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REVIEW

REVIEWS IN Aquaculture

Trained immunity: Perspectives for disease control strategy in marine mollusc aquaculture

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Abstract

Recent evidence has demonstrated the unique properties of the innate immune system, known as innate immune memory, immune priming, or trained immunity. These properties have been described as the ability of the innate immune system to learn from previous microbial experiences, which improves survival after subsequent infection. In this review, we present the state of knowledge on trained immunity in invertebrates and provide a comprehensive overview of these capabilities in cultured marine molluscs, which are currently threatened by recurrent diseases. Studies have shown that exposure to environmental microbiota, pathogens, or derived elements, can provide a stronger response and protection against future infections. These studies highlight common and distinct features of protection, mechanisms, specificity, and duration that vary with immune markers, and methods of stimulation. While the cellular and molecular basis of these responses is only partially understood, effects on phagocytosis, haemocyte populations, apoptosis, oxidative stress, and immune gene expression have been suggested. Finally, we propose a framework for future research to go beyond the current evidence and address potential limitations in the implementation of trained immunity-based strategies to control disease. Immune training may provide a unique opportunity to promote the sustainable development of marine mollusc aquaculture.

KEYWORDS

abalone, clam, innate immunity, marine mollusc, memory, mussel, oyster, priming, trained immunity

1 | INTRODUCTION

Molluscs are one of the most diverse and abundant animal groups in terms of species and biomass.[1] Marine molluscs, in particular, contribute substantially to aquaculture production, with bivalves accounting for ~20% of aquatic animal production by weight.[2,3] Aquaculture is a rapidly growing food production sector and a crucial source of animal protein for human consumption. According to the Food and Agriculture Organization of the United Nations (FAO), worldwide mollusc aquaculture production has increased by ~70 million tonnes over the past three decades.[3] Due to the socio-economic significance of these species, a considerable amount of research has focused on infectious diseases that can occur at different life stages and have repeatedly affected production.[2,4,5] Even more concerning is the increase in the frequency and severity of marine diseases affecting wild and farmed marine species, in association with global changes and anthropogenic disturbances. This represents a major limitation for the sustainability of aquaculture.[6,7] Marine molluscs have been the subject of basic and applied research on ecological issues, and physiological processes (reproduction, growth, metabolism, and immunity) involved in disease mitigation. Research efforts combined with the acquisition of important genomic datasets have significantly enhanced our knowledge of the molecular basis of molluscan immunity, thus providing new opportunities to improve our understanding of mollusc response and resistance to disease.[8–17]

Molluscs exhibit a highly conserved innate immune system, which they use to interact with microorganisms (see Ref. 8, for review). Haemocytes (haemolymph circulating and infiltrating cells) are the main immune response mediators at the cellular level, but epithelial cells have also been implicated in response to pathogens.[18,19] Mollusc genomes contain a wide range of cellular and cytoplasmic recognition molecules and receptors that detect microbe-associated or danger-associated molecular patterns. Some of these recognition receptors (e.g., TLR, RIG-like Receptor (RLR), SR, NLR, and Integrins) and soluble proteins (e.g., FREPs, LBP/BPI, PGRP, GNBP, C1q, lectins) are highly polymorphic or diversified in molluscs and invertebrates.[20] They have been suggested to support specificity in the innate immune response and microbe recognition.[20] Upon recognition, several mechanisms can be induced to eliminate pathogens in the circulating fluids (haemolymph) and tissues or inside immune-competent cells. In haemolymph, proteolytic cascades are conserved in molluscs and primitive prophenoloxidase and complement systems have been found.[21,22] In immune cells, the recognition of pathogens can lead to aggregation, ETosis, and phagocytosis or endocytosis. Pathogens are then engulfed and destroyed by lysosomal enzymes, cytotoxic/cytolytic compounds, and oxidative burst, which is supported by the synthesis of reactive oxygen or nitrogen species (ROS/RNS).[23–25] Upon activation, haemocytes can also trigger signalling pathways (Toll/NF-kB, Interferon (IFN)-like, RLR-STING, Tumor Necrosis Factor pathways, etc.) and well-known cell-autonomous defence mechanisms (such as autophagy or apoptosis) that share striking similarities with pathways of the vertebrate innate immune system.[8,26,27] Antimicrobial activities are mediated by a variety of well-conserved effectors from ROS, RNS, proteases, and antimicrobial peptides (AMPs) and proteins.[28–30] Although the accumulation of genomic data raises many questions, particularly regarding functional conservation and interaction between signalling pathways components, some of these complex mechanisms have been linked to antibacterial and antiviral responses.[31–33]

Recent research has revealed original immune mechanisms in molluscs, in addition to their potent defence systems. The immune system of invertebrates relies on innate mechanisms and has long been thought to lack adaptive mechanisms, unlike the adaptive or acquired immune system of vertebrates, which depends on antibody specificity and T-/B-cell receptor-mediated memory. However, studies on invertebrate and vertebrate species over the last two decades have supported the existence of antigen-independent immunological memory. This demonstrates that the innate immune system can adapt following microbial challenge.[34–36] To avoid any mechanism-based confusion with the vertebrate antibody-dependent adaptive immune system, these immunological memory responses have been called 'immune priming', 'trained immunity', or 'innate immune memory'. Although there is no consensual definition of these phenomena, innate immune memory has been described as the ability of the innate immune system to store or reuse information from a previously encountered non-self-antigen or pathogen, resulting in a more robust response that improves survival upon subsequent exposure to the same or an unrelated pathogen.[36,37] These immunological memory traits, conserved throughout evolution, could provide a survival advantage and greater protection against pathogen infection.[36]

These characteristics have important implications for implementing innovative and sustainable ways to mitigate recurrent diseases in cultured marine molluscs. Currently, there are very few prophylactic or therapeutic treatments to address diseases in marine mollusc aquaculture. Marine molluscs are typically farmed in open environments, which limit the use of antibiotics to larval stages and broodstock conditioning in hatcheries due to feasibility issues and the risk of promoting antimicrobial resistance. Prophylactic treatments, such as probiotics, are also very limited for molluscs. Probiotics have been successfully tested in laboratory settings, demonstrating the potential to improve health and animal depuration in certain species.[38] However, the literature reports several limitations, including inhibitory effects on development.[39] Biosecurity solutions have also been explored to eliminate viral or bacterial pathogens.[40] but these seawater treatments are only suitable for closed hatchery. Young animals are still vulnerable when transferred and cultured in the open sea. Genetic selection is currently the primary strategy being developed to enhance resistance to pathogens.[41–43] These solutions could potentially impair animal genetic diversity, with potential trade-offs which could compromise their resilience to future diseases. For species like oysters, current cultural practices involve immersing larger quantities of oysters in the environment to compensate for losses due to massive mortalities. It is important to note that leaving extensive quantities of dead and sick oysters to decay in farms can have negative consequences on the environment. Studies have shown that it alters the flux of dissolved materials, affects the structure of the planktonic communities and enriches the surrounding seawater with pathogens

(OsHV-1 virus) and opportunistic bacteria, which could facilitate the spread of disease and potentially harm marine biodiversity.[44,45] Overall, current approaches seem inadequate for effectively controlling the emergence or re-occurrence of diseases.[41] They may also be aggravating factors and a major impediment to ensuring sustainable aquaculture development, which requires innovative ways to mitigate these diseases.

In this context, enhancing immune capacities through trained immunity seems like an attractive alternative strategy to prevent disease outbreaks and improve marine mollusc health. To address the application potential of trained immunity, we present in this review an outlook of trained immunity evidence brought forward in invertebrates, describing the main characteristics and mechanisms. We review the latest advances in trained immunity capacities found in marine molluscs of economic interest. Finally, we propose a framework for future research to assess the feasibility of implementing trained immunity for disease control in aquaculture.

2 | TRAINED IMMUNITY IN INVERTEBRATES

The evidence for enhanced protection against either macroparasitic,[46] bacterial, fungal,[47] and viral infections[48–51] has been documented in vertebrates (mammals, Teleostea)[37,52,53] and invertebrate phyla, from Cnidaria, Lophotrochozoa (Spiralia, mostly molluscs), but mostly in Ecdyzoa (Decapoda, Branchiopoda, Lepidoptera, Coleoptera, Diptera, and Hymenoptera).[46,54–59] Studies on trained immunity in invertebrates are quite heterogeneous and largely differ in terms of experimental design, host–pathogen combinations, physiological status of the host (age, developmental stages, sex), elicitors used for training (live vs. inactivated pathogens, non-infectious agents, or synthetic component), dose of stimulus applied (acute infection, repetitive exposition, addition of adjuvants), route of priming (oral, mucosal vs. injection), the degree of demonstrated specificity of the trained response (broad vs. specific), and duration (time between first and second encounter, within and across generations).[35] This improved protection has been observed in different contexts. It can occur within the same developmental stage (within-generation), across life stages (ontogenic), or across generations, also called transgenerational immune priming (TGIP). This prepares the offspring for potential future infectious environments. TGIP has been shown to increase the survival capacities of insects and crustaceans, sometimes over several generations, making it a beneficial survival strategy.[35,60,61] The time between training and challenge can extend over weeks and even the lifetime of the organism (from the larval stage to adulthood). In addition, environmental stressors such as heat or physical stress can affect the immune response and train immunity in invertebrates, leading to increased survival.[62–70] It is worth noting that non-lethal heat shock impacts have been studied in several invertebrate species, revealing a potential role of heat shock proteins (hsp) in enhancing resistance to pathogens.[65,71–75] These proteins may modulate pathogen-associated molecular pattern-induced immune receptor signalling or send endogenous 'danger signals' to the immune system. For instance, they have been used in fish vaccines.[76,77]

Despite an increasing number of reports are shedding light on these phenomena, the biological mechanisms underlying trained immunity are still poorly understood for most invertebrate species. Theoretical models of response have been proposed.[34,36,78–81] Molecular evidence from various studies supports these mechanistic models, demonstrating the potential diversity of response even within the same phylum (Figure 1). They have demonstrated the existence of a biphasic response, called the 'recall response', which is characteristic of vertebrate immune memory. This involves stimulation of the immune response following a primary exposure, followed by an extinction phase and either a similar or stronger and faster secondary response to a subsequent infection (Figure 1). This type of response appears to be very rare in invertebrates, with only a few examples observed in mosquitoes primed with *Plasmodium berghei* or dengue virus.[82,83] Another type of response, known as immune shift, has been shown to exhibit qualitatively distinct primary and secondary responses.[84,85] Immune shift was first observed in the freshwater snail *Biomphalaria glabrata*.[46] It is mediated by snail-soluble immune factors that lead to the degeneration and death of the *Schistosoma mansoni* parasite, following a shift from cellular to humoral response upon secondary infection. Finally, a sustained response was observed. It is activated upon primary exposure, with no extinction phase, and is maintained until the secondary infection.[86–90] The latter response, sometimes dismissed as true memory and compared with immune enhancement,[54] seems to be highly represented in invertebrates.[78] It can lead to increased resistance and potentially transmit trained immunity across generations. In some cases, this response is associated with a gene expression shift called gene frontloading, which is characterized by constitutive changes in gene baseline expression.[88,91] While frontloading has mainly been demonstrated in the context of environmental training and stress response, it has been described as an adaptive mechanism to cope with environmental changes and to drive phenotypic modifications, enhancing robustness in cnidarians and molluscs.[92–95] Interestingly, these mechanisms have been suggested to be supported by epigenetic mechanisms.[88,96]

Many studies in vertebrates, invertebrates, and plants indicate that long-term epigenetic and metabolic reprogramming of the innate immune cells plays a crucial role in the remarkable persistence of immune training. Therefore, these mechanisms emerge as a common denominator of immune training across species.[49,53,54,97] Epigenetic developmental plasticity enables a complex organism to adapt to micro-environmental signals, particularly during early life, thereby increasing its fitness.[98,99] Trained immunity involves epigenetic modifications caused by metabolic reprogramming of innate immune cells (e.g., changes in glycolysis, glutaminolysis, tricarboxylic acid cycle, mevalonate, fumarate, itaconate, and lipid metabolism), since metabolic intermediates (e.g., itaconate, fumarate, and succinate) can act as substrates, cofactors, or inhibitors for chromatin-modifying enzymes.[100–102] These modifications are thought to occur primarily through DNA methylation, histone modification and/or non-coding RNA, which have been shown to alter the expression of genes

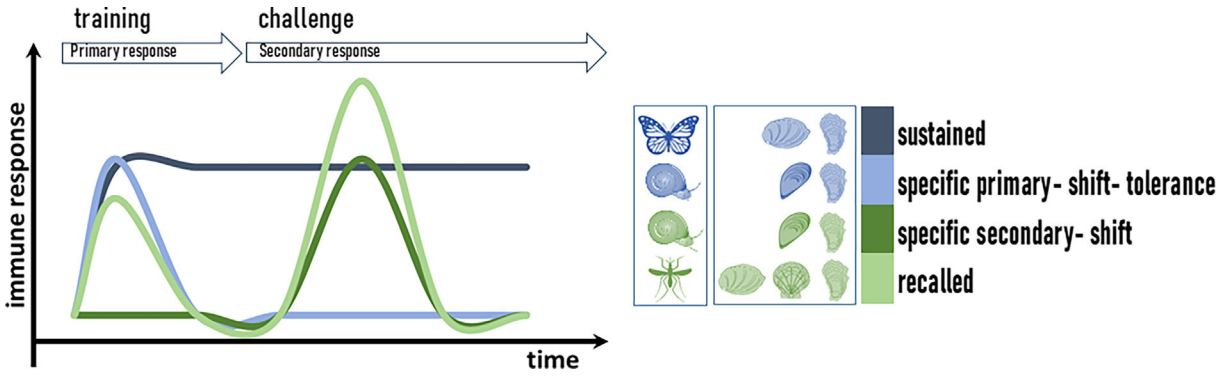

FIGURE 1 Comparative trained immunity response model in invertebrate and aquaculture molluscs. The graph illustrates the diversity of training responses observed in invertebrates and marine molluscs. Immune response over time after training induction (primary response) and challenge (secondary response) is shown. The different response modes described in the literature are indicated by curves in different colours. The legend indicates the species where the different patterns have been observed: a sustained response induced upon training with no extinction phase, that is maintained up to the secondary response (dark blue line); an immune shift displaying qualitatively distinct primary and secondary responses, involving distinct sets of genes (light blue and dark green lines); a tolerance response with a primary response but no secondary response (light blue line). A biphasic response, named recall response with a primary response followed by an extinction phase and either a similar or stronger and faster secondary response to a subsequent challenge (light green line).

encoding key players in the epigenetic regulation machinery of immune gene expression. These modifications can affect the phenotype over time, even without the initial inductive stimulus. In addition, epigenetic inheritance is gaining ground attention as a key mechanism of transgenerational plasticity and an important mediator of genome–microbiome interactions in marine organisms exposed to environmental stress.[103–107] The involvement of epigenetic mechanisms in the transgenerational transmission of trained immunity has also been demonstrated in arthropods.[108–111]

The debate continues on whether specificity is a hallmark of a trained response since trained immunity in vertebrates seems to be less specific than antibody-driven acquired immunity.[53] In humans, trained immunity can cause off-target effects of vaccines, inducing an innate immune response against unrelated pathogens and providing heterologous protection.[112,113] In invertebrates, a wide range of responses have been observed, from highly specific responses that elicit stronger memory when facing closely genetically related bacteria or parasites repeatedly, to cross-protection.[46,56,58,60,114–117] Studies suggested the implication of several classes of multigene families of immune receptors that have the potential for somatic diversification (DSCAMs for Down syndrome cell adhesion molecule, FREPs for fibrinogen-related proteins).[118] These receptors may play a role in enforcing a specific trained response based on their diversity and potential synergistic interactions, as well as mediating an increased cellular response through phagocytosis or haematopoietic proliferation.[119] The transgenerational response can either be specific to the pathogen that induced the training, or non-specific, resulting in more robust offspring that are more resistant to various pathogens (cross-immunity).[61]

Furthermore, immune training has been shown to enhance various immune mechanisms that eliminate pathogens, such as immune cell proliferation and haematopoiesis, phagocytosis, apoptosis, or ROS production.[57,114,120] Trained responses have also been linked to other immune effectors (e.g., AMPs) and stress proteins (hsp). However, further investigation is needed to determine the exact role of these factors.[63,121–124] Like plants, invertebrates can use RNA interference to provide transgenerational protection against viruses.[50,125,126] Studies have suggested that transgenerational protection could also be transmitted through pathogen-derived AMPs or mRNA-encoding immune effectors.[127,128]

Taken together, the accumulating evidence for trained immunity suggests that immunological memory may be a universal feature of all living organisms, from bacteria (CRISPR-Cas system) to humans, with significant implications for both health and disease in invertebrates. However, the available data are incomplete, and a comprehensive overview of this phenomenon across different phyla is necessary. This includes information on duration, specificity, efficiency in a natural context, and the underlying molecular and cellular mechanisms.

3 | TRAINED IMMUNITY IN AQUACULTURE MOLLUSCS

Most cultured molluscan species are bivalves[129] while only a few gastropods are exploited in aquaculture. Accumulating experimental evidence has demonstrated most species possess immune training capacities (Figure 2).

3.1 | Abalones

The most commonly cultured marine gastropod species is the abalone, which is prone to recurrent bacterial and viral infections.[130–132] Evidence of immune training capacities has been found in the European abalone *Haliotis tuberculata (Linnaeus, 1758)*,[133] the New Zealand *Haliotis iris (Gmelin, 1791)*,[134–136] *Haliotis diversicolor (Reeve, 1846)*,[137]

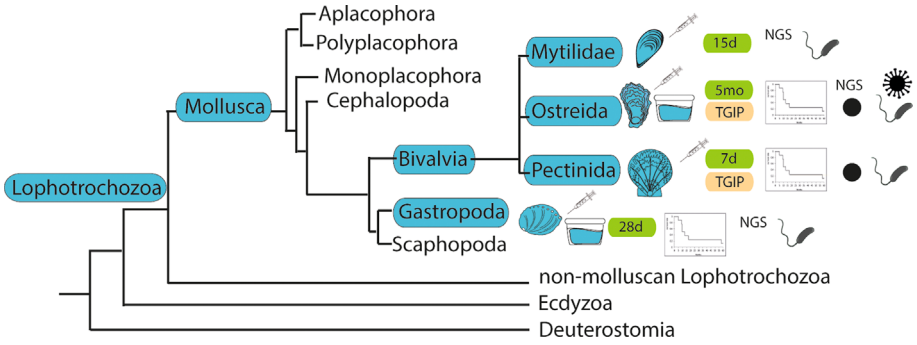

FIGURE 2 Trained immunity evidence in marine molluscs. Illustration of the current knowledge on trained immunity in marine molluscs (marked with blue colour, phylogenetic tree based on Davison and Neiman[217]). The methods used to evidence trained immunity are indicated (injection by a syringe and bath treatment by a tank), as well as the pathogen used (bacteria or virus), as well as the longest training duration observed ('d' for days and 'mo' for months) and when transgenerational immune priming (TGIP) has been observed. A Kaplan–Meier graph indicates when a natural pathogen has been used to induce training and shown enhanced survival capacities observed. 'NGS' indicates when global has been used to explore underlying mechanisms. A dot indicates when specific response has been observed (adapted from Milutinovic et al.[35]).

or *Haliotis discus (Reeve, 1846)*[138] (Table 1). In *H. iris*, several studies have shown that exposure to probiotic-enriched diets coming from bacterial strains isolated from the gastrointestinal tract of healthy abalones could improve immunity, growth, and survival, sometimes several months after exposition.[134–136] This treatment modified some haemocyte-related immune parameters (increased total haemocyte counts and ROS production) as well as the relative abundance of several metabolites that were interpreted as immune training bioindicators.[134] In *H. tuberculata*, the effects of two consecutive infections with a live Gram-negative pathogenic bacterium, *Vibrio harveyi*, were investigated in two abalone populations. The St Malo population appeared to be already resistant to the pathogen (St Malo population, 95% survival), while the Molène population displayed an increased survival rate (51%) after 1 month and lower bacterial detection after the second challenge, which was interpreted as a training effect.[133] Furthermore, a recent study on *H. diversicolor* demonstrated its immune training capacity in response to *V. harveyi* primo-infection.[137] The authors found a significant improvement in survival rates upon secondary challenge with the same pathogen 2 weeks after the first exposure. A global comparative transcriptomic approach on hepatopancreas tissue revealed some molecular changes, reflected by significant upregulations of various pathogen recognition receptors (PGRP; TLR, C1q, scavenger receptors, …) and immune effectors associated with detoxification and antioxidant response, but also of genes involved in phagocytosis, metabolic pathways (glycolysis, fatty acid, and amino acid metabolism) and calcium signalling pathways. Although the experimental scheme in this study could not determine the mode of response involved (analyses performed on a single time point after the second challenge), the results suggest that the protection could rely on a stronger secondary response and/or a sustained but similar immune response. Nevertheless, this study provided a first overview of immune mechanisms that could synergistically lead to immune training in abalones. More recently, in *H. discus hannai*, a transcriptomic study was conducted on haemocytes from trained animals following stimulation with a sub-lethal dose of live *Vibrio parahaemolyticus*.[138] The study showed increased survival rates when facing a second challenge 7 days after priming with a lethal dose of the same pathogen. Gene clusters that could contribute to this enhanced immune protection in haemocytes were identified and classified. There were 1019 genes associated with immune-enhancing regulation and 281 genes classified as immune-enhancing genes. The expression patterns of these genes showed significant up-regulation following re-infection, indicating a recall pattern, and intricate mechanisms involving conserved immune pathways such as NF-kappaB, TLR, NOD-like receptor, and IL-17 signalling pathways. Additionally, the immune effectors involved in detoxification and the mediators of the apoptosis pathway were linked to this training response. In conclusion, immune memory phenomena have been demonstrated in abalone in response to exposure to probiotic or killed bacteria, or to sub-lethal doses of pathogenic bacteria that could increase survival. The specificity of the response was not investigated. The effect of training was observed up to several months after the initial exposure. Molecular mechanisms were identified involving phagocytosis, haemocytes, ROS production, and numerous immune genes. The response in these organisms appears to follow a recalled response profile (Figures 1 and 2).

3.2 | Clams

A few studies have shown that the clam *Chlamys farreri (K.H. Jones & Preston, 1904)*[139–142] (Table 1) displays immune training capacities when exposed to live or heat-killed bacteria. The authors found that injection of pathogenic bacteria (*Vibrio anguillarum* previously known *as Listonella anguillarum*) resulted in a significant increase in immune gene expression (peptidoglycan recognition protein-S1, Cf- PGRP-S1 or C-type lectins) following a second infection (72–168 h after first exposure). Interestingly, Cong et al. reported a faster and stronger

induction of the immune genes after the secondary infection, which is demonstrative of a biphasic response pattern (known as a recall-type response). Originally, Wang et al. found that there was a degree of specificity in the training response, with increased survival when the second injection was performed with the same pathogen.[140] While these studies did not examine duration beyond 7 days, another study suggested transgenerational training capacities in this species.[141] The study demonstrated that maternal stimulation with the heat-killed bacteria *V. anguillarum* induced significant changes in immune protein levels and mRNA expression in the offspring at various developmental stages. These immune proteins (including Cf-LGBP, Cf-LBP/BPI, Cf-LYZ, and Cf-Cu/Zn–SOD) exhibited enhanced agglutination properties and bactericidal activities against Gram-negative bacteria *Escherichia coli* and *Vibro anguillarum*, as well as fungi *Pichia pastoris*. This enhanced immune competence was linked to improved survival in offspring exposed to the same pathogen *V. anguillarum* at the trochophore and D larval stages. Survival capacities in offspring were not investigated at later life stages. Survival beyond these early developmental stages (occurring 24–48 h post-spawning) was not investigated. Further research is needed to confirm whether this increased survival is due to an immune training mechanism, as it was not possible to distinguish the training effect from the genetic effect. Spawning was not replicated for the stimulated and control broodstock to address this issue, and it is common to observe strong genetic bases for disease resistance in shellfish species.[41–43] In conclusion, immune memory phenomena have been demonstrated in clams in response to injection of heat-killed bacteria, which could increase survival. The response showed signs of specificity. The effect of training was observed up to 7 days after the first exposure, but appears to be transgenerational. Molecular mechanisms were identified involving phagocytosis, ROS production, and induction of immune gene expression. The pattern of expression in these organisms appears to follow a recalled response profile (Figures 1 and 2).

3.3 | Mussels

3.3.1 | Biotic factors and enhancement of immune capacities

Recent studies on mussels have attempted to go beyond the simplistic classification of their immunity as non-adaptive and unspecific. The molecular response of mussels after two exposures to *Vibrio tasmaniensis* LGP 32 (formerly named *Vibrio splendidus* LGP32) was explored (Table 1).[143] The mussels were first exposed to a sub-lethal dose of live *V. tasmaniensis* (10^7 UFC/mL), followed by 14 days of rest and a second exposure to a non-lethal dose of the same pathogen. The RNA-seq analysis of haemocytes revealed that the number of differentially expressed genes (DEGs) was significantly lower after the second infection compared with the first, indicating a stronger response to the bacteria during the first encounter. Genes related to pathogen recognition (perlucin-like protein), and the killing and sequestration of invading pathogens (spore cortex-lytic enzyme or henna protein), reached their highest expression levels after the first infection and decreased as the experiment progressed and the second stimulation occurred. Additionally, a set of modulated genes was identified that either increased (primed genes), maintained or decreased (tolerised genes) expression in the context of reinfection. These genes and their functions suggest that haemocytes were activated to control and resolve the inflammatory response, thereby avoiding subsequent DNA damage and cell death. Furthermore, some key immune processes, such as apoptosis or ROS production were clearly contained or reduced when comparing the second exposure with the first one. This suggests either an immunological tolerance or an immune shift profile of mussel immunity in case of reinfection with the Gram-negative bacteria *V. tasmaniensis* LGP32.[143] Whether tolerance should be considered as a response to immune training or not is up for debate. Some studies have interpreted the tolerance phenotype as a compensatory mechanism that results in a reduced response to a secondary stimulus, thus avoiding inflammatory damage.[81] Research on *Mytilus galloprovincialis (Lamarck, 1819)*[143,144] indicates that these animals undergo a reprogramming of immune and stress-related genes. This reprogramming may help prevent damage and excessive responses, ultimately leading to acclimatization to situations of infection or exposure to contaminants. Given that these animals are filter-feeders that continuously internalize particles from the environment; this strategy is likely to be effective. This assessment is supported by the fact that this species is known for being very resilient, with virtually no records of mortality in the natural environment.[145]

Mussels are a promising species for somatic diversification of immune receptors and effector antimicrobial molecules, which could support a trained immune response. They exhibit varied responses to different pathogen species, as evidenced by their distinctive interactions with *Vibrio aestuarianus* 01/032 and *V. tasmaniensis* LGP32. Although mussels can overcome both infections, their responses differ.[146] Some haemolymph molecules have been found to play a role in the sensitive interactions between host haemocytes and specific pathogens.[147,148] The mussel genome contains a diverse array of PGRPs, including Toll-like receptors (TLRs), peptidoglycan receptors, Fibrinogen-like receptors (FREPs), C1q proteins, and immune-related lectins, which enable the recognition of potentially pathogenic species with high specificity.[149] Mussels exhibit a large number of immune-related genes, as reviewed in Ref. 150, and this is highlighted by the variability in their pan-genomic features, where a set of particularly immune-enriched genes varies between individual mussel genomes (this phenomenon is known as presence/absence of variation and has been defined in the recently published Mussel Genome Project[149]).

3.3.2 | Abiotic stress and enhanced immune capacities

The impact of environmental stress on molluscs has not been extensively researched. However, in 2015, Aleng et al.[151] demonstrated that a non-lethal heat shock resulted in a trained status in *Perna viridis (Linnaeus, 1758)*, characterized by thermotolerance and an increased

TABLE 1 Studies evidencing trained immunity in cultured marine molluscs.

mollusc species	Training				time elapse (duration)	Challenge							Ref.
	elicitor	dose	method	immune parameters		species	natural pathogen	dose	survival rate	specificity	across generations	Response	
Abalone *Haliotis iris*	*Exiguobacterium JHEb1, Vibrio JH1, and Enterococcus JHLDc.*	3 × 10exp9 CFU g−1	Oral diet	total hemocyte count and viability	2 days	*Vibrio splendidus*	Yes	50µL @ 5 × 10exp7 cfu	33.3% (non-probiotic-fed) and 22% probiotic-fed mortality	Not investigated	No	no significant differences	Grandiosa, 2020 (134)
				oxydative stress								no significant differences	
				cell apoptosis								significant differences in probiodtic-fed animals	
				metabolomics								no interaction between diet and infection status within the foot muscle metabolome	
Abalone *Haliotis iris*	*Exiguobacterium JHEb1, Vibrio JH1, and Enterococcus JHLDc.*	3 × 10exp9 CFU g−1	Oral diet	total hemocyte count and viability	1- and 3-months diet	*No challenge*			no mortality in probiotic-fed animals after 4 months, and 10% in controls	not investigated	No	no significant differences	Grandiosa, 2018 (136)
				oxydative stress								significantly higher (19.4 ± 23.3%) ROS-positive cells in probiotics-fed abalones	
				hemocyte apoptosis								No significant differences	
				growth								general increase in length, width and weight of the probiotic-fed animals	
				metabolomics								general pattern of enhanced metabolite expressions in probiotics-fed animals	
Abalone *Haliotis iris*	*Exiguobacterium JHEb1, Vibrio JH1, and Enterococcus JHLDc.*	from 2 × 10exp8 CFU/g to 3 × 10oexp9CFU/g	Oral diet	growth	60 days	*No challenge*			no mortality in probiotic-fed-animals and 10% mortality in controls	Not investigated	No	increase in weight of the probiotic-fed animals	Hadi, 2014 (135)
Abalone *Haliotis tuberculate*	*V. harveyi* (ORM4)	10exp4 CFU/mL	immersion	total hemocyte count (THC)	28 days	*V. harveyi* (ORM4)	yes	10exp4 CFU/mL	Significant difference after 1st exposition for St Malo (95%,) and Molène (51%)	not investigated	no	significant difference in Molène population 24h after 1st challenge	Dubief, 2017 (133)
				phagocytosis								significant inhibition of phagocytosis 24h after 1st challenge in both populations and 2 days after 2nd challenge in the Molène population	

TABLE 1 (Continued)

					pathogen load					populations -no further mortalities after 2nd exposition			although not significant, pathogen loads were smaller in Molène population after the 2nd challenge	
					impact of extracellular products of *V. harveyi* on phagocytosis after 2nd challenge								no impact of phagocytosis in St Malo population survivors to the 2nd challenge as opposed to non infected population where phagocytosis was inhibited	
					impact of abalone serum on bacterial growth after 2nd challenge								ability of *V. harveyi* to grow in abalone serum was lower in Saint-Malo (86% of the maximum growth rate observed in LBS for uninfected abalone and 92% for survivors), than in Molène (101% of the rate observed in LBS)	
Abalone	*Haliotis diversicolor*	*V. harveyi*	50 µL of: 1.42 × 10exp3/ 10exp4/ 10exp5/ 10exp6/ 10exp7 CFU/ml	injection	hepathopan-creas RNA-seq analysis	15 days	*V. harveyi*	yes	50 µL of 1.58 × 10exp6 CFU/mL	After the 1st immune stimulation, the survival rate decreased with increased pathogen concentration; after the 2nd stimulation the survival rates in all groups receiving the 1st immune stimulation were significantly higher with no difference between groups	not investigated	no	differential regulation of genes involved in phagocytosis and metabolic related pathways	Yao, 2021 (137)
					Validation on 12 DEGs (qPCR)								the expression trends of selected genes were consistent with the DEG analysis results	
Abalone	*Haliotis discus hannai*	*Vibrio parahaemoyticus*	20µL of 1.0 × 10exp8 CFU/mL solution	injection	RNAseq-analysis from hemolymph	7 days	*V. parahaemolyticus*	yes	100µL of 1.0 × 10exp8 CFU/mL solution	significant increase in survival rate after training	not investigated	no	Identification of 1019 immune-enhancing regulatory genes (ERGs) and 281 essential immune-enhancing genes (EEGs) from conserved immune pathways (NF-kappa B signaling pathway, NOD-like receptor signaling pathway, IL-17 signaling pathway, and TLR signaling)	Zhang, 2022 (138)
					Validation on 11 DEG (qPCR)								the expression trends of selected genes were consistent with the DEG analysis results	
Clam	*Chlamys farreri*	*Listonella (Vibrio) anguillarum*	2.6 x10exp6 CFU	injection adductor muscle	temporal mRNA transcription of CfPGRP-S1 (qPCR)	3 days	*L. (Vibrio) anguillarum*	yes	2,6exp6 CFU	no investigated	not investigated	no	recalled (fatser and stronger response after the second challenge)	Cong, 2009 (139)

(Continues)

TABLE 1 (Continued)

Clam	*Chlamys farreri*	*Vibrio anguillarum* Heat killed	1exp8 CFU/mL-50µL	injection adductor muscle	Expression level of 5 C-lectin genes (PCR)	7 days	*V. anguillarum*	yes	1exp8 CFU/mL-50µL	significant increase in survival rate after training	yes, better survival to a microorganism already encountered	no	recalled (significant upregulation after training of all forms of c-lectin followed by a retsing state and a fatser, stronger response after the secondary challenge)	Wang, 2013 (140)
							M. luteus	no	1exp8 CFU/mL-50µL	no significant difference			up-regulation of C-lectin 1, 2 after challenge and slight up regulation for C-lectin 3, 4, 5 after challenge; all upregulation were smaller that in the Va-Va group	
Clam	*Chlamys farreri*	*Vibrio anguillarum* Heat killed	100µL@ 1 exp8 cell/mL	injection adductor muscle	agglutination assay	7 days before spawning induction	*V. anguillarum*	yes	5exp8cells/L	significantly higher survival until the D-stage larvae	not investigated	yes	agglutination observed with E. coli, V. anguillarum and P. pastoris, none for S. aureus	Yue, 2013 (141)
					qPCR on CfLGPB, CfLec-3, CfLYZ, CfLBP/BPI, Cf SOD								low transcript detection (execpt for SOD) in eggs, impact on BPI, LYZ in trocophore and D-shaped larvae compared to control	
					Western-Blot with CfLGPB, CfLec-3, CfLYZ, CfLBP/BPI antibodies								high detection of proteins except for lec3 in eggs, all immune factors protein concentration increased during development except for LGPB and significance dependent on stage	
					SOD enzymatic activity								SOD activity increased from eggs to trocophrore larvae ;	
					bactericidal activity assay								antibacterial activity increased with a peak at D-stage and then decrease	
Clam	*Chlamys farreri*	*Listonella (Vibrio) anguillarum*	0,1exp8 CFU/mL	Immersion	Phagocytosis	8h	*L. (Vibrio) anuillarum*	yes	0,1exp8 CFU/mL	delayed mortality in trained animals	Not investigated	no	only phagocytosis and ACP activity of the pre-stimulated scallops were significantly higher than those of the un-stimulated scallops in the secondary immersion stimulation	Cong, 2008 (142)
					Phenoloxidase, acid phosphatase, SOD activities									
Mussel	*Mytilus galloprovincialis*	*Vibrio tasmaniensis* LGP32	10exp7 CFU/ml	injection adductor muscle	*V. splendidus* clearance	2 weeks	*V. tasmaniensis* LGP32	no	10exp7 CFU/ml inj 1 and 10exp8 CFU/ml inj 2	not investigated	not investigated	no	*V. splendidus* detection increased 24 hpi and was rapidly controlled 7 days after the injection, returning to control levels	Rey-Campos,2019 (143)
					RNAseq analysis from hemocytes								Identification of 1216 DEG following first challenge and 39 and 31 genes with increased or decreased expression pattern, respectively, after the second encounter	
					Hemocyte Distribution (FACS)								restoration of hemocyte population structure after second encounter	
					Apoptosis assay								second bacteria injection did not induce any further increase in the number of apoptotic cells	
					ROS Analyses								decreased respiratory burst after second exposure	

TABLE 1 (Continued)

Mussel	*Perna viridis*	non-lethal heat shock (NLHS)	38°C- 30 min followed by 6h recovery @28°C	immersion	Hsp70 protein analyses (tissue localization by western-blot, identification using mass spectrometry LC-MS/MS)	10 days	*V. alginolyticus*	yes	immersion in 10exp8 CFU/mL, for 72h	significant increase in survival rates after NHLS	no: NHLS increased thermos-tolerance as well as bacterial tolerance		two Hsp70 isotypes increased after NLHS in all tissues analysed - The enhanced protection correlated with increasing amounts of PvHsp70-1 and PvHsp70-2 in all tissues examined	Aleng, 2015 (151)
Oyster	*C. gigas*	heat killed *V. splendidus* from moribund scallop *Pactinopecten yessoensis*	100µl of 2exp8 CFU/mL	injection adductor muscle	total hemocyte count (THC)	7 days	same strain and concentration - live	no	100µl of 2exp8 CFU/mL	not investigated	Yes: higher phagocytosis response to *V. splendidus* than to *V. anguillarum, V.coralliilyticus, V. lipolytica,* and *M. luteus*	no	significant increase after the primary stimulation and even higher increase (peak earlier at 6 h) after the secondary challenge	Zhang, 2014 (155)
					BrdU incorporation assay								Increase of new generated circulating hemocytes at 6 h after the pre-stimulated oysters received the secondary stimulation	
					phagocytosis assay in hemocytes								the phagocytic rate was also enhanced significantly at 12 h after the secondary stimulation	
					Monitoring of 12 candidate genes related to phagocytosis and hematopoiesis in hemocytes (qPCR)								expression levels of CgIntegrin, CgPI3K, CgRho J, CgMAPKK, CgRab32, CgNADPH, CgRunx1 ,CgBMP7 in the hemocytes of pre-stimulated oysters after the secondary stimulation were higher than that after the primary stimulation	
Oyster	*C. gigas*	heat killed *V. splendidus* from moribund scallop *Pactinopecten yessoensis*	100µl of 2exp8 CFU/mL	injection adductor muscle	Cg-EcSOD gene and protein expression (qPCR and western-blot) in hemocytes	7 days	same strain and concentration - live	no	100µl of 2exp8 CFU/mL	not investigated	not investigated	no	CgEcSOD expresssion was significantly up-regulated at the initial phase and decreased sharply at 48 h post-stimulation; After the secondary stimulation, the mRNA and protein of CgEcSOD were both downregulated significantly	Liu, 2016 (157)
					PAMP binding								CgEcSOD protein could bind LPS, PGN and poly (I:C), and various microorganisms including *Micrococcus luteus, Staphylococcus aureus, Escherichia coli, Vibrio anguillarum, V. splendidus, Pastoris pastoris and Yarrowia lipolytica*	
Oyster	*C. gigas*	formaldehyde -killed *V. splendidus* from moribund scallop *Pactinopecten yessoensis*	100µl of 2exp8 CFU/mL	injection adductor muscle	Immune gene and protein expression (immunohistochemistry (Cg-Clec4, CgGATA3, CgEcSOD)/western-blot (CgGATA3, CgEcSOD)/qPCR	7 days	same strain and concentration - live	no	100µl of 2exp8 CFU/mL	not investigated	not investigated	no	2nd stimulation: expression levels of CgClec-4 and CgIFN in the gill of pre-stimulated oysters were significantly higher than that in controls, while the expression level of CgIL-17 was significantly decreased; the protein level of CgGATA3 and CgEcSOD in gill increased after the secondary challenge	Li, 2017 (156)

(Continues)

TABLE 1 (Continued)

				(Cg-Clec4, CgIFN, CgIL17)) in gills									
				phagocytic activity in gills								The phagocytic rate of pre-stimulated oysters was significantly increased at 6 h after the secondary challenge (faster response than controls)	
				Immune-related enzyme activity (acid phosphatase, catalase) in gills								Significant increase in phosphatase and catalase activity in trained group	
				ROS in gills								ROS production was enhanced significantly at 6 h and 24 h after the secondary challenge	
Oyster	*C. gigas*	heat killed *V. splendidus* from moribund scallop *Pactinopecten yessoensis*	100µl of 2exp8 CFU/mL	injection adductor muscle	RNAseq & Immune gene expression on 10 candidate (qPCR) in hemocytes	7 days	same strain and concentration - live	no	100µl of 2exp8 CFU/mL	not investigated	not investigated	no	potential recall and sustained responses: Identification of 2964 common DEGs up-regulated after both stimulations (metabolic processes and immune related pathways); 187 DEGs higher expressed at resting (0h after stimulation) and55 at activating state (12h after stimulation) of the 2nd immune response (immune recognition receptor signal molecule, immune regulator, apoptosis inhibitor and effector); 13 DEGs were long-lastingly higher expressed at both the resting and activating states after the 2nd immune response (MyD88, anti-virulent tissue inhibitor of metalloproteinase, anti-bacterial proline-rich transmembrane protein)	Wang, 2020 (158)
Oyster	*C. gigas*	POMS	Natural exposure	Immersion in the environment	Survival	21 months	OsHV-1	Yes	injection of approximately 5.07 × 10exp4 OsHV-1 DNA copies per 100 µL	significant decrease in mortality rates in not exposed ranging from 4,2%, 8.3% to 16.7% and 4.2%, compared to control reaching 91.7% and 83.3% mortality	Not investigated	No		Liu, 2023 (180)

TABLE 1 (Continued)

Oyster *C. gigas*	heat killed *V. splendidus* from moribund scallop *Pactinopecten yessoensis*	100µl of 2exp8 CFU/mL	Injection in the adductor muscle	treatment with methyltransferase inhibotor (MTA) and histone demethylases inhibitor (MEF)	7 days	heat killed Vibrio splendidus from moribund scallop Pactinopecten yessoensis	No	100µl of 2exp8 CFU/mL	Not investigated	Not investigated	No	change in the H3K4me3 enrichment of the CgTLR3 promoter after MTA (decrease) and MEF (increase) treatments	Lian, 2024 (159)
				Expression of 4 immune-related genes (qPCR, IL-17, MyD88, Rel, TLR3)								significant differences in the expression of CgTLR3, CgMyd88-2 and CgIL17-1)was found to be elevated in the hemocytes at 6 h after the secondary stimulation	
				CHIP-qPCR assays								significant increase in histone H3K4me3 enrichment was detected in the promoter region of the CgTLR3 gene	
Oyster *C. gigas*	live *Vibrio alginolyticus*	5 × 10exp4, 5 × 10exp5, and 5 × 10exp6 CFU/mL	24h immersion	ROS	7 days	live *Vibrio alginolyticus*	Yes	immersion in 5 × 10exp6 CFU/mL in 30L	no significant differences with control	Not investigated	No	higher levels of ROS after priming and 10 days after challenge except for LV6 and lower levels at 15 days post-challenge	Wang, 2024 (160)
				Vibrio load								no signficant differences of vibrio loads in oysters primed with live Vibrio	
				colonization of bacteria (modified giemsa staining of digestive gland)								no bacteria in digestive gland agter challenge	
				hemocyte morphology								necrotic morphology 15days post-challenge	
	formalin-inactivated *Vibrio alginolyticus*	5 × 10exp4, 5 × 10exp5, and 5 × 10exp6 CFU/mL	24h immersion	ROS	7 days	live *Vibrio alginolyticus*	yes	immersion in 5 × 10exp6 CFU/mL in 30L	significant differences in oysters primed with 5 × 10exp4, 5 × 10exp5 (100% and 92,5%)	Not investigated	no	lower levels of ROS after priming and higher levels 10 days after challenge and lower after 15 days	
				colonization of bacteria (modified giemsa staining of digestive gland)								large number of bacteria after challenge	
				hemocyte morphology								normal morphology 15 days after challenge	
		v 5 × 10exp4 CFU/mL	24h immersion	Growth and survival	7 days before reproduction	live V. *alginolyticus or V. harveyi, V. brasiliensis, V. parahaemolyticus*	yes	immersion 12 days post-fertilization in 5 × 10exp6 CFU/mL	significant differences in umbo larvae 12 days post-fertilization	No specificity-broad spectrum protection	yes	primed larvae suffered mass mortality from 6th to 10th day after fertilization; The proportion of umbo-larvae was always higher in controls on the 6th to 9th day after fertilization; The height of primed larvae was smaller compared to control on the 2nd to 10th day post fertilization but the height of	

(Continues)

TABLE 1 (Continued)

								of Vibrio/10 0mL				primed larvae was bigger on the 14th to 18th day post fertilization.	
Oyster *C. gigas*	poly(I:C)	50µL @ 5mg/mL	injection adductor muscle	pathogen load & Immune gene expression on 9 candidates (qPCR)	1 day	OsHV-1	yes	50µL @ 6.5x 10exp8 copies of C9/C10 gene	not investigated	no impact of a primary stimulation with heat-killed bacteria on OsHV-1 loads	no	significant reduction in OsHV-1 load in poly(I:C) trained oysters	Green, 2013 (166)
	Vibrio tasmaniensis LGP32	50µL@10exp8 CFU/ml										poly I:C injection up-regulated the response of a toll-like receptor, an interferon regulatory factor and Rel/NF-kB, Cg-IFI44 and Cg-PKR	
Oyster *C. gigas*	poly(I:C)	50 µl @ 5 mg/mL	injection adductor muscle	Expression of 15 candidate genes on gill and mantle tissues (qPCR)	7 days	poly(I:C)	no	50 µl @ 5 mg/mL	not investigated	not investigated	no	sustained response: Re-injecting dsRNA 168 h after primary challenge failed to increase mRNA levels of any of the studied target genes above control groups except for PKR and viperin in the mantle tissue	Green, 2014 (164)
				Methyl-DNA immunopre-cipitation on gill and mantle tissues (MeDIP)								Injection of dsRNA did not alter the DNA methylation profile of any of the up-stream regions of housekeeping or anti-viral genes in gill and mantle tissue	
Oyster *C. gigas*	poly(I:C)	100 µl @ 5 mg/mL	injection adductor muscle	pathogen load	3 days before spawning	OsHV-1	yes	immersion in 10exp9 OsHV-1 genome copies (equivalent to 10exp5 OsHV-1 genome copies/larvae)	poly(I:C)-treatment of oyster parents results in offspring with enhanced protection against OsHV-1 infection	not investigated	yes	offspring produced from poly(I:C)-treated parents contained significantly less OsHV-1 DNA at 48h post-inoculation with OsHV-1 compared to control larvae	Green, 2016 (165)
				Expression of 19 immune-related genes (qPCR) in parents (gills & eggs) and D-larvae								Poly(I:C)-treatment of parent oysters did not result in their larvae expressing antiviral-related genes earlier or to a higher magnitude upon exposure to OsHV-1, except for IRF2; Mature eggs collected from poly(I:C)-treated females contained significantly higher transcript abundance of IRF2, ADAR-L and IkappaB2	
Oyster *C. gigas*	*Cg*-IkB2 or GFP dsRNA	100µL@0.5 µg/µL		pathogen load and mRNA expression	2 days	OsHV-1	yes	100µL@ 10exp5 copies of viral genome/ µL	significant increase of survival in dsRNA-treated oysters	not investigated	no	significantly lower to none OsHV-1 detection on trained oysters	Pauletto, 2017 (169)
			injection adductor muscle	Expression of immune-related genes in gonads and gills (qPCR)								24 h after dsRNA injection, all the target genes except SOCS were significantly more expressed in gonads and gills of Cg-IkB2-dsRNA-treated oysters; After infection, candidate genes transcript fold change from dsRNA-treated osyters were lower than in controls	

TABLE 1 (Continued)

Oyster	*C. gigas*	poly(I:C) /dsRNA/ssRNA	19µg/g of oyster for poly(I:C) – 5µg of dsRNA or ssRNA/ oyster	injection adductor muscle/ immersion	pathogen load	24h to 5 months	OsHV-1	yes	20µL @~1x10exp6 copies of DP gene/µL	significant increase in survival rate for oyster trained with poly(I:C) with a dose-response effect	yes, better survival to OsHV-1 than bacteria	no	Reduced pathogen loads and efficient protection through impaired virus replication in oysters trained from 24hrs to 5 months after poly(I:C) injection or immersion	Lafont, 2017 (162)
							V. tasmaniensis LGP32	yes	20µL @3,5x10 exp6 CFU/µL	no significant increase in survival rate for oysters trained with poly(I:C) and infected with bacteria		no		
Oyster	*C. gigas*	poly(I:C)	100µL@0.5 µg/µL	injection adductor muscle	pathogen load	3 days or 10 days before spawning	OsHV-1	yes	immersion of 24hrs larvae in 10exp9 OsHV-1 genome copies or field experiment (natural mortality outbreak)	poly(I:C)-treatment of oyster parents results in offspring with enhanced protection against OsHV-1 infection only in offspring of mothers trained 3 days before spawning	not investigated	yes	no effect of training on OsHV-1 replication in larvae. No difference in the amount of OsHV-1 DNA was observed between the three treatments	Lafont, 2019 (161)
					RNAseq from 3 pools of larvae produced from poly(I:C) treated or control oysters								non-challenged larvae produced from parents trained with poly (I:C) have similar transcriptional profiles to control larvae; a small subset of 47 genes was found to be differentially regulated between offspring of poly(I:C)-treated and non-treated parents	
					Expression of 10 candidate genes (qPCR)								No differences in the expression level of the 10 DEGS	
Oyster	*C. gigas*	poly(I:C)	19 µg/g of oyster	injection adductor muscle	pathogen load	1 day to 5 months	OsHV-1	yes	20µL @1.32x10 exp8 copies of DP gene per oyster	significant increase in survival rate from 1 day to 5 months post-training	not investigated	no	Reduced pathogen loads and efficient protection through impaired virus replication in oysters trained from 24hrs to 5 months after poly(I:C) injection	Lafont, 2020 (88)
					RNAseq whole animals	10 days							Sustained response: only minor transcriptional changes occurred in trained oysters after challenge. Poly(I:C) induced a strong upregulation of gene expression that was mainly maintained throughout the experiment (Interferon-like, NF-kappaB, apoptosis pathways)	
					Expression of immune-related genes in whole animals (qPCR)	5 months			20µL@1.22x10exp8 copies of DP gene/µL				A first set of genes shows a sustained upregulation (RLR, sacsin, IFI44, IFI1, DICER, cGAS, and TLR). A second set of genes shows a sustained response that was significantly lower than in the control (IRF2, IRF8, ADAR, and viperin)	
Oyster	*C. gigas*	environmental microbiota	ND	immersion	Tanscriptomic (RNAseq), epigenetic (BS-seq), metabar-coding analyses	4 months	OsHV-1/POMS	yes	ecologically realistic infection	significant increase in survival within and across generations	not investigated	yes	transcriptomic and epigenetic reprograming	Fallet, 2022 (178)
					pathogen load								reduced pathogen load in trained animals	

Abbreviations: DEGs, differentially expressed genes; dsRNA, double-stranded RNA; EEGs, essential immune-enhancing genes; ERGs, enhancing regulatory genes; FACS, fluorescence-activated cell sorting; IFN, Interferon; LBS, luria-Bertani saline; LC-MS/MS, liquid Chromatography coupled to tandem Mass Spectrometry; LPS, lipopolysaccharide; NF-kB, nuclear Factor kappa B; NLHS, Non-lethal heat shock; POMS, Pacific Oyster Mortality Syndrome; qPCR, quantitative PCR; PGN, peptidoglycane; ROS, reactive oxygen species; SOCS, suppressor of cytokine signaling; STING, stimulator of interferon genes; TLR, Toll-like receptor.

ability to resist *Vibrio alginolyticus* infections. A few years later, *M. galloprovincialis* adults were exposed to microplastics (<5 mm diameter; 4.6E+5 microbeads L^{-1})[144] following the principle of repeated exposure to non-self-molecules. Although not strictly a form of an immune stimulation, the exposure was found to affect the immune and stress response. The results showed that exposure to microplastics resulted in the up-regulation of genes related to stress processes. After the depuration and the second exposure, the expression of those immune and stress-related genes decreased. This suggests that mussels can establish alternative responses that promote acclimation mechanisms to cope with subsequent stress.[144] In a very similar approach, mussels were repeatedly exposed to nanoplastics.[152] This exposure resulted in changes in haemocyte subpopulations, an increase in haemolymph bactericidal activity, and transcription of certain immune-related genes. The authors concluded that these immune parameters may shift to preserve homeostasis upon re-exposure to nanoplastics and train animals to increase their robustness.[152]

In conclusion, immune memory phenomena have been demonstrated in mussels in response to injection of live or heat-killed bacteria or environmental stress for up to 2 weeks after exposure. The specificity of the response was not investigated. Molecular mechanisms have been identified involving phagocytosis, ROS production, apoptosis and alteration of immune gene expression. The response in these organisms appears to follow a tolerance or immune shift response profile (Figures 1 and 2).

3.4 | Oysters

Studies on the Pacific oyster *Crassostrea gigas (Thunberg, 1793)*, recently renamed *Magallana gigas (Salvi & Mariottini, 2016)* provide compelling evidence for the existence of immune training capacities in oysters. Like mussels, oysters possess a large number of immune-related genes characterized by high diversification and polymorphism.[13,20,153] They also exhibit pathogen recognition specificity, displaying distinct responses when infected with bacterial or viral pathogens.[154]

3.4.1 | Antibacterial immune training

Several studies have demonstrated that injection of a heat-killed or formaldehyde-killed bivalve bacterial pathogen *V. splendidus* can induce an enhanced immune response upon a secondary challenge with the same live pathogen[155–159] (Table 1). The authors reported an increase in the total haemocyte count, a higher number of newly generated haemocytes and enhanced cell regeneration in the gills.[155,156] These observations were also associated with an increase in the expression of genes related to haematopoiesis after the secondary challenge suggesting that haematopoiesis may play a role in antibacterial immune training in the Pacific oyster. The gills and haemocytes showed improved phagocytic activity, with a stronger and faster response upon secondary challenge, 7 days after priming.[155,156] Interestingly, the increased phagocytosis of haemocytes appeared to be specific to *V. splendidus*, as the enhanced response was not observed following a secondary challenge with other *Vibrio* species, marine yeast, or gram-positive bacteria.[155] These studies showed discrepancies in the regulation of *Cg*GATA3 and *Cg*SOD genes in haemocytes (not regulated or down-regulated)[155,157] and gills (significant increase).[156] More recently, Lian et al.[159] also demonstrated an enhanced immune response after 7 days of training with the same heat-killed *V. splendidus* strain. This response was associated with significant differences in the expression of Toll-like receptor 3 (CgTLR3), myeloid differentiation factor 88-2 (CgMyd88-2), and interleukin 17-1 (CgIL17-1) in the haemocytes, 6 h after the secondary stimulation. Interestingly, the response was associated with epigenetic modifications with an increase in histone H3 lysine 4 trimethylation (H3K4me3) enrichment at the promoter of the CgTLR3 gene. These results suggest for the first time a role of histone modifications in oyster immune training. Additional studies are necessary to gain a better understanding of the fundamental molecular mechanisms involved and to elucidate the role of these tissues and cells in supporting immune training. Wang et al.[158] enhanced the molecular comprehension of antibacterial immune training in oysters by performing a global transcriptomic approach on haemocytes. They compared the transcriptomic responses between a first exposure to heat-killed *V. splendidus* and a second challenge with the same live bacteria, revealing a series of genes with a recall expression pattern. These genes were associated with metabolic processes and immune-related pathways, including recognition receptors such as TLRs. Interestingly, some DEGs exhibited a higher basal expression level after the first stimulation, which was linked to recognition receptors and signal molecules. This set of genes could contribute to the initiation of an enhanced secondary response. The MyD88 gene from the Toll pathway, and a potential NF-κB target gene, *Cg*TIMP, also showed long-lasting up-regulation, indicating a role of this pathway in this phenomenon. However, this study only focused on the common DEGs between the first and second stimulation. Therefore, we lack information on specific molecular mechanisms that could differ between both stimulations and indicate a shift between responses, which could also contribute to the enhanced immune response. Although the aforementioned studies represent a breakthrough in demonstrating the existence of antibacterial immune training in oysters, the impact on survival was not investigated in these different trials. The use of a *Vibrio* strain isolated from moribund scallops, which is likely non-pathogenic to oysters, may have hindered the evaluation of this aspect. Furthermore, the stimulation time did not exceed 7 days. This period should be extended to observe whether these patterns could be maintained longer in order to evaluate the duration of memory.

However, more recent studies have also tested the induction of immune training responses following a 24-h immersion in formalin-inactivated or live pathogenic *V. alginoluticus*.[160] If pre-exposure to live bacteria didn't affect survival, formalin-inactivated bacteria induced a significant increase in survival 7 days after exposure (from 92.5% to 100% survival). This response was associated with lower levels of ROS after priming and a peak in ROS levels 10 days after challenge. The authors also tested the transmission of the phenotype

to the next generation. Although larvae from trained animals showed greater survival 12 days after fertilization following infection with a wide range of *Vibrio* species, these larvae also suffered massive mortality 6 to 10 days after fertilization. This observation raises the question of the application of a selection filter that may have biased the results.

3.4.2 | Antiviral immune training

Several studies have also reported antiviral immune training capacities in *C. gigas*[26,88,161–168] (Table 1). These studies used a viral mimic, the synthetic double-stranded RNA molecule called poly(I:C), to induce an antiviral immune state that protects oysters from infection by the ostreid herpes virus OsHV-1 µVar. This virus is a triggering pathogen of the Pacific Oyster Mortality Syndrome (POMS), which is currently causing mass mortalities worldwide. The authors demonstrated that poly(I:C), either by injection or bath treatment, could significantly improve long-term protection up to 5 months after primary exposure, increasing oyster survival when faced with OsHV-1 µVar during experimental infections or an environmental disease outbreak.[162] This protection was shown to be specific to antiviral protection. Primary exposure to heat-killed Vibrio bacteria failed to induce protection against OsHV-1 µVar, and poly(I:C) did not provide any protection against a secondary *V. tasmaniensis* infection.[162,166] The induction of antiviral immune training seemed to involve the activation of nucleic acid signalling pathways, which are highly conserved in the *C. gigas* genome.[20,26] Poly(I:C) and other double- and single-stranded RNAs have been shown to increase survival.[162,169] While training has been shown to induce the expression of many conserved antiviral genes,[164,166,169] a whole-animal transcriptomic approach has provided further insight into the molecular pathways involved in this response.[88] The response is characterized by a sustained up-regulation of immune and antiviral genes, particularly genes involved in IFN-like and Toll/NF-kB pathways and apoptosis, which could play a role in the subsequent control of viral infection. This pattern of response suggests that the training relied on pre-conditioning the oyster immune system. In addition to the sustained response, this study reveals other minor gene expression patterns (recalled, shifted), suggesting that the mechanisms behind training may be more complex than previously believed. Furthermore, genes with metabolic and epigenetic functions have been identified in trained oysters. Based on studies of trained mechanisms in mammals and plants,[53,170] epigenetic modifications may explain the observed sustained gene expression pattern and immune protection.

In *C. gigas*, antiviral immune training also seems to protect oysters across generations.[161,165] Offspring of females trained with poly(I:C) 3 days before spawning exhibited enhanced survival capacities when exposed to OsHV-1 µVar.[161,165] This improved survival could not be explained by differential expression profile in the offspring of trained oysters compared with controls. This led the authors to suggest that the enhanced protection may be due to maternal provisioning of antiviral compounds (mRNAs encoding antiviral proteins) in the eggs or reflect epigenetic reprogramming mechanisms. The long-term persistence of the enhanced immune capacities in the offspring needs to be further investigated.

3.4.3 | Environmental stress and enhanced immune capacities

As filter feeders, bivalves evolve in a rich microbial environment with pathogenic and commensal microorganisms that challenge their immune system. This constant interaction challenges their immune system, which may have led to the evolution of immune training mechanisms.[171] Determining the extent to which the natural oyster environment and its microbial content drive immune training would be informative. In mammals and arthropods, commensal microbiota has been shown to shape immune capacities in early life stages and have a systemic effect on the immune response, inducing a form of trained immunity and enhanced resistance to a wide range of unrelated pathogens.[172–176] These findings are reminiscent of the evidence for symbiont-mediated priming, recently reviewed in Refs. 78,177. In *C. gigas*, a recent study showed that larval exposure to a non-infectious environmental microbiota in the laboratory could protect against POMS, both within and across generations.[178] This enhanced immune competence was supported by a long-term reprogramming of immune gene expression and changes in epigenetic marks.[178] Enhanced immune capacities were notably correlated with differential expression of conserved PGRP (lectins, scavenger receptors TLR, RLR, macrophage receptor), innate immune pathways (IFN-TLR-JAK/STAT pathways), and antimicrobial effectors (TNF, proteinases, SOD, interferon-stimulated genes, AMPs). This systemic effect of microbial stimulation conferring protection against a viral disease has also been demonstrated in other vertebrate or invertebrate models.[173–175,179] Interestingly, one study also reported that oysters naturally exposed to a POMS episode in the environment were less susceptible to OsHV-1 21 months later,[180] suggesting a role of immune training in the development of resistance to the disease in the environment.

Regarding abiotic factors, oysters (as sessile organisms) are exposed to constant variations in environmental conditions and especially to thermal stress. Recent studies have shown that high temperatures and harsh environments inhibit the progression of OsHV-1 µVar infection and promote better survival through transcriptomic changes.[181–183] These results suggest that environmental factors could also train immunity at the gene expression level to increase the overall robustness and survival of animals against pathogens.

In conclusion, immune memory phenomena were demonstrated in oysters in response to injection or immersion with heat-killed bacteria, inactivated virus or viral mimic, environmental microbiota, or environmental stress that could increase survival. The response showed no evidence of specificity. The effect of training was observed for up to 5 months after the initial exposure and appears to be transgenerational. Molecular mechanisms have been identified involving phagocytosis, ROS production, apoptosis, and the modification of the expression of many immune genes, but also modifications

of epigenetic marks. The response in these organisms appears to follow a sustained response profile (Figures 1 and 2).

4 | TRAINED IMMUNITY AS A DISEASE CONTROL STRATEGY: RESEARCH PROSPECTS AND POTENTIAL LIMITATIONS

In recent years, these growing number of studies have revealed a new aspect of the immune capacities of cultured marine molluscs. These studies collectively demonstrate that previous exposure to heat-killed or sub-lethal doses of pathogens, environmental microbiota, and microbial-derived compounds can enhance immunity or provide greater protection against future infections. The studies revealed common and distinct features of protection, mechanisms, specificity, or duration, depending on the tissue explored, immune markers, and modes of stimulation used (Table 1 and Figure 2). They also revealed that immune training responses can be as diverse as the species studied, with evidence for either recall, immune shift, sustained, or even tolerance types of responses (Figure 1). Although more reports are emerging on this topic, the cellular and molecular mechanisms behind these responses in marine molluscs are not yet fully understood and remain mostly speculative (see for review Refs. 54,184). The authors have highlighted the functional role of phagocytosis (abalones, oysters), effects on haemocyte populations (abalones, mussels, oysters), apoptosis (mussels, oysters), oxidative stress (oysters, mussels), and the involvement of multiple immune genes from recognition receptors, conserved signalling pathways (IFN-like, Toll/NF-κB pathways), cytokines, and effectors (AMPs; Table 1). These findings are consistent with studies in snails, insects, and mammals where trained immunity leads to more effective antimicrobial responses. This involves phagocytosis, modifications in the density of circulating immune cells, increased cytokine and ROS production, and the involvement of the Toll signalling pathway.[53,114,185–189]

The combined data allow us to improve our knowledge of molluscan immune capacities, and to consider new strategies for disease mitigation in marine mollusc aquaculture. Table 1 highlights the discrepancies between studies exploring these phenomena and illustrates the need to adopt common experimental schemes to draw common or specific patterns between species. In the following sections, we present a framework for future research aimed at developing trained immunity-based applications and addressing its potential limitations (Figure 3). We discuss issues and propose ways to understand (i) how trained immunity can be induced and implemented in aquaculture systems, (ii) the underlying mechanisms of this memory trait, (iii) its potential limitations, and (iv) associated socio-economic issues.

4.1 | Demonstrating and implementing immune training

Various methods have been used to demonstrate trained immunity in invertebrate species, particularly using inactivated bacteria, viruses, non-pathogenic bacteria, or microbial-derived compounds as training agents. While trained immunity appears to be a conserved property in molluscs, it may not be relevant to all pathogen interactions or all mollusc species. These capacities may depend on life histories and the co-evolution of host–pathogen interactions. Therefore, the choice of immuno-stimulant used to test the phenomenon may be crucial for its detection. Trained immunity may be more likely to be detected when exposed to natural pathogens and using natural routes of infection. Studies have also shown that various factors can influence the outcome of immune training and should be considered when comparing results between species or methodologies. The susceptibility to pathogens and immune competence in marine molluscs can vary depending on age, genetics, feeding habits, and environmental factors, as well as sex, circadian cycle, or the presence of other infectious agents or symbionts.[185]

To fully characterize a 'trained state' in different mollusc species, it is important to consider the various markers. Survival against natural pathogens as well as pathogen loads after training should be systematically assessed. It is also important to study immune capacities in different genetic backgrounds to determine whether different populations respond similarly to immune stimulation. As trained immunity appears to depend on the specificity and longevity to recognize and 'remember' pathogens previously encountered, it is crucial to investigate the specificity and persistence of protection over time. Some studies have reported non-specific protective effects. When invertebrates (crustaceans, insects) were injected with an inactivated pathogen or its derived elements, this treatment could confer increased protection against other pathogens (cross-protection).[51,190] This broad response may be advantageous in dealing with polymicrobial or emerging diseases. As for the duration of protection, immune training has been shown to persist throughout the life of the animal and across generations. Studies on insects have shown that the acquisition of training capacities can depend on a specific set point and time of exposition, suggesting higher survival benefits when trained early in life.[191,192] Early development is recognized as a window of vulnerability and opportunity that can affect the developing immune system and lay the foundation for lifelong immunity.[175,176,193,194] Furthermore, there is compelling evidence that early life environments can induce long-lasting changes in the immune system of offspring and have critical impacts on health and disease.[195] Early life stages may thus represent a critical window to imprint the immune system with long-lasting protection from disease.[196]

After establishing the potential to induce trained immunity, the next step towards application is to consider how to produce and expose animals to training agents on a farming scale. The optimization of training induction should evaluate the process of pathogen inactivation, the optimal dosage, and the delivery method. All of these parameters have the potential to affect the immunogenicity of the training agent, its application, and the efficiency and durability of the induced training response. For biosecurity reasons, it is recommended to use inactivated natural pathogens or microbial-derived elements. A recent study showed

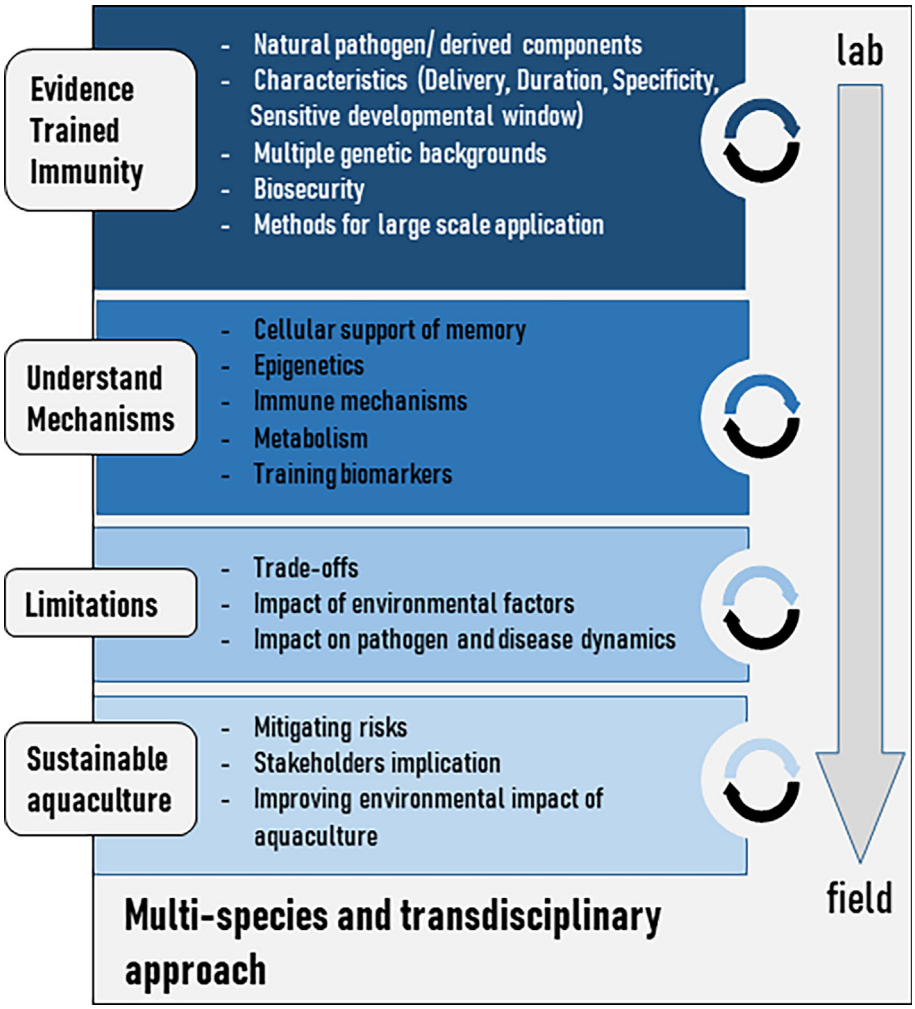

FIGURE 3 A proposed framework for the development of trained immunity investigation and application in cultured marine molluscs. Schematic representation of the different steps proposed to access a comprehensive view of trained immunity in marine molluscs to help develop new strategies for disease mitigation (from laboratory to field). We will need to investigate several species and use a transdisciplinary approach to go from evidencing these phenomena to understanding all its possible limitations to warrant a sustainable development of aquaculture.

that a wide range of potential inactivation methods could be used to induce antiviral immune training in oysters.[197] In addition, it could be necessary to identify the immunogenic fractions (e.g., proteic or nucleic acid components of pathogens) of the training agent, which could be more easily produced and preserved over time. Large-scale methods of treatment should also be investigated. If the majority of training experiments performed in the laboratory were carried out by injecting the animals (filing the shells or after anaesthesia), this method of administration would be difficult to apply on a large scale. Early immune training would greatly benefit future applications in aquaculture, especially when batch exposition through immersion is more practical. For example, several dozens of oysters can produce hundreds of millions of progenies in commercial hatcheries. Although this is a very large number of progenies, it represents a relatively small volume in litres. Investigating trained immunity inheritance over generations could also be a key asset to produce large quantities of trained progenies. Implementing this method in cultured and hatchery-reared animals would be advantageous in terms of practicality, food safety, and ethics.

4.2 | Understanding and characterizing trained immunity mechanisms

Studies on the molecular and cellular foundations of trained immunity in invertebrates indicate that its mechanistic underpinnings might be as diverse as the host–pathogen systems and forms of immunological memory.[34,36] These systems may have co-evolved in response to environmental conditions and cost–benefit trade-offs. They could also be the result of different immune strategies that are not necessarily genetically related but serve a similar general function, allowing individuals to learn from their own immunological experiences. Investigating the molecular and cellular underpinnings of trained immunity could help determine whether the innate immune response of marine molluscs is

a true adaptive response or as some authors have suggested, 'just' due to a 'loitering' immune response.[186] Studying these mechanisms will also help to identify cellular, molecular, metabolic, or epigenetic biomarkers of a trained phenotype that could predict the probability of survival and provide indicators of efficient training in populations.

Recently developed cutting-edge 'omics' technologies could be used to perform integrative approaches and to obtain a comprehensive picture of the mechanisms supporting within-generational or trans-generational trained immunity. These approaches could help to envision the nature of the training response, from receptors to effectors that mediate the protection phenotype, but also to identify original or conserved trained responses and immune factors supporting them. Depending on the training agent and immune pathways involved, diverse responses are likely to be identified.[185] The relative impact of the up-regulation of immune genes or through the poising of the enhanced response can be deciphered. To this end, research on trained immunity must investigate and compare the basal activation state during the initial stimulation and the response developed after the removal of the initial stimulation. Recent studies on several molluscan species have shown that invertebrates have plastic immune effectors that can provide an efficient and specific response to pathogen exposure. In particular, numerous studies have suggested a role for proteins bearing immunoglobulin superfamily domains like DSCAMS or FREPs that function as hypervariable PGRPs.[119,198] Those candidate proteins have been identified in different bivalve species and transcriptomic approaches should help to characterize their role in trained immunity.[32,199]

In contrast to 'classical' adaptive immune memory, trained immunity is not antigen-specific. Instead of being mediated through gene rearrangements, it involves epigenetic and metabolic reprogramming. The rapidly growing fields of epigenetics and metabolomics will allow to further investigate these mechanisms in invertebrates and to determine whether they can target specific immune pathways and cells. Although immune cells have been shown to play a role in trained immunity, particularly in vertebrates, evidence for their role in the induction and long-term storage of memory information in invertebrates is still lacking and requires further investigation. The recent single-cell RNAseq technology should help determine whether trained immunity can differentiate or activate specific haemocyte populations, as well as its impact on cellular functions. The use of proven techniques, such as the adoptive transfer of immune cells and the study of cellular activities (phagocytosis) should also help to decipher the cellular mechanisms and cell types that support memory. Haemocytes are the cells of choice to investigate memory carriers, but the impact of training in other organs and cell tissues should not be overlooked. In mammals, trained immunity depends on the reprogramming of bone marrow immune cells (called central trained immunity) but also on functional changes in peripheral long-lived immune cells or non-immune epithelial and endothelial cells (called peripheral-trained immunity).[53] Different tissues may exhibit varying responses and susceptibility to pathogens and damage, resulting in different immune reactions.

Elucidating the mechanisms of trained immunity could help to design multiple strategies to induce or enhance training by applying biological modifiers that regulate specific immune, metabolic, or epigenetic pathways.

4.3 | Potential limitations of trained immunity

Before implementing trained immunity-based strategies in farms, several potential issues should be addressed.

The potentially profound effects of trained immunity on cellular and metabolic mechanisms and other physiological responses raise the question of possible trade-offs between trained immunity and other physiological traits. Long-lived organisms are likely to face repeated exposure to the same or similar pathogens. Therefore, sustained immune protection and an overall increase in host defence capacity should be beneficial and provide a survival advantage. Despite its benefits in the context of infection, long-term activation of the innate immune system may be a double-edged sword, inducing maladaptive and detrimental effects. Moreover, the phenotypic traits that benefit an organism during one developmental stage may have negative consequences in subsequent stages of life. This is especially true for molluscs, whose developmental stages can vary greatly in form, physiology, and environmental conditions. It has been suggested that trained immunity mechanisms may contribute to the pathogenesis of auto-inflammatory and/or autoimmune diseases in vertebrates.[53] In invertebrates, studies have suggested potential trade-offs between training response and various fitness traits, notably nutrient-demanding processes, such as reproduction, larval development, or other immune defences.[61,200,201] In oysters, enhanced immune capacities in larvae from poly(I:C)-treated females seem to display trade-offs with fitness traits. Transgenerational training seems to impair growing capacities and impact the oyster microbiome. These results call for further research on the effects of trained immunity on physiological responses.[168] This trade-off issue should be considered to understand the adaptive significance of these mechanisms and to anticipate potential limitations for future applications in cultured molluscs.

In addition, previous studies in invertebrates have shown that immune training induction is not restricted to pathogen exposure but can also be modulated by a wide range of environmental factors, including non-pathogenic microbes[62,172,178] and environmental stress conditions.[62–64] A question arises as to whether various natural environments and their microbial content (commensal non-pathogenic microbes or sub-lethal exposure to pathogens) may influence the acquisition of a trained phenotype or the efficiency of protection strategies for animals farmed in the open environment. Cross-talk between immune pathways has indeed been reported, where stimulation by one class of pathogen influences the response to another.[62,172] Studies in vertebrates have also reported impaired

antiviral immunity in the lung after manipulation of commensal bacteria.[174] In oysters, early exposure to environmental microbiota has been shown to durably train the immune response and to increase resistance to a polymicrobial disease.[178] It could be speculated that commensal bacteria modulate responsiveness to pathogens as well as trained capacities and should be further investigated.

Trained immunity could also have ecological and epidemiological impacts by modifying host–pathogen interactions and co-evolution. It can alter epidemiologically relevant parameters such as disease-induced mortality and recovery from infection. Additionally, it can impact pathogen shedding rates, transmission probabilities, and the persistence of diseases in wild and cultured populations.[201–203] Theoretical models have tried to assess the impact of within-generational and trans-generational trained immunity on the evolutionary ecology of host–pathogen interactions by predicting their effects on disease prevalence, but also on the age structure and population dynamics of insects.[204] The developed interaction models suggest that immune training may or may not affect pathogen persistence and disease dynamics under different scenarios. Epidemiological studies in human populations have shown that the effects of non-specific protection induced by vaccination could last for over 5 years.[112] The effects of trained immunity on host–pathogen interactions and disease dynamics could significantly influence trained immunity-based strategies and their implementation in farms. To address how disease can be influenced by control strategies, we need to develop data-driven epidemiological models that account for the trained status. These models should simulate the impact of training on disease transmission dynamics and the output of implementing protection strategies in mollusc farms. On the one hand, trained immunity should lead to a reduction in pathogen circulation in the environment, inducing a virtuous circle. On the other hand, sup-optimal protection could negatively influence disease dynamics in populations. Studies on adaptive immunity have shown that imperfect or 'leaky' vaccination can increase disease prevalence and microbial virulence evolution. It is crucial to prevent these situations from occurring.

4.4 | Socio-economic challenges of solutions based on trained immunity

Trained immunity could significantly contribute to sustainable marine mollusc aquaculture by providing prophylactic approaches to manage disease impact on socio-ecological systems. These emerging techniques could therefore lead to major changes in farming practices and the organization of the aquaculture sector. In this context, the socio-anthropological and socio-economic aspects of this research will be of great importance.

According to the sociology of innovation,[205] one of the main obstacles to technological innovation is its appropriation by the population. This challenge is even greater when changes affect the living world.[206–208] Biotechnological developments can generate controversies that last long after their implementation in the market.[209] These controversies do not only involve ethical issues related to the domestication or artificialization of the living world but also indirect socio-economic impacts such as market structures and the use of territories (e.g., professional and recreative fisheries, tourism).[210,211] In the case of pathogen control in particular, the controversies surrounding biotechnological solutions such as vaccination or the use of antibiotics illustrate the potentially irrational questions that may arise from the lack of involvement of various stakeholders.

Implementing trained immunity-based solutions in the aquaculture sector is not only about gaining acceptance by highlighting the positive outcomes of this technique (improved biosecurity, fewer infected animals transmitting the disease, less impact on the environment) and increasing the control and prevention of pathologies. Instead, scientists need to rethink their approach by involving various stakeholders (from the general population, to public institutions, to farmers and aquaculture farmer organizations) in the early stages of the technological innovation process and involving them in the selection of the most suitable solutions that match their specific needs and resources. Stakeholders, with their diverse backgrounds, are increasingly aware of the complexity of environmental issues and more involved in aquaculture policy-making and management. They are rightfully demanding safer products and coherent global solutions. Their knowledge is a valuable resource for developing innovative solutions and promoting lasting changes in perception and behaviour.

To support the development of technologies in local aquacultural socio-ecological systems, researchers can develop a hybrid network. This network would be composed of aquaculture professional organizations (such as shellfish farming committees and professional hatchery associations), shellfish farming facilities, health and veterinary institutions, and policymakers who follow the steps identified by actor-network theory (Problematization, Interest, Enlistment, and Mobilization).[212] By doing this, they can create and maintain consensus when controversies arise and ensure the *naturalization*[213] of novelty within the various collectives concerned. This may require modifying the initially planned research agendas to meet stakeholder expectations or to consider unexpected factors within aquaculture socio-ecosystems resulting from the application of new technologies, To achieve this, it is necessary to engage with all stakeholders in order to support the development of these innovations and to address the questions and concerns of producers and consumers.

5 | CONCLUSIONS

Over the past two decades, it has become increasingly clear that marine molluscs exhibit incredibly plastic immune responses and that their innate immune system also retains elements of immune memory. Although significant progress has been made in discovering trained immunity capacities in marine molluscs, this review has highlighted the scarcity of studies considering the recent emergence of these concepts in these species. Understanding this novel aspect of immunology is crucial in light of the increasing epizootic disease outbreaks currently affecting marine invertebrates, for which no treatments are currently available. A trained immunity-based strategy

could be a viable alternative or complement to current genetic selection strategies,[214] with the potential for with a possibility of rapid implementation to combat future emerging diseases. This review also emphasizes the need for more comprehensive information in several grey areas. There is still much to learn about how the innate immune system of marine mollusc acquires memory, both within and across generations. We need to understand all the factors that influence its effects, its relationship with animal fitness, and its impact on epidemiology. Future research will shed light on the adaptive strategies and evolutionary history adopted by different species to control pathogens. There is still a long way to go before trained immunity can be applied on a large scale in marine molluscs. However, recent evidence of the development of a vaccination strategy in insects[215] and the potential for vaccine-like approaches in shrimps (see Ref.216, for review) pave the way for future applications in other invertebrates. We anticipate that this field of research will represent an important new approach for developing more efficient prophylactic measures and ensuring sustainable and environmentally sound disease management in marine mollusc aquaculture.

AUTHOR CONTRIBUTIONS

Caroline Montagnani: Conceptualization; visualization; writing—original draft; writing—review and editing; funding acquisition. **Benjamin Morga:** Writing—review and editing. **Beatriz Novoa:** Writing—review and editing; writing—original draft. **Benjamin Gourbal:** Writing—review and editing. **Amaro Saco:** Writing—review and editing. **Magali Rey-Campos:** Writing—review and editing. **Marion Bourhis:** Writing—original draft; writing—review and editing. **Fabien Riera:** Writing—original draft; writing—review and editing. **Emmanuel Vignal:** Writing—review and editing; visualization. **Charlotte Corporeau:** Writing—review and editing. **Guillaume M. Charrière:** Writing—review and editing. **Marie-Agnès Travers:** Writing—review and editing. **Lionel Lionel Dégremont:** Writing—review and editing. **Yannick Gueguen:** Writing—review and editing. **Céline Cosseau:** Writing—review and editing. **Antonio Figueras:** Conceptualization; writing—original draft; writing—review and editing.

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The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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