune response.³⁸ Diverse technologies aim to create a K-selected microbial community that counteracts r-strategic opportunists. These microbial management strategies have resulted in improved performance and increased viability of larvae.^{39–41} Otherwise, in a broader context, several studies have evidenced the critical role of microbiota during the early developmental steps to educate and durably imprint the host innate immune system in mammals^{37,42,43}, in fish⁴⁴ in crustaceans^{45,46} and in molluscs.^{47,48} Despite these evidence, antagonistic approaches toward microbial communities, such as water sterilisation and antibiotic treatment, have been favoured so far.^{49,50}

In this review, we promote the potential application of natural microorganisms as a treatment during the early developmental stage (larval rearing) to shape and sustain lifelong innate immune competence in aquaculture. First, we report recent studies which emphasise the critical role played by the microbiota in molluscs and crustaceans of economic interest for the aquaculture field. Then, we discuss the

possibility of using the growing amount of data describing the host associated microbiota to predict health promoting bacteria. Finally, we argue that the application of these new generation probiotics for microbial education in early developmental steps (larval rearing) could be a novel strategy to induce long-term beneficial effects in aquaculture.

2 | HOST-MICROBIOTA INTERACTIONS IN HEALTH AND DISEASE OF MOLLUSC AND CRUSTACEAN SPECIES

2.1 | Definition of the holobiont concept

Numerous studies have provided evidence that host associated microbial communities can influence multiple facets of animal physiology encompassing pathogen resistance, immunity, development, behaviour and ecological functions such as stress tolerance.^{51–60} These impacts imply that microbiota can profoundly influence animal performance, fitness and adaptation capacities in changing environmental conditions.^{59,61} These observations fall into the widely accepted holobiont concept which considers multicellular organisms as a complex community of species. Initially, this concept took into account only the mutualistic obligate associations between the host and its symbionts.⁶² However, with the advancements in sequencing methodologies and progress in microbiota research, the concept of holobiont was expanded to encompass the association between the host and all its associated microorganisms (bacteria, Archaea, viruses and yeasts).⁶³ Building on the holobiont concept, the hologenome theory of evolution considers the holobiont with its hologenome (all genomic entities in interaction) as the unit of selection during evolution.^{64,65} This theory implies that the host and the symbiont do not interact only for food or ecological niche, but also constitute the true unit of selection, thus co-evolving together and shaping the genetic composition of each other. These concepts have enabled a re-evaluation of host-associated microorganisms beyond their association with diseases and have permitted the consideration of their role in host physiology (see⁶⁶). The influence of the microbiota on the host immunity is one of the best examples illustrating the contribution of associated microorganisms to host fitness. Interestingly, commensal microbiota has multiple ways to cooperate with the host immune system to prevent pathogen infection. This has been well described in model species, especially in mice where experiments on germ free animals is feasible. Commensal bacteria can boost the immune system either locally or systemically (e.g., in distant tissues).^{67–70} Additionally, commensals can also directly impact bacteria (either true pathogens or opportunistic ones) through interference with their entry and establishment via direct competition or production of antimicrobial compounds. The aquatic invertebrate model organism, the cnidarian *Hydra vulgaris* extensively addresses the beneficial effect of the microbiota. A substantial body of work clearly shows that mucosal innate immunity is shaped by host-microbiota interactions.⁷¹

2.2 | Influence of the microbiota for health and disease outcomes in crustaceans and molluscs of aquaculture interests

Several studies focusing on host-associated microbiota have highlighted the beneficial effects of microorganisms for invertebrate species relevant to aquaculture. Beneficial properties of the microbiota have been observed in several context relevant for aquaculture such as enhanced growth, feed efficiency, increased survival in early life stages, settlement, shell quality, size or organoleptic quality.^{36,72–74} We have focused our review work on host associated microorganisms which are beneficial in a context of health and infectious disease outcomes (Table 1). Several studies have indicated that higher microbiota diversity is associated with the host health status in both crustaceans and molluscs (Table 1, part 1). This may result from the capacity of a highly diversified microbial community to provide a greater number of beneficial functions. In different shrimp species, a greater diversity of metabolites with potential beneficial properties is produced by half of the core microbiota likely contributing to their immune homeostasis.⁷⁶ Additionally, functional redundancy has been described in the more diverse microbial community of the yellow abalone compared to the blue abalone correlating with a lower prevalence of the Withering Syndrome disease.^{78,79} However, it's essential to note that while the mentioned studies are mostly correlative, only two of them have demonstrated a direct causality between healthy or diseased microbiota and health status. In penaeid shrimp, intestinal microbiota transfer (IMT) from WFS diseased shrimp to healthy ones led to the development of symptoms similar to those of diseased shrimp.¹⁶ In *C. gigas*, a microbiota transfer from healthy juvenile donor oysters to healthy recipient larval oysters for 10 days, resulted in enhanced immune response at juvenile stages and a greater capacity to prevent OsHV-1 viral proliferation associated with reduced mortality to the POMS disease.⁴⁷

Beyond the beneficial properties induced by a highly diversified microbial community, specific strains within the microbiota may also contribute to the host health through their direct antagonistic effect on pathogens (Table 1, part 2). For instance, the addition of Alterin-producing *Pseudoalteromonas* hCg-42 correlates with lower mortality in *Vibrio* challenged *C. gigas*.^{80,81,84} Injection of BALOs (*Bdellovibrio*-like organisms) in lobster juveniles results in lower *Vibrio* abundance compared to non-injected control group.⁸² BALOs are a group of Gram-negative bacteria that prey on other Gram-negative bacteria and have been reported in the haemolymph microbiota of diverse shrimp species and the spiny lobster *Panulirus ornatus*. Moreover, in a broader context, extensive correlation analysis have revealed associations between the presence or abundance of specific bacteria and varying disease prevalence.^{21,72,86} Nevertheless, the precise role of these bacteria in contributing to the advantageous properties to their associated host has yet to be accurately delineated.

In summary, evidence suggests that the host-associated microbiota significantly contributes to the health of molluscs and crustaceans relevant to aquaculture. Disruption of a healthy and diversified microbiota can lead to disease states. Understanding the dynamic interplay of this interaction in these species is essential to implement disease mitigation strategies.

TABLE 1 Studies which support that host-associated microbiota influences the health of molluscs and crustaceans of aquaculture interest.

Part 1: Studies which highlight how the microbiota diversity influences health and disease outcomes.				
Species	Disease	Microbiota change	Effect on the Host	Reference
<i>L. vannamei</i>	WSSV	Increased microbial diversity induced by dietary supplementation with brown seaweeds composition	Reduction of the mortality rates after a WSSV challenge in the diet-fed group compared to the control shrimp	75
<i>M. japonicus</i> , <i>L. vannamei</i> , <i>Macrobrachium rosenbergii</i> , <i>Procambarus clarkii</i>	WSSV	Production of metabolites with antiviral potential by half of the core microbiota in response to viral challenge	Likely contributes to a better immune homeostasis and potential host resistance	76
<i>Homarus gammarus</i>	Infection with <i>Homarus gammarus nudivirus</i> (HgNV)	Increased species richness and diversity observed in sea-based container culture compared to land-based culture	Lower viral prevalence in sea-based container culture compared to land-based culture	77
Penaeid shrimp	WFS	Decreased in microbial richness and diversity during the WFS disease	Intestinal microbiota transplants from WFS diseased to healthy shrimp lead to symptoms similar to those of the diseased ones	16
<i>Haliotis fulgens</i> <i>Haliotis corrugata</i>	WS	Higher microbial diversity in the yellow abalone (<i>Haliotis corrugata</i>) compared to blue abalone (<i>Haliotis fulgens</i>)	Increased susceptibility to the WS in the blue abalone correlates with structural and functional alterations in their microbiota, in contrast to yellow abalone which maintains a stable microbiota under WS stress	78,79
<i>C. gigas</i>	POMS	Higher microbial diversity in larvae after a microbiota transfer compared to control group	Lower mortality in the microbiota transferred group compared to control	47
Part 2: Studies which highlight the beneficial contribution of a specific bacterial species for the health of its autochthonous host by antagonistic effects.				
Species	Disease	Microbiota change	Effect on the Host	Reference
<i>C. gigas</i>	Vibriosis	Addition of <i>Pseudoalteromonas</i> (hCg-42 and hCg-6) (producer of antimicrobial molecules (Alterins)) to <i>C. gigas</i>	In vitro antimicrobial effect against vibrio and decrease of the mortality rates after a vibrio challenge in <i>C. gigas</i> incubated with hCg-42	(80,81; Not published results)
<i>P. ornatus</i>	Vibriosis	Addition of BALOs (<i>Bdellovibrio</i> and like Organisms) to <i>P. ornatus</i>	Abundance decreases of vibrio in haemolymph of host injected with <i>Halobacteriovorax</i> sp. (BALOs)	82
<i>Litopenaeus vannamei</i>	Vibriosis	Addition of <i>Bacillus</i> sp. YC5-2 initially isolated from the guts of healthy wild adult shrimp	In vitro antibacterial effect against vibrio and decreased mortality after a vibrio challenge	83
<i>C. gigas</i>	<i>V. coralliilyticus</i> infection	Addition of <i>Pseudoalteromonas</i> (D16 and DM14), and <i>Epibacterium</i> (B11) initially isolated from <i>C. gigas</i> oysters	Improving survival of oysters during <i>V. coralliilyticus</i> infection	85

Note: Studies that underscore the microbiota's impact on health and disease outcomes in invertebrates relevant to aquaculture. The part 1 comprises studies demonstrating how a shift in the microbiota diversity (reported in column 3) impacts the host health status (reported in column 4). The part 2 comprises studies demonstrating how the introduction of antimicrobial-producing strains into the host microbiota (indicated in column 3) counteracts pathogens and contributes to the health benefits of the host (indicated in column 4).

2.3 | Innate immune effectors are key players which shape the microbiota of molluscs and crustaceans of interest to aquaculture

In many species, host-microbiota associations appear relatively stable within an individual showing a strong correlation between host

phylogeny and microbiota that co-evolved for beneficial outcomes, a concept known as phyllosymbiosis.⁸⁷ This concept implies that the host can selectively shape its microbiota, leading to convergence of host phylogeny and microbiota composition. The correlation between host genetic background and microbiota composition has been clearly observed in marine molluscs. In *C. gigas*, microbiota specific to oyster

TABLE 2 Studies which support direct consequences of the innate immune system for the host associated microbiota structure.

Organisms	Innate immune effectors	Influence on the microbiota structure	Reference
<i>M. japonicus</i>	MjHeCL (protein that combines a C-type lectin domain, and an antimicrobial peptide)	The down-regulation of MjHeCL expression by RNAi leads to the proliferation of the haemolymph microbiota, ultimately resulting in shrimp death	102
<i>M. japonicus</i>	The catalase enzyme by regulating the reactive oxygen species (ROS) level	High level of ROS in shrimp intestine (obtained after inactivation of MjCAT which encodes for the catalase) correlates with a reduction in bacterial load of the shrimp intestinal lumen	103
<i>M. japonicus</i>	Nitric Oxide (possibly through its positive regulation of AMPs production)	After RNA interference of the nitric oxide synthase (NOS) or treatment with an inhibitor of NOS, NO production decreased, and the gut bacterial load increased significantly in shrimp	104
<i>L. vannamei</i>	Antimicrobial peptides of the Crustin family	Inactivation of the gene encoding for the antimicrobial peptide LvCrustin II-1 leads to change in the microbiota composition of the shrimp gills	105
<i>L. vannamei</i>	Antimicrobial peptides of the Crustin family	Inactivation of the gene encoding for the antimicrobial peptide LvCrustin I-1 leads to change in the microbiota composition of shrimp intestines	106
<i>A. purpuratus</i>	Antimicrobial peptide from the big defensin family (ApBD1)	Inactivation of the gene encoding for ApBD1 leads to changes in bacterial composition of the hemolymph microbiota	107
<i>A. purpuratus</i>	The bactericidal/permeability increasing protein ApLBP/BP1	Inactivation of the gene encoding for ApLBP/BP1 leads to changes in bacterial composition of the hemolymph microbiota	107
<i>A. purpuratus</i>	g-type lysozyme ApGlys	Inactivation of the gene encoding for ApGlys leads to higher diversity in hemolymph bacterial community and imbalance in certain bacterial groups	108
<i>C. gigas</i>	Antimicrobial peptide from the big defensin family (Cg-BigDef1)	Injection of synthetic Cg-BigDef1 into the oyster tissues induced a significant shift in oyster microbiota β -diversity	109

Note: Studies indicating that certain host innate immune effectors (specified in column 2) directly influence microbiota composition. The repercussions of the inactivation (or addition) of these effectors on host microbiota structure are detailed in column 3.

families has been described and perfectly segregates with the oyster's genetic background.^{86,88,89}

In a broader context, evidence from invertebrate models reveals that immune systems can control microbial communities and promote its long-term colonisation through diverse strategies such as mechanical barriers provided by chitine or mucus layers, or mediated by conserved non-self recognition and antimicrobial mechanisms.⁹⁰⁻⁹³ Among all these mechanisms, antimicrobial peptides are major players identified as key regulators of a well-balanced interaction between commensal microbes and host tissues. Implication of these peptides for microbiota shaping has been well characterised in the two noteworthy invertebrate aquatic models, the squid *Euprymna scolopes*⁹⁴ and the cnidaria *Hydra vulgaris*.^{95,96} In molluscs of aquaculture interest, several studies have shown an indirect link between microbiota and host immunity, especially in the context of abiotic stresses^{27,48,97-101} and disease-triggered dysbiosis.^{20,26} However, in these studies, whether the stress triggers a change in the microbiota resulting in an immune shift or conversely, whether the stress triggers an immune shift resulting in a change of microbiota structure remains to be determined. The few studies that have addressed the direct influence of the host innate immunity on microbiota composition in

molluscs and crustaceans are listed in the Table 2. A substantial number of studies have been conducted in the kuruma shrimp *Marsupenaeus japonicus* and the king prawn *Litopenaeus vannamei* where RNAi invalidation has pointed to a causal link between innate immune effectors (reactive oxygen/nitrogen species—ROS and RNS—and antimicrobial peptides) and the balance of the bacterial microbiome (Table 2). In *L. vannamei*, similar studies involving inactivation of genes encoding for AMPs of the crustin family have shown that the type I and type II crustins were involved in regulating the host bacterial community.¹⁰⁶

In molluscs, to our knowledge, studies have investigated the direct influence of the host innate immunity for microbiota composition in scallops and oysters. In the scallop *Argopecten purpuratus*, the big defensin ApBD1 and the bactericidal/permeability increasing protein ApLBP/BP1 have been shown to regulate the proliferation of specific bacterial groups in the haemolymph.^{107,110} In the same species, a g-type lysozyme is shown to participate in the microbial homeostasis.¹⁰⁸ In *C. gigas*, big defensins have the potential to shape oyster microbiota. Cg-BigDef1 induced a significant shift in oyster microbiota β -diversity 6 and 24 h after its injection into oyster tissues.¹⁰⁹

In summary, a body of research in molluscs and crustaceans has revealed an interaction between host immunity and microbiota composition, particularly in response to abiotic stresses and dysbiosis triggered by diseases. Additionally, a direct causal link between innate immune effectors and the balance of the bacterial microbiome has been demonstrated in kuruma shrimp, king prawn, scallops, and oysters, where gene function invalidation serves as a method to establish causality between host innate immunity and microbiota composition.

3 | TOWARD THE USE OF NEXT GENERATION PROBIOTICS FOR AN OPTIMISED MICROBIAL EDUCATION

3.1 | Probiotic usage: definition and limitation for shellfish aquaculture

In this article, we reviewed recent studies that emphasise the critical role played by the microbiota on health and disease resistance for economically important marine invertebrates. There is an increasing body of evidence that points toward the beneficial advantage conferred by the microbiota for crustaceans and molluscs in an aquaculture context and how a fine-tuned setting is necessary to ensure an optimal host-microbiota balance. Disruption of this balance by external stressors or pathogens induces a decrease in the fitness of the holobiont, eventually leading to death. In this sense, it is evident that we should now consider each organism as an holobiont where associated microorganisms not only provide a beneficial advantage in adverse conditions but are also essential players in maintaining host health. Experimentation with the microbial component of the holobiont has been active for decades using probiotics. According to the definition of the Food and Agriculture Organization (FAO), a probiotic is 'live microorganisms, which when consumed in adequate amounts, confer a health benefit on the host'.¹¹¹ Probiotics have been successfully applied for decades in human health, focusing on beneficial bacteria isolated from natural human microbiota. For instance, lactic acid bacteria (LAB) have been extensively studied for their beneficial properties (as reviewed by¹¹²) and were recently used for potential application in depuration in *C. gigas*.¹¹³ This has led to several commercial applications with proved beneficial effect in human health and in agro-industries. The use of probiotic strains has further expanded to animal production, and is considered a promising eco-responsible and prophylactic alternative to antibiotics.¹¹⁴ The extensive use of antibiotics has been identified as a major cause for emergence of antibiotic resistant pathogens in shrimp aquaculture.¹¹⁵ In this field, the application of probiotics has been used for decades as an environmentally-friendly alternative strategy to fight pathogens.^{29,116} The use of probiotics has also been successfully tested on oysters.¹¹⁷⁻¹²⁰ The mode of action of probiotics and their beneficial effects on disease resistance and species performance in aquaculture have been extensively reviewed.^{29,116,121,122} Several limitations regarding the application of probiotics, which is not always beneficial, have been

TABLE 3 Current limitations of probiotic application in mollusc and crustacean aquaculture.

The probiotics do not persist in the endogenous microbiota	
Exposure of the European abalone <i>Haliotis tuberculata</i> to the <i>Pseudoalteromonas</i> hCg-6 exogenous strain leads to a transient establishment of this probiotic strain in the haemolymph rather than the establishment of a long-term interaction	31
Exposure of <i>C. gigas</i> larvae to bacteriocin-like inhibitory substance (BLIS)—producing <i>Aeromonas</i> , showed that the probiotic strain concentration decreased right after it was added to the oyster and was not detectable 72 h after its addition	123
The probiotics display short-term beneficial effects	
In <i>L. vannamei</i> , larval exposure to <i>Bacillus subtilis</i> E20 or to <i>Bacillus</i> sp. YC5-2 is beneficial to the shrimp during larval stages but subsequent beneficial effects on further stages have not been tested.	83,124
Immunomodulation is observed in <i>L. stillostris</i> after a larval exposure to <i>Pseudoalteromonas</i> probiotic candidates but it has not been tested on later stages.	125
Addition of <i>Bacillus pumilus</i> R106-95 and <i>Phaeobacter inhibens</i> S4 during larval development of the eastern oyster <i>C. virginica</i> for either 6 or 24 h prior to pathogen challenge leads to an effective immune response and protection against <i>V. coralliilyticus</i> and <i>Vibrio tubiashii</i> . The beneficial effect was observed immediately after exposure, but no significant protection was observed when the larvae were challenged 48 and 96 h after removal of the probiotic.	120,126
The exposure of <i>C. cortezinsis</i> larvae to a mix of two bacteria <i>Pseudomonas aeruginosa</i> , strain YC58 and <i>Burkholderia cepacia</i> , strain Y021, leads to a better survival during the larval rearing stages.	127
A 20-h larval exposure to <i>Pseudoalteromonas</i> sp. D41 or <i>Phaeobacter gallaeciensis</i> , has conferred a significant protection to <i>C. gigas</i> larvae against <i>V. coralliilyticus</i>	119
An immunomodulation was also observed on the Yesso scallop <i>Patinopecten yessoensis</i> fed with a diet of microalgae supplemented with the strain <i>Pseudoalteromonas</i> sp. F15 during larval stages	128
In the sea cucumber <i>Apostichopus japonicus</i> , an exposure to bacterial strains <i>Pseudoalteromonas elyakovii</i> HS1, <i>Shewanella japonica</i> HS7, or <i>Vibrio tasmaniensis</i> HS10 leads to an enhanced cellular and humoral immune response and to an improved survival and growth rate	129

reported. One major limitation is that probiotic strains do not persist in the endogenous microbiota (Table 3). The addition of non-autochthonous strains (bacteria isolated from other host species) is a possible explanation considering the tight association between the host and its microbiota, as explained in the first part of this review. Regular addition of probiotics supplemented with prebiotics (compounds in food that induce the growth or activity of the probiotics) has been a strategy applied so far to maintain the long-term beneficial properties of the added strains. However, such an approach increases the economic cost of the probiotic solution.²⁸ Another major

limitation in the shellfish farms is the impossibility of using probiotics in open sea culture. In this sense, the use of probiotics in molluscs industry is limited to hatcheries during larval rearing or necessitate land-based facility to apply the treatment on spat or adult stages. Except for the study performed by Fallet *et al.*,⁴⁷ the literature on probiotics added during larval stages do not report long-term beneficial effect for molluscs and crustacean health (Table 3). Several reasons might explain this potential caveat: (1) The long-term beneficial properties have not been addressed in the published study mentioned in Table 3 and the authors have overlooked the potential of their probiotics to confer long-term benefits to their host (2) The author have added a single probiotic strain and considering the importance of the microbial diversity for host fitness, the use of multi-strain probiotics (MSP) should lead to more beneficial effects. MSP consist of a mix of two or more strains, that have proven to offer various beneficial effects for their host. The improved performance of MSP exposure compared to single strain has been intensively described in fish^{130,131} and is starting to be described in crustaceans and molluscs.^{132,133} For example, the use of a mix of four different probiotics has more efficiently improved the growth and immune status of *L. vannamei* in comparison with single-strain probiotics.¹³⁴ The addition of MSP during the larval stage conferred a protective effect in Spiny Lobster (*P. ornatus*) larvae against *Vibrio owensii* infections.¹³⁵

In summary, probiotics have proven to be promising tools for mitigating disease risks in mollusc and crustacean aquaculture. However, there are challenges that still need to be addressed, such as the limited persistence in the endogenous microbiota, the impossibility of applying them in open sea culture, and the associated economic costs. The use of multi-strain probiotics isolated from the endogenous microbiota is a promising way to enhance host performance. Additionally, exploring the long-term effects on immune status after probiotics addition during larval rearing is another avenue that deserves further exploration.

3.2 | Next generation probiotics: emerging tools for a better prediction of health promoting bacteria

The recent advances on host-microbiota interactions have brought new insights, that should broaden the use of probiotics in the most updated strategies. A substantial number of large scale analyses applied to diverse crustacean¹³⁶ or mollusc species¹²² has led to multiple descriptions of bacteria associated with different tissues, at several life stages, related to different health statuses, in different environments. These datasets constitute tremendously valuable resources for predicting stage and species-specific beneficial bacteria. Correlation studies that identify association between health status and microbiota composition should lead to deciphering consortia of beneficial microorganisms. This approach has been applied to microbial communities associated with health and disease in shrimp in order to identify stage specific disease or bioindicators of health.¹³⁷ Based on a linear discriminant analysis (LDA) effect size (LEfSe),¹³⁸ Zheng *et al.* identified that the genus *Meridianimaribacter* was enriched in

healthy shrimp and with good water quality. In agreement with other culture-dependent study,¹³⁹ this genus was identified as a beneficial bacterium for shrimp larvae and was suggested as a probiotic candidate for shrimp larval rearing. Similarly, based on the LEfSe method applied to the bacterial community associated with POMS-sensitive and resistant oysters, we identified stage specific bacterial taxa that are overrepresented in more resistant oysters during experimental infection.⁴⁷ In another study, we performed a differential analysis of taxa abundance between conditions in microbiome and identified three bacterial families (*Colwelliaceae*, *Cyanobacteria* (Subsection III, family I), and *Rhodobacteraceae*) significantly associated with oysters resistant to POMS disease.⁸⁶ Overall, we suggest that predictive analysis based on correlation studies in combination with literature information, should be an upstream step to identify putative beneficial bacteria that can be then isolated by culture-based approaches (Figure 1). We anticipate an improved benefit of the probiotics designed in such a way considering the concept of the holobiont and the long co-evolutionary history between the host and the bacteria (Box 1). However, this is only a predictive strategy, and the beneficial effect of these probiotics requires validation in an experimental framework, as we describe in Figure 1.

3.3 | Microbial education during early stages: An opportunity to sustain long-term beneficial effects

Here, we also explore another possibility, which involves educating the host immune system by applying beneficial microorganisms throughout the course of immune system development. This 'microbial education' plan is not just another probiotic strategy but is a real concept rooted in robust recent scientific studies that span several fields, as summarised in the Box 1: (1) the microbial community management in aquaculture, (2) the holobiont theory, (3) the biological embedding concept, (4) the possibility to prime the innate immune system of invertebrate species to induce long-term immune protection. The 'microbial education' outline is to add host specific microorganisms during the larval development. This strategy aims to influence the immune system toward better defence capacities. The possibility to influence the developmental trajectory of individuals through environmental manipulation during early development has been previously discussed by others.^{149,150} This strategy can be used to produce a desired phenotype and applied to critical issues in aquaculture with some benefits but also, some barriers as summarised in Table 4. The possibility to educate the host immune system through a microbial education has been recently demonstrated in oysters.⁴⁷ In this study, we showed that it was possible to increase *C. gigas* immune competence until the juvenile stage, 4 months after a 10 days natural microbial exposure during the larval stages. This study clearly highlighted that an interaction with a diversified and autochthonous microbiota during the early developmental stages has lifelong consequences, even transgenerational impact, and could be instrumental in fighting against POMS disease. Such an approach remains to be investigated for other pathogens commonly described from the larval to adult stages in *C. gigas*,¹⁵¹ and other oyster

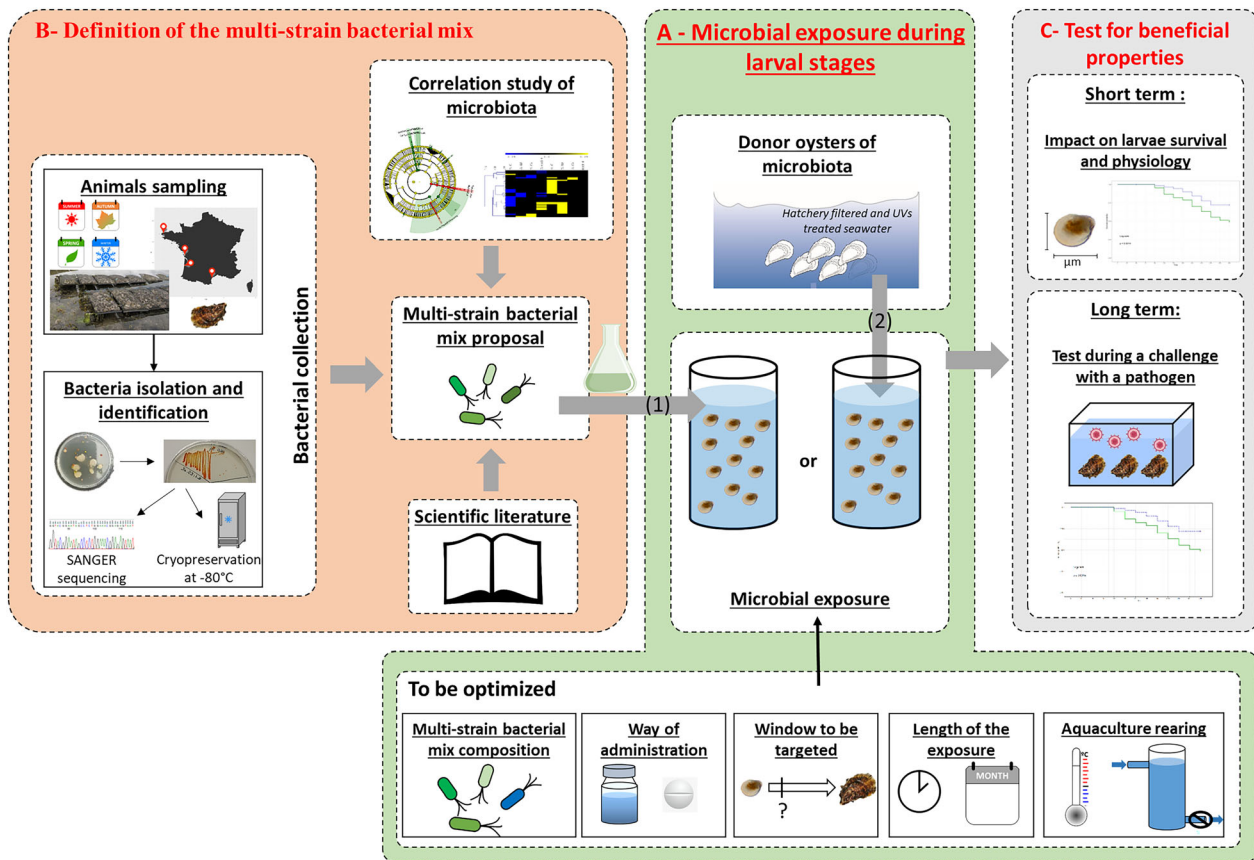


FIGURE 1 Full scale experimental design to identify beneficial bacteria for microbial education. For a long-term beneficial effect, it is recommended to perform the microbial education during larval stages (Part A, green coloured). The microorganisms to be added to the seawater during larval rearing can be introduced either (1) by pathogen-free donor oysters that were always kept in controlled facilities using UV-treated seawater, strict biosecurity zoning and management procedures or (2) by adding cultured based multi-strain bacterial mix that has been carefully selected. The method of administration of the mix and its composition has to be optimised to maximise its absorption by the larvae (Dipping or in freeze-dried form, delayed or simultaneous to the feeding, biofloc form). The exposure window (from embryogenesis to larval stages) and the duration of the exposure to the bacterial cocktails must be adjusted. Rearing conditions are other parameters that should be tested (Temperature, continuous flow or batch rearing system). The definition of the multi-strain bacterial mix (Part B, orange coloured) is a necessary upstream step to better anticipate beneficial properties. First, a library of cultivable bacteria must be created. These bacteria will be preferentially isolated from the host to be targeted. Pathogen-resistant animals (if probiotics aims at improving resistance toward a particular infectious disease) must be collected from several geographical sites and at different seasons in order to maximise the bacterial diversity. The bacteria thus obtained will then be cultivated, purified, and cryopreserved. Several physicochemical parameters (culture media, temperature) for bacterial culture may be tested to increase the potential biodiversity in the bacterial library. Identification of each cultivated strain from the collection will be performed by SANGER sequencing of the 16S rRNA coding gene. In parallel, *in silico* predictive analysis must be performed to predict which bacteria are generally associated to resistant phenotype in the host (if probiotics aims at improving resistance toward a particular infectious disease). This correlative study will necessitate that several (meta)barcoding analyses have been previously generated on microbiota sampled from resistant and sensitive animals toward a specified disease. These correlative analyses, coupled with an exhaustive study of the scientific literature, should make it possible to predict bacteria from the collection that could be beneficial probiotic candidates. Then, beneficial effects of microorganism exposure will have to be tested (Part C, grey coloured). Short term effect will be tested during the larval stages. Particular attention should be paid to the effect of the multi-strain bacterial mix on the survival and physiology of the larvae, to test whether the exposure is deleterious, beneficial, or neutral for larval development and growth properties. Sampling for molecular analysis (i.e., transcriptomic, barcoding, metabolic, epigenomic analysis) may be worth it to decipher molecular basis of the microbial effect. Finally, long-term beneficial effects will be tested on subsequent life cycle stages: juveniles and adults will be challenged by pathogens.

species.¹⁵² This early life microbial education is somewhat in contradiction with some hatchery current practices, which seek to limit the introduction of both non-pathogenic and pathogenic bacteria, viruses and eukaryotes as well as to eliminate unwanted plankton and zooplankton in seawater, from the conditioning of the broodstock to the larval and spat stages.^{49,50,153,154} Additionally, the depletion of bacteria in the

rearing environment and in farmed animals is often accentuated by the addition of antibiotics. This process was tested in response to the risk of mortality in livestock^{153,155,156} and was quickly associated with the risk of selecting antibiotic-resistant bacteria.^{153,157} Today, however, this use remains a common practice, mainly at the larval stage of various species of marine invertebrates, as well as their broodstock.

BOX 1 Scientific rationale behind the microbial education.

	Description of the concept
(1) Microbial community management in aquaculture	The microbial management in aquaculture consist in controlling the water microbiota in rearing system according to ecological selection principles. Methods which favour K-selection in the rearing environment have been shown to select against <i>r</i> -strategic microbes and promote healthy microbe-larvae interactions. ^{39–41,140}
(2) Holobiont concept	Host associated microbial communities can influence multiple facets of animal physiology including development, behaviour, ecological functions such as stress tolerance as well as pathogen resistance and immunity. ^{51–60} The beneficial effect of the microbiota for host immune homeostasis is clearly one of the best illustration of the holobiont theory. ^{67–70}
(3) Biological embedding	The biological embedding is the process by which the life history of an individual during ontogeny impacts its development and modifies its phenotype. ¹⁴¹ The environment has a long-lasting influence on the functions of biological systems and this environmental imprint has major consequences for the behaviour and health of individuals. The early stages of development are sensitive windows for recording this environmental information. ^{142,143} Microbial colonisation during early life is an example of biological embedding and many studies have emphasised its critical role in educating and durably imprinting the immune system. ^{42,43}
(4) Immune priming	The immunological memory as described in the adaptive immune system of vertebrates does not exist per se in invertebrates and the fight against pathogens relies on innate immune mechanisms. It has been recently shown that this innate immunity can be trained to retain the memory of a primary exposure to better respond to infections encountered later. ¹⁴⁴ In molluscs and crustaceans, recent studies have shown that it is possible to train the innate immune system. ^{145–148} The possibility of using the trained immunity as a potential strategy to fight disease in shrimp aquaculture has been recently reviewed by Roy et al. ⁴⁵

TABLE 4 Benefits and barriers for practicability in aquaculture.

Benefits
The microbial education is a probiotic strategy that must be applied during the larval stages in hatcheries/nurseries. This plan is feasible for shellfish as it represents small volume of animals to be treated using bath, even those which will be transferred in open sea at later stages.
The use of donors of microorganisms is the most promising strategy, it is easy to implement, and it is not costly.
The probiotic exposure can be performed on numerous animals (several hundreds of millions of larvae) at the same time by bath or on their diet.
The microbial education aims at providing individuals with an increased robustness against infectious diseases. It is a strategy to better face the epizooties without using antibiotics.
The exposure benefice during larval stages is expected to be lifelong but also multigenerational.
Barriers
The larval stage is identified as the most sensitive rearing phase, and inappropriate probiotic exposure during this stage may be more detrimental than beneficial.
The microbial education approach is empirical and require time for optimisation. The optimal setup for one species might not be applicable to another species. Similarly, the response of the host to the exposure of probiotics could depend on their broodstock, used to produce the shellfish.
The exposure to multi-strain probiotic could lead to increase resistance toward particular pathogens and may not protect against other diseases.
The use of donors of microorganisms is the most promising strategy, but the transferred microbiota is not controlled and there is a risk for pathogen transmission.

Globally, research efforts remain to be performed for microbial education to be applied during larval rearing. The microbial management plan aligns with our proposal, aiming to increase the performance and viability of larvae by applying ecological theories and optimising larvae-microbiota interactions.^{40,140} Diverse methods (MMS: Microbially Matured Water System, BFT: Bio Floc Technology, RAS: Recirculating Aquaculture System) allow for the stable presence of naturally K-selected microbial community into the rearing tanks. It clearly has a long-term beneficial impact and favours the production of high quality juveniles.^{39,41} The immunomodulatory properties and health benefits of such practices have been demonstrated.³⁵ However, using these methods, the added microflora is not controlled and not specific to the raised species and it is hazardous since it may contain pathogenic microorganisms. In perspective of this review, we propose a simple conceptual model to summarise the possible strategy that could be applied to perform the most appropriate early-stage microbial education (Figure 1). Full-scale trials will be necessary and should consider several parameters that could impact hatching and larval growth/survival. The parameters should include: the definition of the multi-strain bacterial mix to be applied, the way the

bacterial mix should be administered, the most appropriate window to be targeted, the duration of the exposure, and the aquaculture rearing conditions. In Fallet et al.,⁴⁷ the microorganisms to which recipient oyster larvae were exposed came from healthy *C. gigas* spat sampled in their natural environment and used as microbiota donors. Those donor oysters were held upstream of the larval tanks but downstream the filter systems. This strategy allowed transferring the whole oyster associated microorganisms' community to recipient larvae and accordingly, the immunomodulatory potential of the overall microbial diversity (including viral, prokaryotic, eukaryotic and uncultivable microorganisms) was exploited. Such a strategy could be applied to hatcheries. However, a major limitation of such a practice should be considered: the microorganism community is not controlled and remains hazardous. A real health risk remains since donor oysters of microbiota may also carry opportunistic or latent pathogens while appearing to be healthy. In this sense, a controlled environment rich in microorganisms used during the larval rearing is recommended. This can be achieved using donor oysters of microbiota which were always kept in biosecured facilities (Figure 1). In this way, the oysters were shown to be devoid of the three main pathogens of *C. gigas* from larvae to juveniles.^{151,158} Alternatively, a controlled culture-based bacterial formulation, a multi-strain bacterial mix can be added during the larval rearing (Figure 1). For this purpose, it will be necessary to first build a collection of host-associated bacteria and screen several bacterial cocktails for their ability to induce long term immunomodulatory effects. Early development is the preferential stage to be targeted to induce long lasting effects, as previously reported.^{42-44,47} Targeting this stage also offers the possibility to treat several millions of larvae at the same time.

In summary, we discuss here an alternative approach to mitigate infectious disease in aquaculture. We propose to educate the host immune system by introducing host beneficial microorganisms during the maturation of the innate immune system. This concept of 'microbial education' goes beyond conventional probiotics strategies and is grounded in recent scientific studies that intersect various fields, such as the management of microbial communities in aquaculture, the holobiont theory, the biological embedding concept, and the ability to prime the invertebrate innate immune system. We further suggest a potential strategy for early-stage microbial education, with an emphasis on controlled environments and health considerations when transferring microorganisms.

4 | CONCLUSIONS

Diseases of farmed shellfish induce significant economic losses. To address these recurring threats, the exploration of alternative and environmentally friendly solutions has been ongoing for a long time. In this review, we delve into how host-associated microorganisms not only greatly contribute to improving the fitness of their host but also constitute a much more complex evolutionary unit called the holobiont. We discuss the possibility of implementing advanced

techniques and the concept of the holobiont field as potential new strategies to mitigate diseases in farmed animals. We suggest (1) using the increasingly expansive data describing microbiota in diverse species as a tool to predict the most natural beneficial bacteria for their associated host and (2) applying an exposure of these predicted beneficial microorganisms during the larval rearing step in the hatchery to benefit from biological embedding. If properly applied, this microbial education is anticipated to induce lifelong protection and could be a natural and sustainable strategy for safeguarding aquaculture efforts from infectious disease.

AUTHOR CONTRIBUTIONS

Céline Cosseau: Conceptualization; investigation; funding acquisition; writing – original draft; writing – review and editing; validation; methodology; project administration; supervision. **Luc Dantan:** Conceptualization; investigation; funding acquisition; writing – original draft; methodology; validation; visualization; writing – review and editing; software; formal analysis; data curation. **Eve Toulza:** Conceptualization; investigation; funding acquisition; writing – original draft; methodology; validation; writing – review and editing; project administration; supervision. **Bruno Petton:** Conceptualization; writing – original draft; writing – review and editing. **Caroline Montagnani:** Writing – original draft; writing – review and editing. **Lionel Degremont:** Writing – original draft; conceptualization; investigation; writing – review and editing. **Benjamin Morga:** Conceptualization; investigation; writing – review and editing. **Yannick Fleury:** Conceptualization; investigation; validation. **Guillaume Mitta:** Conceptualization; investigation; writing – review and editing. **Yannick Gueguen:** Writing – original draft; writing – review and editing; funding acquisition; project administration. **Jeremie Vidal-Dupiol:** Conceptualization; investigation; funding acquisition; writing – original draft; writing – review and editing; project administration.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The articles that support the content of this review are available and a DOI of each of the mentioned article in the text is provided in the references section.

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REFERENCES

- Anderson JL, Asche F, Garlock T. Economics of aquaculture policy and regulation. *Ann Rev Resour Econ*. 2019;11:101-123. doi:10.1146/annurev-resource-100518-093750
- Food and Agriculture Organisation. The state of world fisheries and aquaculture 2022. *State World Fish Aquac*. 2022;2022. doi:10.4060/cc0461en
- Altizer S, Ostfeld RS, Johnson PTJ, Kutz S, Harvell CD. Climate change and infectious diseases: from evidence to a predictive framework. *Science*. 2013;80(341):514-519. doi:10.1126/science.1239401
- Behringer DC, Duermit-Moreau E. *Crustaceans*. *J Invertebr Pathol*; 2021. doi:10.1016/j.jip.2020.107500
- Burge CA, Mark Eakin C, Friedman CS, et al. Climate change influences on marine infectious diseases: implications for management and society. *Annu Rev Mar Sci*. 2014;6:249-277. doi:10.1146/annurev-marine-010213-135029
- Harvell CD, Mitchell CE, Ward JR, et al. Climate warming and disease risks for terrestrial and marine biota. *Science*. 2002;80(296):2158-2162. doi:10.1126/science.1063699
- Jones KE, Patel NG, Levy MA, et al. Global trends in emerging infectious diseases. *Nature*. 2008;451:990-993. doi:10.1038/nature06536
- Kibenge FS. Emerging viruses in aquaculture. *Curr Opin Virol*. 2019; 34:97-103. doi:10.1016/j.coviro.2018.12.008
- Lafferty KD, Harvell CD, Conrad JM, et al. Infectious diseases affect marine fisheries and aquaculture economics. *Annu Rev Mar Sci*. 2015;7:471-496. doi:10.1146/annurev-marine-010814-015646
- Soudant PE, Chu FL, Volety A. Host-parasite interactions: marine bivalve molluscs and protozoan parasites, *Perkinsus* species. *J Invertebr Pathol*. 2013;114:196-216. doi:10.1016/j.jip.2013.06.001
- Travers MA, Boettcher Miller K, Roque A, Friedman CS. Bacterial diseases in marine bivalves. *J Invertebr Pathol*. 2015;131:11-31. doi:10.1016/j.jip.2015.07.010
- Sánchez-Paz A. White spot syndrome virus: an overview on an emergent concern. *Vet Res*. 2010;41:43. doi:10.1051/vetres/2010015
- Pradeep B, Rai P, Mohan SA, Shekhar MS, Karunasagar I. Biology, host range, pathogenesis and diagnosis of white spot syndrome virus. *Indian J Virol*. 2012;23:161-174. doi:10.1007/s13337-012-0079-y
- Hong X, Lu L, Xu D. Progress in research on acute hepatopancreatic necrosis disease (AHPND). *Aquac Int*. 2016;24:577-593. doi:10.1007/s10499-015-9948-x
- Crosson LM, Friedman CS. Withering syndrome susceptibility of northeastern Pacific abalones: a complex relationship with phylogeny and thermal experience. *J Invertebr Pathol*. 2018;151:91-101. doi:10.1016/j.jip.2017.11.005
- Huang Z, Zeng S, Xiong J, et al. Microecological Koch's postulates reveal that intestinal microbiota dysbiosis contributes to shrimp white feces syndrome. *Microbiome*. 2020;8:32. doi:10.1186/s40168-020-00802-3
- Barbosa Solomieu V, Renault T, Travers MA. Mass mortality in bivalves and the intricate case of the Pacific oyster, *Crassostrea gigas*. *J Invertebr Pathol*. 2015;131:2-10. doi:10.1016/j.jip.2015.07.011
- Petton B, Destoumieux-Garzón D, Pernet F, et al. The Pacific oyster mortality syndrome, a polymicrobial and multifactorial disease: state of knowledge and future directions. *Front Immunol*. 2021;12. doi:10.3389/fimmu.2021.630343
- Petton B, Bruto M, James A, Labreuche Y, Alunno-Bruscia M, Le Roux F. *Crassostrea gigas* mortality in France: the usual suspect, a herpes virus, may not be the killer in this polymicrobial opportunistic disease. *Front Microbiol*. 2015;6. doi:10.3389/fmicb.2015.00686
- de Lorgeril J, Lucasson A, Petton B, et al. Immune-suppression by OsHV-1 viral infection causes fatal bacteraemia in Pacific oysters. *Nat Commun*. 2018;9:4215. doi:10.1038/s41467-018-06659-3
- King WL, Jenkins C, Go J, Siboni N, Seymour JR, Labbate M. Characterisation of the Pacific oyster microbiome during a summer mortality event. *Microb Ecol*. 2019;77:502-512. doi:10.1007/s00248-018-1226-9
- Lasa A, di Cesare A, Tassistro G, et al. Dynamics of the Pacific oyster pathobiota during mortality episodes in Europe assessed by 16S rRNA gene profiling and a new target enrichment next-generation sequencing strategy. *Environ Microbiol*. 2019;21:4548-4562. doi:10.1111/1462-2920.14750
- Green TJ, Siboni N, King WL, Labbate M, Seymour JR, Raftos D. Simulated marine heat wave alters abundance and structure of *Vibrio* populations associated with the Pacific oyster resulting in a mass mortality event. *Microb Ecol*. 2019;77:736-747. doi:10.1007/s00248-018-1242-9
- Lokmer A, Wegner KM. Hemolymph microbiome of Pacific oysters in response to temperature, temperature stress and infection. *ISME J*. 2015;9:670-682. doi:10.1038/ismej.2014.160
- Li YF, Chen YW, Xu JK, et al. Temperature elevation and *Vibrio cyclitrophicus* infection reduce the diversity of haemolymph microbiome of the mussel *Mytilus coruscus*. *Sci Rep*. 2019;9:16391. doi:10.1038/s41598-019-52752-y
- Milan M, Smits M, Dalla Rovere G, et al. Host-microbiota interactions shed light on mortality events in the striped venus clam *Chamelea gallina*. *Mol Ecol*. 2019;28:4486-4499. doi:10.1111/mec.15227
- Liu H, Zha S, Yang Z, et al. Acute sulfide exposure induces hemocyte toxicity and microbiota dysbiosis in blood clam *Tegillarca granosa*. *Aquat Toxicol*. 2022;249:106224. doi:10.1016/j.aquatox.2022.106224
- Pérez-Sánchez T, Mora-Sánchez B, Balcázar JL. Biological approaches for disease control in aquaculture: advantages, limitations and challenges. *Trends Microbiol*. 2018;26:896-903. doi:10.1016/j.tim.2018.05.002
- Goh JXH, Tan LTH, Law JWF, et al. Harnessing the potentialities of probiotics, prebiotics, synbiotics, paraprobiotics, and postbiotics for shrimp farming. *Rev Aquac*. 2022;00:1-80. doi:10.1111/raq.12659
- Kiymaci ME, Altanlar N, Gumustas M, Ozkan SA, Akin A. Quorum sensing signals and related virulence inhibition of *Pseudomonas aeruginosa* by a potential probiotic strain's organic acid. *Microb Pathog*. 2018;121:190-197. doi:10.1016/j.micpath.2018.05.042
- Offret C, Rochard V, Laguerre H, et al. Protective efficacy of a *Pseudoalteromonas* strain in European abalone, *Haliotis tuberculata*,

- infected with *Vibrio harveyi* ORM4. *Probiotics Antimicrob Proteins*. 2018;11:239-247. doi:10.1007/s12602-018-9389-8
32. Touraki M, Karamanlidou G, Karavida P, Chrysi K. Evaluation of the probiotics *Bacillus subtilis* and *Lactobacillus plantarum* bioencapsulated in artemia nauplii against vibriosis in European sea bass larvae (*Dicentrarchus labrax*, L.). *World J Microbiol Biotechnol*. 2012;28:2425-2433. doi:10.1007/s11274-012-1052-z
 33. Zhao W, Yuan T, Piva C, et al. The probiotic bacterium *Phaeobacter inhibens* downregulates virulence factor transcription in the shellfish pathogen *Vibrio corallilyticus* by N-acyl homoserine lactone production. *Appl Environ Microbiol*. 2019;85:1-14. doi:10.1128/AEM.01545-18
 34. Khanjani MH, Mohammadi A, Emerenciano MGC. Microorganisms in biofloc aquaculture system. *Aquac Reports*. 2022;26:101300. doi:10.1016/j.aqrep.2022.101300
 35. Kumar V, Roy S, Behera BK, Swain HS, Das BK. Biofloc microbiome with bioremediation and health benefits. *Front Microbiol*. 2021;12. doi:10.3389/fmicb.2021.741164
 36. Rajeev R, Adithya KK, Kiran GS, Selvin J. Healthy microbiome: a key to successful and sustainable shrimp aquaculture. *Rev Aquac*. 2021;13:238-258. doi:10.1111/raq.12471
 37. Renz H, Holt PG, Inouye M, Logan AC, Prescott SL, Sly PD. An exposure perspective: Early-life events and immune development in a changing world. *Journal of Allergy and Clinical Immunology*, 2017;140(1):24-40. <https://doi.org/10.1016/j.jaci.2017.05.015>
 38. Khanjani MH, Sharifinia M, Emerenciano MGC. A detailed look at the impacts of biofloc on immunological and hematological parameters and improving resistance to diseases. *Fish Shellfish Immunol*. 2023;137:1050-4648. doi:10.1016/j.fsi.2023.108796
 39. Bossier P, De Schrijver P, Defoirdt T, et al. Microbial community management in aquaculture. *Procedia Food Sci*. 2016;6:37-39. doi:10.1016/j.profoo.2016.02.007
 40. De Schryver P, Defoirdt T, Sorgeloos P. Early mortality syndrome outbreaks: a microbial management issue in shrimp farming? *PLoS Pathog*. 2014;10:e1003919. doi:10.1371/journal.ppat.1003919
 41. Vadstein O, Attramadal KJK, Bakke I, Olsen Y. K-selection as microbial community management strategy: a method for improved viability of larvae in aquaculture. *Front Microbiol*. 2018;9. doi:10.3389/fmicb.2018.02730
 42. Arrieta MC, Stiemsma LT, Amenyogbe N, Brown E, Finlay B. The intestinal microbiome in early life: health and disease. *Front Immunol*. 2014;5. doi:10.3389/fimmu.2014.00427
 43. Gensollen T, Iyer SS, Kasper DL, Blumberg RS. How colonization by microbiota in early life shapes the immune system. *Science*. 2016;352(6175):539-544. doi:10.1126/science.1250037
 44. Galindo-Villegas J, García-Moreno D, De Oliveira S, Meseguer J, Mulero V. Regulation of immunity and disease resistance by commensal microbes and chromatin modifications during zebrafish development. *Proc Natl Acad Sci USA*. 2012;109(39). doi:10.1073/pnas.1209920109
 45. Roy S, Bossier P, Norouzitallab P, Vanrompay D. Trained immunity and perspectives for shrimp aquaculture. *Rev Aquac*. 2020;12:2351-2370. doi:10.1111/raq.12438
 46. Ziaei-Nejad S, Rezaei MH, Takami GA, Lovett DL, Mirvaghefi AR, Shakouri M. The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*. *Aquaculture*. 2006;252:516-524. doi:10.1016/j.aquaculture.2005.07.021
 47. Fallet M, Montagnani C, Petton B, et al. Early life microbial exposures shape the *Crassostrea gigas* immune system for lifelong and intergenerational disease protection. *Microbiome*. 2022;10:85. doi:10.1186/s40168-022-01280-5
 48. Yang MJ, Song H, Yu ZL, et al. Changes in symbiotic microbiota and immune responses in early development stages of *Rapana venosa* (Valenciennes, 1846) provide insights into immune system development in gastropods. *Front Microbiol*. 2020;11:1265. doi:10.3389/fmicb.2020.01265
 49. Cordier C, Voulgaris A, Stavrakakis C, Sauvade P, Coelho F, Moulin P. Ultrafiltration for environmental safety in shellfish production: a case of bloom emergence. *Water Sci Eng*. 2021;14:46-53. doi:10.1016/j.wse.2021.03.003
 50. Eljaddi T, Ragueneau S, Cordier C, et al. Ultrafiltration to secure shellfish industrial activities: culture of microalgae and oyster fertilization. *Aquac Eng*. 2021;95:102204. doi:10.1016/j.aquaceng.2021.102204
 51. Gilbert SF, Bosch TCG, Ledón-Rettig C. Eco-evo-devo: developmental symbiosis and developmental plasticity as evolutionary agents. *Nat Rev Genet*. 2015;16:611-622. doi:10.1038/nrg3982
 52. Gould AL, Zhang V, Lamberti L, et al. Microbiome interactions shape host fitness. *Proc Natl Acad Sci USA*. 2018;115:E11951-E11960. doi:10.1073/pnas.1809349115
 53. Heijtz RD, Wang S, Anuar F, et al. Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci USA*. 2011;108:3047-3052. doi:10.1073/pnas.1010529108
 54. Kohl KD, Yahn J. Effects of environmental temperature on the gut microbial communities of tadpoles. *Environ Microbiol*. 2016;18:1561-1565. doi:10.1111/1462-2920.13255
 55. Kostic AD, Howitt MR, Garrett WS. Exploring host-microbiota interactions in animal models and humans. *Genes Dev*. 2013;27:701-718. doi:10.1101/gad.212522.112
 56. McFall-Ngai M, Hadfield MG, Bosch TCG, et al. Animals in a bacterial world, a new imperative for the life sciences. *Proc Natl Acad Sci USA*. 2013;110:3229-3236. doi:10.1073/pnas.1218525110
 57. Schmidt VT, Smith KF, Melvin DW, Amaral-Zettler LA. Community assembly of a euryhaline fish microbiome during salinity acclimation. *Mol Ecol*. 2015;24:2537-2550. doi:10.1111/mec.13177
 58. Sharon G, Segal D, Ringo JM, Hefetz A, Zilber-Rosenberg I, Rosenberg E. Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *Proc Natl Acad Sci USA*. 2010;107:20051-20056. doi:10.1073/pnas.1009906107
 59. Webster NS, Reusch TB. Microbial contributions to the persistence of coral reefs. *ISME J*. 2017;11:2167-2174. doi:10.1038/ismej.2017.66
 60. Webster NS, Botté ES, Soo RM, Whalan S. The larval sponge holobiont exhibits high thermal tolerance. *Environ Microbiol Rep*. 2011;3:756-762. doi:10.1111/j.1758-2229.2011.00296.x
 61. Rosenberg E, Sharon G, Zilber-Rosenberg I. The hologenome theory of evolution contains Lamarckian aspects within a Darwinian framework. *Environ Microbiol*. 2009;11:2959-2962. doi:10.1111/j.1462-2920.2009.01995.x
 62. Rohwer F, Seguritan V, Azam F, Knowlton N. Diversity and distribution of coral-associated bacteria. *Mar Ecol Prog Ser*. 2002;243:1-10. doi:10.3354/meps243001
 63. Bordenstein SR, Theis KR. Host biology in light of the microbiome: ten principles of holobionts and hologenomes. *PLoS Biol*. 2015;13:1-23. doi:10.1371/journal.pbio.1002226
 64. Theis KR, Dheilly NM, Klassen JL, et al. Getting the hologenome concept right: an eco-evolutionary framework for hosts and their microbiomes. *mSystems*. 2016. 1(2). doi:10.1128/msystems.00028-16
 65. Zilber-Rosenberg I, Rosenberg E. Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiol Rev*. 2008;32:723-735. doi:10.1111/j.1574-6976.2008.00123.x
 66. Laukens D, Brinkman BM, Raes J, De Vos M, Vandenabeele P. Heterogeneity of the gut microbiome in mice: guidelines for optimizing experimental design. *FEMS Microbiol Rev*. 2015;40:117-132. doi:10.1093/femsre/fuv036
 67. Abt MC, Artis D. The dynamic influence of commensal bacteria on the immune response to pathogens. *Curr Opin Microbiol*. 2013;16:4-9. doi:10.1016/j.mib.2012.12.002

68. Chung H, Pamp SJ, Hill JA, et al. Gut immune maturation depends on colonization with a host-specific microbiota. *Cell*. 2012;149:1578-1593. doi:10.1016/j.cell.2012.04.037
69. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science*. 2012;80(336):1268-1273. doi:10.1126/science.1223490
70. Sommer F, Bäckhed F. The gut microbiota-masters of host development and physiology. *Nat Rev Microbiol*. 2013;11:227-238. doi:10.1038/nrmicro2974
71. Schröder K, Bosch TCG. The origin of mucosal immunity: lessons from the holobiont hydra. *MBio*. 2016;7(6). doi:10.3389/fmicb.2022.852460
72. Infante-Villamil S, Huerlimann R, Jerry DR. Microbiome diversity and dysbiosis in aquaculture. *Rev Aquac*. 2021;13:1077-1096. doi:10.1111/raq.12513
73. Paillard C, Gueguen Y, Wegner KM, et al. Recent advances in bivalve-microbiota interactions for disease prevention in aquaculture. *Curr Opin Biotechnol*. 2022;73:225-232. doi:10.1016/j.copbio.2021.07.026
74. Yu W, Lu Y, Shen Y, et al. Exploring the intestinal microbiota and metabolome profiles associated with feed efficiency in Pacific abalone (*Haliotis discus hannai*). *Front Microbiol*. 2022;13. doi:10.3389/fmicb.2022.852460
75. Schleder DD, Blank M, Peruch LGB, et al. Impact of combinations of brown seaweeds on shrimp gut microbiota and response to thermal shock and white spot disease. *Aquaculture*. 2020;519:734779. doi:10.1016/j.aquaculture.2019.734779
76. Zhang S, Sun X. Core gut microbiota of shrimp function as a regulator to maintain immune homeostasis in response to WSSV infection. *Microbiol Spectr*. 2022;10:e0246521. doi:10.1128/spectrum.02465-21
77. Holt CC, van der Giezen M, Daniels CL, Stentiford GD, Bass D. Spatial and temporal axes impact ecology of the gut microbiome in juvenile European lobster (*Homarus gammarus*). *ISME J*. 2020;14:531-543. doi:10.1038/s41396-019-0546-1
78. Cicala F, Cisterna-Céliz JA, Moore JD, Rocha-Olivares A. Structure, dynamics and predicted functional role of the gut microbiota of the blue (*Haliotis fulgens*) and yellow (*H. corrugata*) abalone from Baja California sur, Mexico. *PeerJ*. 2018;6:e5830. doi:10.7717/peerj.5830
79. Cicala F, Cisterna-Céliz JA, Paolinelli M, Moore JD, Sevigny J, Rocha-Olivares A. The role of diversity in mediating microbiota structural and functional differences in two sympatric species of abalone under stressed withering syndrome conditions. *Microb Ecol*. 2022;1-11:277-287. doi:10.1007/s00248-022-01970-5
80. Defer D, Desriac F, Henry J, et al. Antimicrobial peptides in oyster hemolymph: the bacterial connection. *Fish Shellfish Immunol*. 2013;34:1439-1447. doi:10.1016/j.fsi.2013.03.357
81. Desriac F, El Harras A, Simon M, et al. Alterins produced by oyster-associated *Pseudoalteromonas* are antibacterial Cyclolipopeptides with LPS-binding activity. *Mar Drugs*. 2020;18:630. doi:10.3390/md18120630
82. Ooi MC, Goulden EF, Smith GG, Bridle AR. Predatory bacteria in the haemolymph of the cultured spiny lobster *Panulirus ornatus*. *Microbiol (United Kingdom)*. 2021;167:1113. doi:10.1099/MIC.0.001113
83. Luis-Villaseñor IE, Macías-Rodríguez ME, Gómez-Gil B, Ascencio-Valle F, Campa-Córdova ÁL. Beneficial effects of four *Bacillus* strains on the larval cultivation of *Litopenaeus vannamei*. *Aquaculture*. 2011;321:136-144. doi:10.1016/j.aquaculture.2011.08.036
84. Dantan L. *Caractérisation des effets bénéfiques de la microflore naturelle de l'huître du Pacifique *Crassostrea gigas* en vue d'applications en conchyliculture*. Université de Perpignan. 2023. <https://theses.hal.science/tel-0438280>
85. Madison D, Schubiger C, Lunda S, Mueller RS, Langdon C. A marine probiotic treatment against the bacterial pathogen *Vibrio coralliilyticus* to improve the performance of Pacific (*Crassostrea gigas*) and Kumamoto (*C. sikamea*) oyster larvae. *Aquaculture*. 2022;560:738611. doi:10.1016/j.aquaculture.2022.738611
86. Clerissi C, de Lorgeril J, Petton B, et al. Microbiota composition and evenness predict survival rate of oysters confronted to Pacific oyster mortality syndrome. *Front Microbiol*. 2020;11:1-11. doi:10.3389/fmicb.2020.00311
87. Lim SJ, Bordenstein SR. An introduction to phyllosymbiosis. *Proc R Soc B Biol Sci*. 2020;287:20192900. doi:10.1098/rspb.2019.2900
88. Dupont S, Lokmer A, Corre E, et al. Oyster hemolymph is a complex and dynamic ecosystem hosting bacteria, protists and viruses. *Anim Microbiome*. 2020;2:12. doi:10.1186/s42523-020-00032-w
89. King WL, Siboni N, Williams NLR, et al. Variability in the composition of pacific oyster microbiomes across oyster families exhibiting different levels of susceptibility to OsHV-1 μ var disease. *Front Microbiol*. 2019;10:1-12. doi:10.3389/fmicb.2019.00473
90. Broderick NA. Friend, foe or food? Recognition and the role of antimicrobial peptides in gut immunity and drosophila-microbe interactions. *Philos Trans R Soc B Biol Sci*. 2016;371:20150295. doi:10.1098/rstb.2015.0295
91. Chu H, Mazmanian SK. Innate immune recognition of the microbiota promotes host-microbial symbiosis. *Nat Immunol*. 2013;14:668-675. doi:10.1038/ni.2635
92. Dishaw LJ, Leigh B, Cannon JP, et al. Gut immunity in a protochordate involves a secreted immunoglobulin-type mediator binding host chitin and bacteria. *Nat Commun*. 2016;7(1). doi:10.1038/ncomms10617
93. Rosenstiel P, Philipp EER, Schreiber S, Bosch TCG. Evolution and function of innate immune receptors—insights from marine invertebrates. *J Innate Immun*. 2009;1:291-300. doi:10.1159/000211193
94. Heath-Heckman EAC, Foster J, Apicella MA, Goldman WE, McFall-Ngai M. Environmental cues and symbiont microbe-associated molecular patterns function in concert to drive the daily remodelling of the crypt-cell brush border of the *Euprymna scolopes* light organ. *Cell Microbiol*. 2016;18:1642-1652. doi:10.1111/cmi.12602
95. Augustin R, Schröder K, Rincón APM, et al. A secreted antibacterial neuropeptide shapes the microbiome of hydra. *Nat Commun*. 2017;8:1-8. doi:10.1038/s41467-017-00625-1
96. Fraune S, Bosch TCG. Long-term maintenance of species-specific bacterial microbiota in the basal metazoan Hydra. *Proc Natl Acad Sci USA*. 2007;104:13146-13151. doi:10.1073/pnas.0703375104
97. Auguste M, Lasa A, Pallavicini A, Gualdi S, Vezzulli L, Canesi L. Exposure to TiO₂ nanoparticles induces shifts in the microbiota composition of *Mytilus galloprovincialis* hemolymph. *Sci Total Environ*. 2019;670:129-137. doi:10.1016/j.scitotenv.2019.03.133
98. Auguste M, Lasa A, Balbi T, Pallavicini A, Vezzulli L, Canesi L. Impact of nanoplastics on hemolymph immune parameters and microbiota composition in *Mytilus galloprovincialis*. *Mar Environ Res*. 2020;159:105017. doi:10.1016/j.marenvres.2020.105017
99. Dang X, Huang Q, He YQ, Gaitán-Espitia JD, Zhang T, Thiagarajan V. Ocean acidification drives gut microbiome changes linked to species-specific immune defence. *Aquat Toxicol*. 2023;256:106413. doi:10.1016/j.aquatox.2023.106413
100. Li X, Yang B, Shi C, et al. Synergistic interaction of low salinity stress with *Vibrio* infection causes mass mortalities in the oyster by inducing host microflora imbalance and immune dysregulation. *Front Immunol*. 2022;13:1. doi:10.3389/fimmu.2022.859975
101. Zhao J, Zhao B, Kong N, et al. Increased abundances of potential pathogenic bacteria and expressions of inflammatory cytokines in the intestine of oyster *Crassostrea gigas* after high temperature stress. *Dev Comp Immunol*. 2023;141:104630. doi:10.1016/j.dci.2022.104630
102. Wang XW, Xu JD, Zhao XF, Vasta GR, Wang JX. A shrimp C-type lectin inhibits proliferation of the hemolymph microbiota by maintaining the expression of antimicrobial peptides. *J Biol Chem*. 2014;289:11779-11790. doi:10.1074/jbc.M114.552307
103. Yang HT, Yang MC, Sun JJ, et al. Catalase eliminates reactive oxygen species and influences the intestinal microbiota of shrimp. *Fish Shellfish Immunol*. 2015;47:63-73. doi:10.1016/j.fsi.2015.08.021

104. Hong PP, Zhu XX, Yuan WJ, Niu GJ, Wang JX. Nitric oxide synthase regulates gut microbiota homeostasis by ERK-NF- κ B pathway in shrimp. *Front Immunol.* 2021;12. doi:10.3389/fimmu.2021.778098
105. Lv X, Li S, Yu Y, Zhang X, Li F. Characterization of a gill-abundant crustin with microbiota modulating function in *Litopenaeus vannamei*. *Fish Shellfish Immunol.* 2020;105:393-404. doi:10.1016/j.fsi.2020.07.014
106. Lv X, Li S, Yu Y, Xiang J, Li F. The immune function of a novel crustin with an atypical WAP domain in regulating intestinal microbiota homeostasis in *Litopenaeus vannamei*. *Dev Comp Immunol.* 2020;111:103756. doi:10.1016/j.dci.2020.103756
107. González R, Gonçalves AT, Rojas R, Brokordt K, Rosa RD, Schmitt P. Host defense effectors expressed by hemocytes shape the bacterial microbiota from the scallop Hemolymph. *Front Immunol.* 2020;11:1. doi:10.3389/fimmu.2020.599625
108. González R, González D, Stambuk F, et al. A g-type lysozyme from the scallop *Argopecten purpuratus* participates in the immune response and in the stability of the hemolymph microbiota. *Fish Shellfish Immunol.* 2022;123:324-334. doi:10.1016/j.fsi.2022.03.015
109. de San Nicolas N, Asokan A, Rosa RD, et al. Functional diversification of oyster big defensins generates antimicrobial specificity and synergy against members of the microbiota. *Mar Drugs.* 2022;2022:745. doi:10.3390/md20120745
110. Muñoz K, Flores-Herrera P, Gonçalves AT, et al. The immune response of the scallop *Argopecten purpuratus* is associated with changes in the host microbiota structure and diversity. *Fish Shellfish Immunol.* 2019;91:241-250. doi:10.1016/j.fsi.2019.05.028
111. Food and Agriculture Organisation of the United Nation. Probiotics in food. Health and nutritional properties and guidelines for evaluation. Rome/Roma. 2006.
112. Mousavi Khaneghah A, Abhari K, Eş I, et al. Interactions between probiotics and pathogenic microorganisms in hosts and foods: a review. *Trends Food Sci Technol.* 2020;95:205-218. doi:10.1016/j.tifs.2019.11.022
113. Sorée M, Kolypczuk L, Hadjiev E, et al. Screening of marine lactic acid bacteria for *Vibrio parahaemolyticus* inhibition and application to depuration in Pacific oysters (*Crassostrea gigas*). *J Appl Microbiol.* 2023;134(2). doi:10.1093/jambio/ixac081
114. Getachew T. A review on effects of probiotic supplementation in poultry performance and cholesterol levels of egg and meat. *Getachew T J World Poult Res J Homepage J World Poult Res.* 2016;6:31-36.
115. Thornber K, Verner-Jeffreys D, Hinchliffe S, Rahman MM, Bass D, Tyler CR. Evaluating antimicrobial resistance in the global shrimp industry. *Rev Aquac.* 2020;12:966-986. doi:10.1111/raq.12367
116. Akhter N, Wu B, Memon AM, Mohsin M. Probiotics and prebiotics associated with aquaculture: a review. *Fish Shellfish Immunol.* 2015;45:733-741. doi:10.1016/j.fsi.2015.05.038
117. Aguilar-Macias OL, Ojeda-Ramirez JJ, Campa-Córdova AI, Saucedo PE. Evaluation of natural and commercial probiotics for improving growth and survival of the pearl oyster, *Pinctada mazatlanica*, during Late hatchery and early field culturing. *J World Aquacult Soc.* 2010;41:447-454. doi:10.1111/j.1749-7345.2010.00386.x
118. García-Bernal M, Medina-Marrero R, Campa-Córdova AI, Mazón-Suástegui JM. Growth and antioxidant response of juvenile oysters *Crassostrea sikamea* and *Crassostrea corteziensis* treated with *Streptomyces* strains. *Arq Bras Med Vet e Zootec.* 2019;71:1993-1998. doi:10.1590/1678-4162-11225
119. Kesarcodi-Watson A, Miner P, Nicolas JL, Robert R. Protective effect of four potential probiotics against pathogen-challenge of the larvae of three bivalves: Pacific oyster (*Crassostrea gigas*), flat oyster (*Ostrea edulis*) and scallop (*Pecten maximus*). *Aquaculture.* 2012;344-349:29-34. doi:10.1016/j.aquaculture.2012.02.029
120. Modak TH, Gomez-Chiari M. Contrasting immunomodulatory effects of probiotic and pathogenic bacteria on eastern oyster, *Crassostrea virginica*, larvae. *Vaccine.* 2020;8:1-23. doi:10.3390/vaccines8040588
121. Sumon TA, Hussain MA, Sumon MAA, et al. Functionality and prophylactic role of probiotics in shellfish aquaculture. *Aquac Reports.* 2022;25:101220. doi:10.1016/j.aqrep.2022.101220
122. Yeh H, Skubel SA, Patel H, Cai Shi D, Bushek D, Chikindas ML. From farm to fingers: an exploration of probiotics for oysters, from production to human consumption. *Probiotics Antimicrob Proteins.* 2020;12:351-364. doi:10.1007/s12602-019-09629-3
123. Gibson LF, Woodworth J, George AM. Probiotic activity of *Aeromonas media* on the Pacific oyster, *Crassostrea gigas*, when challenged with *Vibrio tubiashii*. *Aquaculture.* 1998;169:111-120. doi:10.1016/S0044-8486(98)00369-X
124. Liu KF, Chiu CH, Shiu YL, Cheng W, Liu CH. Effects of the probiotic, *Bacillus subtilis* E20, on the survival, development, stress tolerance, and immune status of white shrimp, *Litopenaeus vannamei* larvae. *Fish Shellfish Immunol.* 2010;28:837-844. doi:10.1016/j.fsi.2010.01.012
125. Pham D, Ansquer D, Chevalier A, et al. Selection and characterization of potential probiotic bacteria for *Litopenaeus stylirostris* shrimp hatcheries in New Caledonia. *Aquaculture.* 2014;432:475-482. doi:10.1016/j.aquaculture.2014.04.031
126. Karim M, Zhao W, Rowley D, Nelson D, Gomez-Chiari M. Probiotic strains for shellfish aquaculture: protection of eastern oyster, *Crassostrea virginica*, larvae and juveniles against bacterial challenge. *J Shellfish Res.* 2013;32:401-408. doi:10.2983/035.032.0220
127. Campa-Córdova AI, Luna-González A, Mazón-Suástegui JM, Aguirre-Guzmán G, Ascencio F, González-Ocampo HA. Efecto de bacterias probióticas en el cultivo larvario del ostión de placer *Crassostrea corteziensis* (Bivalvia: Ostreidae). *Rev Biol Trop.* 2011;59:183-191. doi:10.15517/rbt.v59i1.3188
128. Ma YX, Liu JC, Li M, Tao W, Yu ZC, Bin LY. The use of *Pseudoalteromonas* sp. F15 in larviculture of the Yesso scallop, *Patinopecten yessoensis*. *Aquac Res.* 2019;50:1844-1850. doi:10.1111/are.14066
129. Chi C, Liu JY, Fei SZ, et al. Effect of intestinal autochthonous probiotics isolated from the gut of sea cucumber (*Apostichopus japonicus*) on immune response and growth of *A. japonicus*. *Fish Shellfish Immunol.* 2014;38:367-373. doi:10.1016/j.fsi.2014.04.001
130. Puvanasundram P, Chong CM, Sabri S, Yusoff MS, Karim M. Multi-strain probiotics: functions, effectiveness and formulations for aquaculture applications. *Aquac Reports.* 2021;21:100905. doi:10.1016/j.aqrep.2021.100905
131. Padeniya U, Larson ET, Septriani S, et al. Probiotic treatment enhances pre-feeding larval development and early survival in zebrafish *Danio rerio*. *J Aquat Anim Health.* 2022;34:3-11. doi:10.1002/aah.10148
132. Grandiosa R, Young T, Van Nguyen T, Mérien F, Alfaro AC. Immune response in probiotic-fed New Zealand black-footed abalone (*Haliotis iris*) under *Vibrio splendidus* challenge. *Fish Shellfish Immunol.* 2020;104:633-639. doi:10.1016/j.fsi.2020.06.007
133. Kesarcodi-Watson A, Kaspar H, Lategan MJ, Gibson L. Performance of single and multi-strain probiotics during hatchery production of Greenshell™ mussel larvae, *Perna canaliculus*. *Aquaculture.* 2012;354-355:56-63. doi:10.1016/j.aquaculture.2012.04.026
134. Wang YC, Hu SY, Chiu CS, Liu CH. Multiple-strain probiotics appear to be more effective in improving the growth performance and health status of white shrimp, *Litopenaeus vannamei*, than single probiotic strains. *Fish Shellfish Immunol.* 2019;84:1050-1058. doi:10.1016/j.fsi.2018.11.017
135. Goulden EF, Hall MR, Pereg LL, Høj L. Identification of an antagonistic probiotic combination protecting ornate spiny lobster (*Panulirus ornatus*) larvae against *vibrio owensii* infection. *PLoS One.* 2012;7:e39667. doi:10.1371/journal.pone.0039667
136. Holt CC, Bass D, Stentiford GD, van der Giezen M. Understanding the role of the shrimp gut microbiome in health and disease. *J Invertebr Pathol.* 2021;186:107387. doi:10.1016/j.jip.2020.107387

137. Zheng Y, Yu M, Liu J, et al. Bacterial community associated with healthy and diseased Pacific white shrimp (*Litopenaeus vannamei*) larvae and rearing water across different growth stages. *Front Microbiol.* 2017;8:1362. doi:10.3389/fmicb.2017.01362
138. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. *Genome Biol.* 2011;12:R60. doi:10.1186/gb-2011-12-6-r60
139. Zheng Y, Yu M, Liu Y, et al. Comparison of cultivable bacterial communities associated with Pacific white shrimp (*Litopenaeus vannamei*) larvae at different health statuses and growth stages. *Aquaculture.* 2016;451:163-169. doi:10.1016/j.aquaculture.2015.09.020
140. De Schryver P, Vadstein O. Ecological theory as a foundation to control pathogenic invasion in aquaculture. *ISME J.* 2014;8:2360-2368. doi:10.1038/ismej.2014.84
141. Aristizabal MJ, Anreiter I, Halldorsdottir T, et al. Biological embedding of experience: a primer on epigenetics. *Proc Natl Acad Sci USA.* 2020;117:23261-23269. doi:10.1073/pnas.1820838116
142. Dolinoy DC, Anderson OS, Rozek LS. Epigenetic manifestation of environmental exposures. *Nutrition in Epigenetics.* John Wiley & Sons, Ltd; 2011:287-307.
143. Fellous A, Wegner KM, John U, Mark FC, Shama LNS. Windows of opportunity: ocean warming shapes temperature-sensitive epigenetic reprogramming and gene expression across gametogenesis and embryogenesis in marine stickleback. *Glob Chang Biol.* 2022;28:54-71. doi:10.1111/gcb.15942
144. Netea MG, Domínguez-Andrés J, Barreiro LB, et al. Defining trained immunity and its role in health and disease. *Nat Rev Immunol.* 2020;20:375-388. doi:10.1038/s41577-020-0285-6
145. Zhang T, Qiu L, Sun Z, et al. The specifically enhanced cellular immune responses in Pacific oyster (*Crassostrea gigas*) against secondary challenge with *Vibrio splendidus*. *Dev Comp Immunol.* 2014;45:141-150. doi:10.1016/j.dci.2014.02.015
146. Norouzitallab P, Baruah K, Biswas P, Vanrompay D, Bossier P. Probing the phenomenon of trained immunity in invertebrates during a transgenerational study, using brine shrimp artemia as a model system. *Sci Rep.* 2016;6(1). doi:10.1038/srep21166
147. Lafont M, Petton B, Vergnes A, et al. Long-lasting antiviral innate immune priming in the Lophotrochozoan Pacific oyster, *Crassostrea gigas*. *Sci Rep.* 7(1), 2017. doi:10.1038/s41598-017-13564-0
148. Lafont M, Vergnes A, Vidal-Dupiol J, et al. A sustained immune response supports long-term antiviral immune priming in the pacific oyster, *Crassostrea gigas*. *MBio.* 11(2) 2020. doi:10.1128/mBio.02777-19
149. Eirin-Lopez JM, Putnam H. Marine environmental epigenetics. *In Annual Review of Marine Science.* Annual Reviews; 2019;11:335-368. doi:10.3389/fmars.2021.685075
150. Gavery MR, Roberts SB. Epigenetic considerations in aquaculture. *PeerJ.* 2017;2017:e4147. doi:10.7717/peerj.4147
151. Dégremont L, Morga B, Maurouard E, Travers MA. Susceptibility variation to the main pathogens of *Crassostrea gigas* at the larval, spat and juvenile stages using unselected and selected oysters to OsHV-1 and/or *V. aestuarianus*. *J Invertebr Pathol.* 2021;183:107601. doi:10.1016/j.jip.2021.107601
152. Dégremont L, Garcia C, Allen SK. Genetic improvement for disease resistance in oysters: a review. *J Invertebr Pathol.* 2015;131:226-241. doi:10.1016/j.jip.2015.05.010
153. Bourne N, Hodgson CA, Whyte JNC. A manual for scallop culture in British Columbia. *Canadian Technical Report of Fisheries and Aquatic Sciences No 1694;* 1989. Fisheries and Oceans Canada, Pacific Biological Station, Nanaimo, BC, Canada. 215 pp.
154. Helm MM, Bourne N, Lovatelli A. Hatchery culture of bivalves. A practical manual. *FAO Fish Tech Pap.* 2004;471:203.
155. Le Pennec M, Prieur D, Chardy P. Développement larvaire de *Mytilus edulis* (L) en présence d'antibiotiques. *Rev Int Ocean Med.* 1973;30:115-137.
156. Nicolas JL, Comps M, Cochennec N. Herpes-like virus infecting Pacific-oyster larvae, *Crassostrea gigas*. *Bull Eur Assoc Fish Pathol.* 1992;12:11-13. <https://eafp.org/download/1992-Volume12/Issue%201/3-Nicolas%20et%20al.pdf>
157. Martin YP, Mengus BM. Utilisation de souches bactériennes sélectionnées dans l'alimentation des larves de *Mytilus galloprovincialis* Imk (*Mollusque bivalve*) en élevages expérimentaux. *Aquaculture.* 1977;10:253-262.
158. Azéma P, Lamy JB, Boudry P, Renault T, Travers MA, Dégremont L. Genetic parameters of resistance to *Vibrio aestuarianus*, and OsHV-1 infections in the Pacific oyster, *Crassostrea gigas*, at three different life stages. *Genet Sel Evol.* 2017;49:1-16. doi:10.1186/s12711-017-0297-2

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