



HAL
open science

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

Caroline Montagnani, Benjamin Morga, Beatriz Novoa, Benjamin Gourbal, Amaro Saco, Magali Rey-campos, Marion Bourhis, Fabien Riera, Emmanuel Vignal, Charlotte Corporeau, et al.

► To cite this version:

Caroline Montagnani, Benjamin Morga, Beatriz Novoa, Benjamin Gourbal, Amaro Saco, et al.. Trained immunity: Perspectives for disease control strategy in marine mollusc aquaculture. *Reviews in Aquaculture*, 2024, 10.1111/raq.12906 . hal-04565659

HAL Id: hal-04565659

<https://hal.science/hal-04565659>

Submitted on 2 May 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.


















Distributed under a Creative Commons Attribution 4.0 International License

REVIEW

REVIEWS IN Aquaculture

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

Caroline Montagnani¹  | Benjamin Morga²  | Beatriz Novoa³  |
 Benjamin Gourbal¹  | Amaro Saco³  | Magali Rey-Campos³  |
 Marion Bourhis⁴ | Fabien Riera⁴  | Emmanuel Vignal¹  |
 Charlotte Corporeau⁵  | Guillaume M. Charrière¹  | Marie-Agnès Travers¹  |
 Lionel Dégremont²  | Yannick Gueguen⁶  | Céline Cosseau¹  |
 Antonio Figueras³ 

¹IHPE, Université de Montpellier, CNRS, Ifremer, Université de Perpignan, Montpellier, France

²Ifremer, ASIM, La Tremblade, France

³Institute of Marine Research (IIM), National Research Council (CSIC), Vigo, Spain

⁴Géoarchitecture, Université Bretagne Sud, Lorient, France

⁵Ifremer, Université de Brest, CNRS, IRD, LEMAR, Plouzané, France

⁶MARBEC, Univ Montpellier, CNRS, IRD, Ifremer, Sète, France

Correspondence

Caroline Montagnani, UMR 5244 IHPE, CC80, Université de Montpellier- 2, place Eugène Bataillon, 34095 Montpellier, France.
 Email: cmontagn@ifremer.fr

Funding information

Agence Nationale de la Recherche, Grant/Award Number: ANR-22-CE20-0017

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

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Authors. *Reviews in Aquaculture* published by John Wiley & Sons Australia, Ltd.

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- κ B, 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 macro-parasitic,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

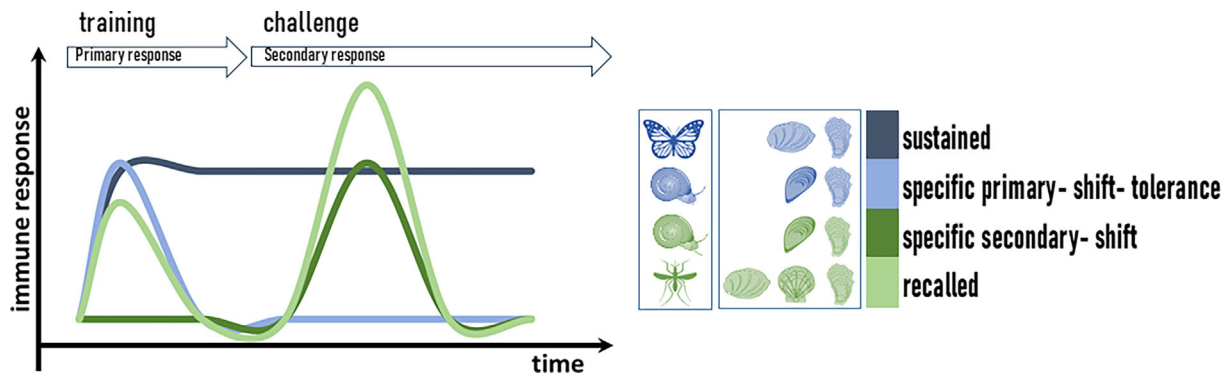


FIGURE 1 Comparative trained immunity response model in invertebrate and aquaculture molluscs. The graph illustrates the diversity of training responses observed in invertebrates and marine molluscs. Immune response over time after training induction (primary response) and challenge (secondary response) 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 bivalves¹²⁹ 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 abalone *Haliotis iris* (Gmelin, 1791),^{134–136} *Haliotis diversicolor* (Reeve, 1846),¹³⁷

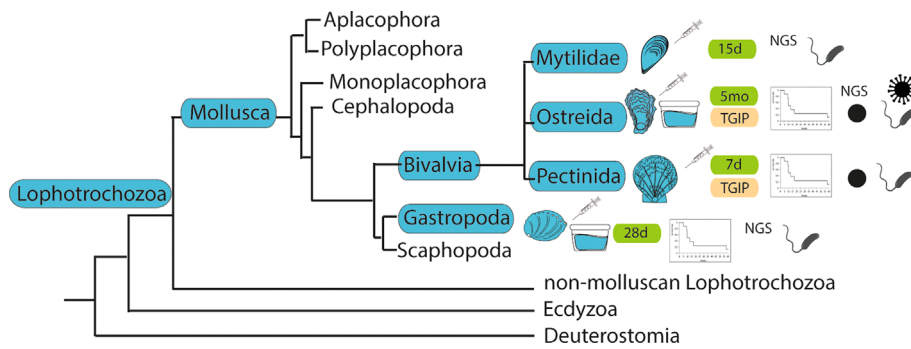


FIGURE 2 Trained immunity evidence in marine molluscs. Illustration of the current knowledge on trained immunity in marine molluscs (marked with blue colour, phylogenetic tree based on Davison and Neiman²¹⁷). The methods used to evidence trained immunity are indicated (injection by a syringe and bath 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 *Vibrio 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 (tolerated 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 Project¹⁴⁹).

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 |
| Abalone <i>Haliotis iris</i> | <i>Exiguobacterium JHEb1</i> , <i>Vibrio JH1</i> , and <i>Enterococcus JHLdC</i> | 3×10^{exp9} CFU g ⁻¹ | Oral diet | total hemocyte count and viability | <i>Vibrio splendidus</i> | Yes | $50 \mu\text{L} @ 5 \times 10^{exp7}$ cfu | 33.3% (non-probiotic-fed) and 22% probiotic-fed mortality | Not investigated | No | no significant differences |
| | | | | oxidative stress | | | | | | | no significant differences |
| | | | | cell apoptosis | | | | | | | significant differences in probiotic-fed animals |
| | | | | metabolomics | | | | | | | no interaction between diet and infection status within the foot muscle metabolome |
| Abalone <i>Haliotis iris</i> | <i>Exiguobacterium JHEb1</i> , <i>Vibrio JH1</i> , and <i>Enterococcus JHLdC</i> | 3×10^{exp9} CFU g ⁻¹ | Oral diet | total hemocyte count and viability | No challenge | | | no mortality in probiotic-fed animals after 4 months, and 10% in controls | not investigated | No | no significant differences |
| | | | | oxidative stress | | | | | | | significantly higher ($19.4 \pm 23.3\%$) ROS-positive cells in probiotic-fed abalones |
| | | | | hemocyte apoptosis | | | | | | | No significant differences |
| | | | | growth | | | | | | | general increase in length, width and weight of the probiotic-fed animals |
| Abalone <i>Haliotis iris</i> | <i>Exiguobacterium JHEb1</i> , <i>Vibrio JH1</i> , and <i>Enterococcus JHLdC</i> | from 2×10^{exp8} CFU/g to 3×10^{exp9} CFU/g | Oral diet | growth | No challenge | | | no mortality in probiotic-fed animals and 10% mortality in controls | Not investigated | No | increase in weight of the probiotic-fed animals |
| | | | | metabolomics | | | | | | | general pattern of enhanced metabolite expressions in probiotic-fed animals |
| Abalone <i>Haliotis tuberculata</i> | <i>V. harveyi</i> (ORM4) | 10^{exp4} CFU/mL | immersion | total hemocyte count (THC) | <i>V. harveyi</i> (ORM4) | Yes | 10^{exp4} CFU/mL | Significant difference after 1 st exposition for St Malo (95%) and Malène (5%) | not investigated | no | significant difference in Malène population 24h after 1 st challenge |
| | | | | phagocytosis | | | | | | | significant inhibition of phagocytosis 24h after 1 st challenge in both populations and 2 days after 2 nd challenge in the Malène population |

(Continues)

TABLE 1 (Continued)

| | | | | | | | | | | | |
|----------------|------------------------------|---------------------------|--|--|-----|---------------------------------------|--|------------------|----|-------------------|--|
| Abalone | <i>Halitatis diversicola</i> | injection | 50 µL of: 1.42 × 10exp3/ 10exp4/ 10exp5/ 10exp6/ 10exp7 CFU/ml | <i>V. harveyi</i> | yes | 50 µL of 1.58 × 10exp6 CFU/mL | After the 1 st immune stimulation, the survival rate decreased with increased pathogen concentration; after the 2 nd stimulation the survival rates in all groups receiving the 1 st immune stimulation were significantly higher with no difference between groups | not investigated | no | Yac, 2021 (137) | although not significant, pathogen loads were smaller in Molène population after the 2 nd challenge no impact of phagocytosis in St. Malo population survivors to the 2 nd challenge as opposed to non infected population where phagocytosis was inhibited ability of <i>V. harveyi</i> 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 | <i>Halitatis hannai</i> | injection | 20µL of 1.0 × 10exp8 CFU/mL solution | <i>V. parahaemolyticus</i> | yes | 100µL of 1.0 × 10exp8 CFU/mL solution | significant increase in survival rate after training | not investigated | no | Zhang, 2022 (138) | Identification of 1019 immune-enhancing regulatory genes (ERGs) and 281 essential immune-enhancing genes (EEGs) from conserved immune pathways (NF-κappa B signaling pathway, NOD-like receptor signaling pathway, IL-17 signaling pathway, and TLR signaling) |
| Clam | <i>Chlamys farreii</i> | injection adductor muscle | 2.6 × 10exp6 CFU | <i>Listonella (Vibrio) anguillarum</i> | yes | 2.6exp6 CFU | no investigated | not investigated | no | Cong, 2009 (139) | recalled (faster and stronger response after the second challenge) |

TABLE 1 (Continued)

| | | | | | | | | | | | | | | |
|---------------|----------------------------------|--|-----------------------|---------------------------|--|----------------------------------|--------------------------------|-----|---|--|---|-----|---|------------------------|
| Clam | <i>Chlamys farreri</i> | <i>Vibrio anguillarum</i> Heat killed | 10exp8 CFU/mL-50µL | injection adductor muscle | Expression level of 5 C-lectin genes (PCR) | 7 days | <i>V. anguillarum</i> | yes | 10exp8 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 resting state and a faster, stronger response after the secondary challenge) 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 than in the Va-Va group | Wang, 2013 (140) |
| | | | | | | | | no | 10exp8 CFU/mL-50µL | no significant difference | | | | |
| Clam | <i>Chlamys farreri</i> | <i>Vibrio anguillarum</i> Heat killed | 100µL@ 1 exp8 cell/mL | injection adductor muscle | agglutination assay qPCR on CtLCPB, CtLec-3, CtLYZ, CtLBP/BPI, Ct SOD Western-Blot with CtLCPB, CtLec-3, CtLYZ, CtLBP/BPI antibodies SOD enzymatic activity bacteriocidal activity assay | 7 days before spawning induction | <i>V. anguillarum</i> | yes | 5exp8el Is/L | significantly higher survival until the D-stage larvae | not investigated | yes | agglutination observed with <i>E. coli</i> , <i>V. anguillarum</i> and <i>P. pastoris</i> , none for <i>S. aureus</i> low transcript detection (except for SOD) in eggs, impact on BPI, LYZ in trocophore and D-shaped larvae compared to control high detection of proteins except for lect3 in eggs, all immune factors protein concentration increased during development except for LCPB and significance dependent on stage SOD activity increased from eggs to trocophore larvae; antibacterial activity increased with a peak at D-stage and then decrease | Yue, 2013 (141) |
| | | | | | | | | | | | | | | |
| Clam | <i>Chlamys farreri</i> | <i>Listonella (Vibrio) anguillarum</i> | 0.1exp8 CFU/mL | Immersion | Phagocytosis Phenoloxidase, acid phosphatase, SOD activities | 8h | <i>L. (Vibrio) anguillarum</i> | 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) |
| | | | | | | | | | | | | | | |
| Mussel | <i>Mytilus galloprovincialis</i> | <i>Vibrio tasmaniensis</i> LCP32 | 10exp7 CFU/mL | injection adductor muscle | <i>V. splendius</i> clearance RNAseq analysis from hemocytes Hemocyte Distribution (FACS) Apoptosis assay ROS Analyses | 2 weeks | <i>V. tasmaniensis</i> LCP32 | no | 10exp7 CFU/mL inj 1 and 10exp8 CFU/mL inj 2 | not investigated | not investigated | no | <i>V. splendius</i> detection increased 24 hpi and was rapidly controlled 7 days after the injection, returning to control levels Identification of 1216 DEG following first challenge and 39 and 31 genes with increased or decreased expression pattern, respectively, after the second encounter restoration of hemocyte population structure after second encounter second bacteria injection did not induce any further increase in the number of apoptotic cells decreased respiratory burst after second exposure | Rey-Campos, 2019 (143) |
| | | | | | | | | | | | | | | |

(Continues)

TABLE 1 (Continued)

| | | | | | | | | | | | | | | |
|---------------|-----------------|--|-----------------------|---------------------------|---|-----------|--------------------------------------|-----|---|--|------------------|----|--|------------------|
| Oyster | <i>C. gigas</i> | heat killed <i>V. splendidus</i> from moribund scallop <i>Pactinopacten yessoensis</i> | 100µl of 2exp8 CFU/mL | injection adductor muscle | RNAseq & immune gene expression on 10 candidate (pPCR) in hemocytes | 7 days | same strain and concentration - live | no | 100µl of 2exp8 CFU/mL | not investigated | not investigated | no | The phagocytic rate of pre-stimulated oysters was significantly increased at 6 h after the secondary challenge (faster response than controls) Significant increase in phosphatase and catalase activity in trained group ROS production was enhanced significantly at 6 h and 24 h after the secondary challenge potential recall and sustained responses: Identification of 2944 common DEGs up-regulated after both stimulations (metabolic processes and immune related pathways); 187 DEGs higher expressed at resting (0h after stimulation) and 55 at activating state (2h after stimulation) of the 2 nd 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 2 nd immune response (MyD88, anti-virulent tissue inhibitor of metalloproteinase, anti-bacterial proline-rich transmembrane protein) | Wang, 2020 (158) |
| | | | | | | | | | | | | | | |
| Oyster | <i>C. gigas</i> | | | Natural exposure | | 21 months | DshV-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) | |

(Continues)

TABLE 1 (Continued)

| | | | | | | | | | | | | | |
|---------------------------|--|---|----------------------------------|---|--------|--|-----|------------------|------------------|--|--|---|---|
| Oyster <i>C. gigas</i> | heat killed <i>V. splendidus</i> from moribund scallop <i>Pactinopecten yessoensis</i> | 100µl of 2exp8 CFU/mL | Injection in the adductor muscle | treatment with methyltransferase inhibitor (MTA) and histone demethylases inhibitor (MEF) Expression of 4 immune-related genes (gPCR, IL-17, MyD88, Rel, TLR3) ChIP-qPCR assays | 7 days | heat killed <i>V. splendidus</i> from moribund scallop <i>Pactinopecten yessoensis</i> | No | Not investigated | Not investigated | No | change in the H3K4me3 enrichment of the CgTLR3 promoter after MTA (decrease) and MEF (increase) treatments | Lian, 2024 (159) | |
| | | | | | | | | | | | | | 100µl of 2exp8 CFU/mL |
| Oyster <i>C. gigas</i> | live <i>V. alginolyticus</i> | 5 × 10exp4, 5 × 10exp5, and 5 × 10exp6 CFU/mL | 24h immersion | ROS | 7 days | live <i>V. alginolyticus</i> | Yes | Not investigated | No | higher levels of ROS after priming and 10 days after challenge except for L16 and lower levels at 15 days post-challenge | Wang, 2024 (160) | | |
| | | | | Vibrio load | | | | | | | | immersion on in 5 × 10exp6 CFU/mL in 30L | no significant differences of vibrio loads in oysters primed with live <i>Vibrio</i> |
| | | | | colonization of bacteria (modified giemsa staining of digestive gland) | | | | | | | | no significant differences with control | no bacteria in digestive gland after challenge |
| | | | | hemocyte morphology | | | | | | | | immersion on in 5 × 10exp6 CFU/mL in 30L | necrotic morphology 15days post-challenge |
| Oyster <i>C. gigas</i> | formalin-inactivated <i>V. alginolyticus</i> | 5 × 10exp4, 5 × 10exp5, and 5 × 10exp6 CFU/mL | 24h immersion | ROS | 7 days | live <i>V. alginolyticus</i> | yes | Not investigated | no | lower levels of ROS after priming and higher levels 10 days after challenge and lower after 15 days | Wang, 2024 (160) | | |
| | | | | colonization of bacteria (modified giemsa staining of digestive gland) | | | | | | | | immersion on in 5 × 10exp4, 5 × 10exp5, 5 × 10exp6 and 92.5% | large number of bacteria after challenge |
| | | | | hemocyte morphology | | | | | | | | immersion on in 5 × 10exp6 CFU/mL in 30L | normal morphology 15 days after challenge |
| | | | | Growth and survival | | | | | | | | immersion on 12 days post-fertilization on in 5 × 10exp6 CFU/mL | primed larvae suffered mass mortality from 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 |

TABLE 1 (Continued)

| | | | | | | | | | | | | | |
|----------------------------------|----------------------------------|---------------------------|---------------------------|---|------------------------|-----------|-----|---|--|--|-----|--|---|
| Oyster <i>C. gigas</i> | poly(I:C) | 50 µL @ 5mg/mL | injection adductor muscle | pathogen load & immune gene expression on 9 candidates (qPCR) | 1 day | OshV-1 | yes | 50 µL @ 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) treated oysters | Green, 2013 (166) |
| | <i>Vibrio tasmaniensis</i> LBP32 | 50 µL @ 10exp8 pfu CFU/ml | | | | | | | | | | poly(I:C) injection up-regulated the response of a toll-like receptor, an interferon regulatory factor and Rel/NF-κB, Cg-IR144 and Cg-PKR | |
| Oyster <i>C. gigas</i> | 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 immunoprecipitation on gill and mantle tissues (MeIP) | | | | | | | | 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 <i>C. gigas</i> | 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-1 and IkappaB2 | |
| Oyster <i>C. gigas</i> | Cg-MB2 or GFP dsRNA | 100 µL @ 0.5 µg/µL | | pathogen load and mRNA expression | 2 days | OshV-1 | yes | 100 µL @ 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 treated oysters | Pauletto, 2017 (169) |
| | | | injection adductor muscle | Expression of immune-related genes in gonads and gills (qPCR) | | | | | | | | | genes except SOCS were significantly more expressed in gonads and gills of Cg-MB2-dsRNA-treated oysters; After infection, candidate genes transcript fold change from dsRNA-treated oysters were lower than in controls |

(Continues)

TABLE 1 (Continued)

| | | | | | | | | | | | | | | | |
|----------------------------------|---------------------------|--|--------------------------------------|--|-----------------------------------|--|-----|---|--|--|-----|---|--------------------|--|--|
| Oyster <i>C. gigas</i> | poly(I:C) /dsRNA/ssRNA | 19 µg/g of oyster for poly(I:C) - 5 µg of dsRNA or ssRNA/ oyster | injection adductor muscle/ immersion | pathogen load | 24h to 5 months | OshV-1 / <i>tasmaniensis</i> LCP32 | yes | 20 µL @ 1x10 ⁶ 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) | | |
| | | | | | | | yes | 20 µL @ 3.5x10 ⁶ exp6 CFU/µL | no significant increase in survival rate for oysters trained with poly(I:C) and infected with bacteria | | no | | | | |
| Oyster <i>C. gigas</i> | poly(I:C) | 100 µL @ 0.5 µg/µL | injection adductor muscle | pathogen load | 3 days or 10 days before spawning | OshV-1 | yes | immersion of larvae in 10exp9 OshV-1 genome copies or field experiment (natural mortality outbreak) | poly(I:C)-treatment of oyster parents results in offspring 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 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 | Lafont, 2019 (161) | | |
| | | | | RNAseq from 3 pools of larvae produced from poly(I:C) treated or control oysters | | | | | | | | | | | |
| | | | | Expression of 10 candidate genes (qPCR) | | | | | | | | | | | |
| Oyster <i>C. gigas</i> | poly(I:C) | 19 µg/g of oyster | injection adductor muscle | pathogen load | 1 day to 5 months | OshV-1 | yes | 20 µL @ 1.32x10 ⁸ 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 | | | | | | | | | | | |
| | | | | Expression of immune-related genes in whole animals (qPCR) | | | | | | | | | | | |
| Oyster <i>C. gigas</i> | environmental microbiota | ND | immersion | Transcriptomic (RNAseq), epigenetic (BS-seq), metabolite-coding analyses | 4 months | OshV-1/PPOMS | yes | 20 µL @ 1.22x10 ⁸ exp8 copies of DP gene/µL | significant increase in survival within and across generations | not investigated | yes | 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-κappaB, apoptosis pathways) A first set of genes shows a sustained upregulation (RLR, sarsin, IFI44, 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) | Fallet, 2022 (178) | | |
| | | | | pathogen load | | | | | | | | | | | |

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-κB, nuclear Factor kappa B; NLHS, Non-lethal heat shock; POMS, Pacific Oyster Mortality Syndrome; qPCR, quantitative PCR; 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 CgGATA3 and CgSOD 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- κ B target gene, CgTIMP, 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. alginolyticus*.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 μ Var. 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 μ Var 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 μ Var, 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- κ B 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 μ Var.^{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 μ Var 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- κ B 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

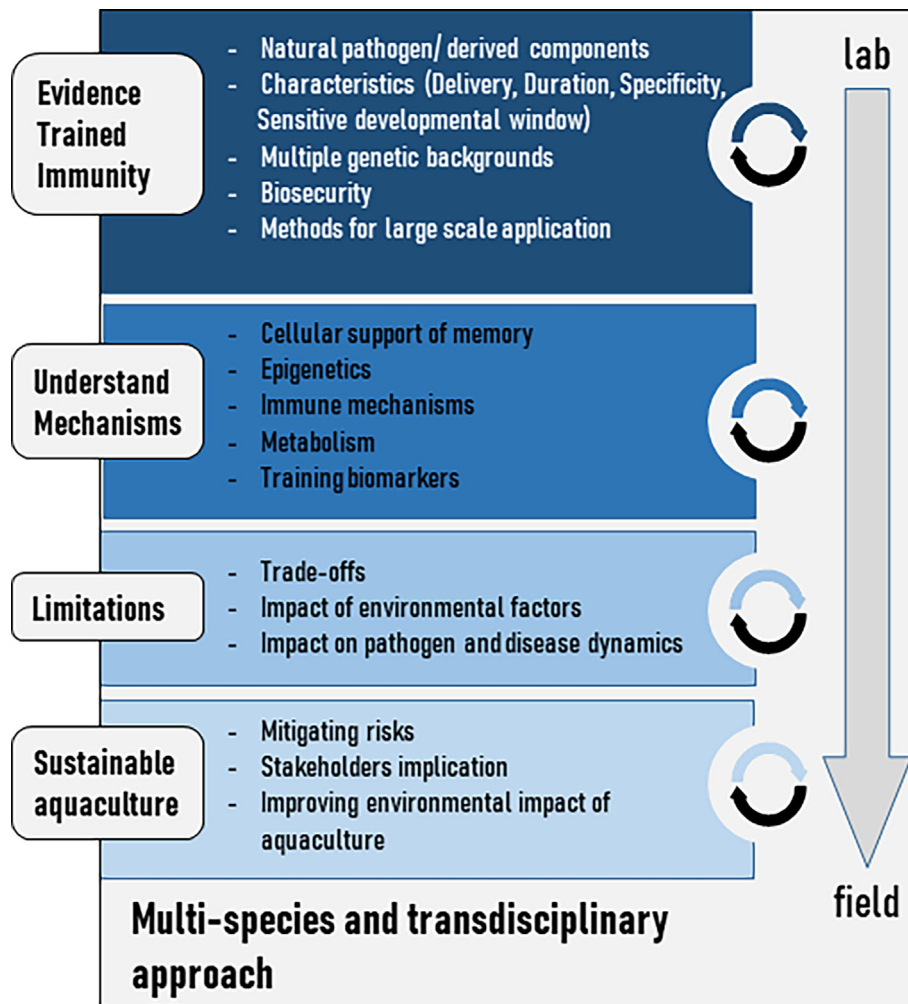


FIGURE 3 A proposed framework for the development of trained immunity investigation and application in cultured marine molluscs. Schematic representation of the different steps proposed to access a comprehensive view of trained immunity in marine molluscs to help develop new strategies for disease mitigation (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, sub-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 insects²¹⁵ 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.

ACKNOWLEDGEMENTS

This work was supported by the project ‘Primoyster’ funded by the French Research Agency (ANR-22-CE20-0017). We thank Maude Jacquot for fruitful revision of the article.

CONFLICT OF INTEREST STATEMENT

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.

ORCID

Caroline Montagnani  <https://orcid.org/0000-0002-0861-7967>

Benjamin Morga  <https://orcid.org/0000-0002-3196-4537>

Beatriz Novoa  <https://orcid.org/0000-0003-4888-8419>

Benjamin Gourbal  <https://orcid.org/0000-0003-2097-2563>

Amaro Saco  <https://orcid.org/0000-0001-7310-9267>

Magali Rey-Campos  <https://orcid.org/0000-0003-3711-8187>

Fabien Riera  <https://orcid.org/0000-0002-9198-7028>

Emmanuel Vignal  <https://orcid.org/0000-0002-2585-119X>

Charlotte Corporeau  <https://orcid.org/0000-0003-1187-358X>

Guillaume M. Charrière  <https://orcid.org/0000-0002-4796-1488>

Marie-Agnès Travers  <https://orcid.org/0000-0001-5340-7435>

Lionel Dégremont  <https://orcid.org/0000-0001-6759-6791>

Yannick Gueguen  <https://orcid.org/0000-0002-8749-9582>

Céline Cosseau  <https://orcid.org/0000-0002-0780-8980>

Antonio Figueras  <https://orcid.org/0000-0002-0617-0030>

REFERENCES

1. Bar-On YM, Phillips R, Milo R. The biomass distribution on Earth. *Proc Natl Acad Sci U S A*. 2018;115:6506–6511.
2. Stentiford GD, Neil DM, Peeler EJ, et al. Disease will limit future food supply from the global crustacean fishery and aquaculture sectors. *J Invertebr Pathol*. 2012;110:141–157.
3. FAO. *Aquaculture Production (Quantities and Values) 1950–2021. Global Aquaculture Production*. Fisheries and Aquaculture Division; 2023.
4. Pernet F, Lupo C, Bacher C, Whittington RJ. Infectious diseases in oyster aquaculture require a new integrated approach. *Philos Trans R Soc Lond B Biol Sci*. 2016;371:20150213.
5. Burge CA, Mark Eakin C, Friedman CS, et al. Climate change influences on marine infectious diseases: implications for management and society. *Ann Rev Mar Sci*. 2014;6:249–277.
6. Rodrigues LC, Van den Bergh JC, Massa F, Theodorou JA, Ziveri P, Gazeau F. Sensitivity of Mediterranean bivalve mollusc aquaculture to climate change, ocean acidification, and other environmental pressures: findings from a producer survey. *J Shellfish Res*. 2015;34:1161–1176.
7. Naylor RL, Williams SL, Strong DR. Ecology. Aquaculture—a gateway for exotic species. *Science*. 2001;294:1655–1656.
8. Escoubas JM, Destoumieux-Garçon D, Montagnani C, et al. Immunity in molluscs. In: Hein WR, ed. *Encyclopedia of Immunobiology*. Elsevier; 2015.
9. de Lorgeril J, Zenagui R, Rosa RD, Piquemal D, Bachere E. Whole transcriptome profiling of successful immune response to vibrio infections in the oyster *Crassostrea gigas* by digital gene expression analysis. *PLoS One*. 2011;6:e23142.
10. Takeuchi T, Kawashima T, Koyanagi R, et al. Draft genome of the pearl oyster *Pinctada fucata*: a platform for understanding bivalve biology. *DNA Res*. 2012;19:117–130.
11. Deleury E, Dubreuil G, Elangovan N, et al. Specific versus non-specific immune responses in an invertebrate species evidenced by a comparative de novo sequencing study. *PLoS One*. 2012;7:e32512.
12. Dheilly NM, Adema C, Raftos DA, et al. No more non-model species: the promise of next generation sequencing for comparative immunology. *Dev Comp Immunol*. 2014;45:56–66.
13. Zhang G, Adema C, Raftos DA, Gourbal B, Grunau C, Du Pasquier L. The oyster genome reveals stress adaptation and complexity of shell formation. *Nature*. 2012;490:49–54.
14. Tomita K, Yokobori S, Oshima T, Ueda T, Watanabe K. The cephalopod *Loligo bleekeri* mitochondrial genome: multiplied noncoding regions and transposition of tRNA genes. *J Mol Evol*. 2002;54:486–500.
15. Cunha RL, Grande C, Zardoya R. Neogastropod phylogenetic relationships based on entire mitochondrial genomes. *BMC Evol Biol*. 2009;9:210.
16. Yuan Y, Li Q, Yu H, Kong L. The complete mitochondrial genomes of six heterodont bivalves (Tellinoidea and Solenoidea): variable gene

- arrangements and phylogenetic implications. *PLoS One*. 2012;7:e32353.
17. Medina M, Lal S, Vallès Y, et al. Crawling through time: transition of snails to slugs dating back to the Paleozoic, based on mitochondrial phylogenomics. *Mar Genomics*. 2011;4:51-59.
 18. Allam B, Espinosa EP. Bivalve immunity and response to infections: are we looking at the right place? *Fish Shellfish Immunol*. 2016;53:4-12.
 19. Bachere E, Rosa RD, Schmitt P, et al. The new insights into the oyster antimicrobial defense: cellular, molecular and genetic view. *Fish Shellfish Immunol*. 2015;46(1):50-64. doi:10.1016/j.fsi.2015.02.040
 20. Zhang L, Li L, Guo X, Litman GW, Dishaw LJ, Zhang G. Massive expansion and functional divergence of innate immune genes in a protostome. *Sci Rep*. 2015;5:8693.
 21. Hellio C, Bado-Nilles A, Gagnaire B, Renault T, Thomas-Guyon H. Demonstration of a true phenoloxidase activity and activation of a ProPO cascade in Pacific oyster, *Crassostrea gigas* (Thunberg) in vitro. *Fish Shellfish Immunol*. 2007;22:433-440.
 22. Wang L, Zhang H, Wang L, et al. The RNA-seq analysis suggests a potential multi-component complement system in oyster *Crassostrea gigas*. *Dev Comp Immunol*. 2017;76:209-219.
 23. Anderson RS. Reactive oxygen species and antimicrobial defenses of invertebrates: a bivalve model. *Adv Exp Med Biol*. 2001;484:131-139.
 24. Lambert C, Soudant P, Jegaden M, et al. In vitro modulation of reactive oxygen and nitrogen intermediate (ROI/RNI) production in *Crassostrea gigas* hemocytes. *Aquaculture*. 2007;270:413-421.
 25. Romero A, Novoa B, Figueras A. Extracellular traps (ETosis) can be activated through NADPH-dependent and -independent mechanisms in bivalve mollusks. *Dev Comp Immunol*. 2020;106:103585.
 26. Green TJ, Raftos D, Speck P, Montagnani C. Antiviral immunity in marine molluscs. *J Gen Virol*. 2015;96:2471-2482.
 27. Wang L, Song X, Song L. The oyster immunity. *Dev Comp Immunol*. 2018;80:99-118.
 28. Gerdol M, Schmitt P, Venier P, Rocha G, Rosa RD, Destoumieux-Garçon D. Functional insights from the evolutionary diversification of big defensins. *Front Immunol*. 2020;11:758.
 29. Destoumieux-Garçon D, Canesi L, Oyanedel D, et al. *Vibrio*-bivalve interactions in health and disease. *Environ Microbiol*. 2020;22:4323-4341.
 30. Green TJ, Speck P, Geng L, et al. Oyster viperin retains direct antiviral activity and its transcription occurs via a signalling pathway involving a heat-stable haemolymph protein. *J Gen Virol*. 2015;96:3587-3597.
 31. Rey-Campos M, Moreira R, Valenzuela-Muñoz V, Gallardo-Escárate C, Novoa B, Figueras A. High individual variability in the transcriptomic response of Mediterranean mussels to *Vibrio* reveals the involvement of myticins in tissue injury. *Sci Rep*. 2019;9:3569.
 32. Wang L, Zhang H, Wang M, et al. The transcriptomic expression of pattern recognition receptors: insight into molecular recognition of various invading pathogens in Oyster *Crassostrea gigas*. *Dev Comp Immunol*. 2019;91:1-7.
 33. Travers MA, Meistertzheim AL, Cardinaud M, et al. Gene expression patterns of abalone, *Haliotis tuberculata*, during successive infections by the pathogen *Vibrio harveyi*. *J Invertebr Pathol*. 2010;105:289-297.
 34. Pradeu T, Du Pasquier L. Immunological memory: what's in a name? *Immunol Rev*. 2018;283:7-20.
 35. Milutinovic B, Kurtz J. Immune memory in invertebrates. *Semin Immunol*. 2016;28:328-342.
 36. Gourbal B, Pinaud S, Beckers GJM, Van Der Meer JWM, Conrath U, Netea MG. Innate immune memory: an evolutionary perspective. *Immunol Rev*. 2018;283:21-40.
 37. Netea MG, Schlitzer A, Placek K, Joosten LAB, Schultze JL. Innate and adaptive immune memory: an evolutionary continuum in the Host's response to pathogens. *Cell Host Microbe*. 2019;25:13-26.
 38. Prado S, Romalde JL, Barja JL. Review of probiotics for use in bivalve hatcheries. *Vet Microbiol*. 2010;145:187-197.
 39. Madison D, Schubiger C, Lunda S, Mueller RS, Langdon C. A marine probiotic treatment against the bacterial pathogen *Vibrio coralliilyticus* to improve the performance of Pacific (*Crassostrea gigas*) and Kumamoto (*C. sikamea*) oyster larvae. *Aquaculture*. 2022;560.
 40. Cordier C, Stavarakakis C, Morga B, et al. Removal of pathogens by ultrafiltration from sea water. *Environ Int*. 2020;142:105809.
 41. Degremont L, Garcia C, Allen SK Jr. Genetic improvement for disease resistance in oysters: a review. *J Invertebr Pathol*. 2015;131:226-241.
 42. Holland OJ, Toomey M, Ahrens C, et al. Whole genome resequencing reveals signatures of rapid selection in a virus-affected commercial fishery. *Mol Ecol*. 2022;31:3658-3671.
 43. Smits M, Enez F, Ferrareso S, et al. Potential for genetic improvement of resistance to *Perkinsus olseni* in the Manila clam, *Ruditapes philippinarum*, using DNA parentage assignment and mass spawning. *Front Vet Sci*. 2020;7:579840.
 44. Richard M, Bourreau J, Montagnani C, et al. Influence of OSHV-1 oyster mortality episode on dissolved inorganic fluxes: an ex situ experiment at the individual scale. *Aquaculture*. 2017;475:40-51.
 45. Richard M, Bec B, Vanhuyse C, et al. Changes in planktonic microbial components in interaction with juvenile oysters during a mortality episode in the Thau lagoon (France). *Aquaculture*. 2019;503:231-241.
 46. Portela J, Duval D, Rognon A, et al. Evidence for specific genotype-dependent immune priming in the lophotrochozoan *Biomphalaria glabrata* snail. *J Innate Immun*. 2013;5:261-276.
 47. Huang XW, Xu MN, Zheng HX, et al. Pre-exposure to *Candida glabrata* protects *Galleria mellonella* against subsequent lethal fungal infections. *Virulence*. 2020;11:1674-1684.
 48. Tidbury HJ, Pedersen AB, Boots M. Within and transgenerational immune priming in an insect to a DNA virus. *Proc Biol Sci*. 2011;278:871-876.
 49. Mondotte JA, Gausson V, Frangeul L, et al. Evidence for long-lasting transgenerational antiviral immunity in insects. *Cell Rep*. 2020;33:108506.
 50. Rechavi O, Minevich G, Hobert O. Transgenerational inheritance of an acquired small RNA-based antiviral response in *C. elegans*. *Cell*. 2011;147:1248-1256.
 51. Chang YH, Kumar R, Ng TH, Wang HC. What vaccination studies tell us about immunological memory within the innate immune system of cultured shrimp and crayfish. *Dev Comp Immunol*. 2018;80:53-66.
 52. Netea MG. Training innate immunity: the changing concept of immunological memory in innate host defence. *Eur J Clin Invest*. 2013;43:881-884.
 53. Netea MG, Domínguez-Andrés J, Barreiro LB, et al. Defining trained immunity and its role in health and disease. *Nat Rev Immunol*. 2020;20:375-388.
 54. Lanz-Mendoza H, Contreras-Garduno J. Innate immune memory in invertebrates: concept and potential mechanisms. *Dev Comp Immunol*. 2022;127:104285.
 55. Kurtz J. Memory in the innate and adaptive immune systems. *Microbes Infect*. 2004;6:1410-1417.
 56. Kurtz J, Franz K. Innate defence: evidence for memory in invertebrate immunity. *Nature*. 2003;425:37-38.
 57. Rodrigues J, Brayner FA, Alves LC, Dixit R, Barillas-Mury C. Hemocyte differentiation mediates innate immune memory in *Anopheles gambiae* mosquitoes. *Science*. 2010;329:1353-1355.
 58. Sadd BM, Schmid-Hempel P. Insect immunity shows specificity in protection upon secondary pathogen exposure. *Curr Biol*. 2006;16:1206-1210.
 59. Cooper EL, Rinkevich B, Uhlenbruck G, Valembos P. Invertebrate immunity: another viewpoint. *Scand J Immunol*. 1992;35:247-266.

60. Roth O, Sadd BM, Schmid-Hempel P, Kurtz J. Strain-specific priming of resistance in the red flour beetle, *Tribolium castaneum*. *Proc Biol Sci*. 2009;276:145-151.
61. Tetreau G, Dhinaut J, Gourbal B, Moret Y. Trans-generational immune priming in invertebrates: current knowledge and future prospects. *Front Immunol*. 2019;10:1938.
62. Eggert H, Diddens-de Buhr MF, Kurtz J. A temperature shock can lead to trans-generational immune priming in the red flour beetle, *Tribolium castaneum*. *Ecol Evol*. 2015;5:1318-1326.
63. Norouzitallab P, Baruah K, Vandegehuchte M, et al. Environmental heat stress induces epigenetic transgenerational inheritance of robustness in parthenogenetic *Artemia* model. *FASEB J*. 2014;28:3552-3563.
64. Mydlarz LD, Jones LE, Harvell CD. Innate immunity environmental drivers and disease ecology of marine and freshwater invertebrates. *Annu Rev Ecol Evol S*. 2006;37:251-288.
65. Encomio VG, Chu FLE. Heat shock protein (hsp70) expression and thermal tolerance in sublethally heat-shocked eastern oysters *Crassostrea virginica* infected with the parasite *Perkinsus marinus*. *Dis Aquat Organ*. 2007;76:251-260.
66. Mowlds P, Barron A, Kavanagh K. Physical stress primes the immune response of *Galleria mellonella* larvae to infection by *Candida albicans*. *Microbes Infect*. 2008;10:628-634.
67. Mowlds P, Kavanagh K. Effect of pre-incubation temperature on susceptibility of *Galleria mellonella* larvae to infection by *Candida albicans*. *Mycopathologia*. 2008;165:5-12.
68. Browne N, Surlis C, Kavanagh K. Thermal and physical stresses induce a short-term immune priming effect in *Galleria mellonella* larvae. *J Insect Physiol*. 2014;63:21-26.
69. Wojda I, Jakubowicz T. Humoral immune response upon mild heat-shock conditions in *Galleria mellonella* larvae. *J Insect Physiol*. 2007; 53:1134-1144.
70. Anyanful A, Easley KA, Benian GM, Kalman D. Conditioning protects *C. elegans* from lethal effects of enteropathogenic *E. coli* by activating genes that regulate lifespan and innate immunity. *Cell Host Microbe*. 2009;5:450-462.
71. Roberts RJ, Agius C, Saliba C, Bossier P, Sung YY. Heat shock proteins (chaperones) in fish and shellfish and their potential role in relation to fish health: a review. *J Fish Dis*. 2010;33:789-801.
72. Baruah K, Ranjan J, Sorgeloos P, Bossier P. Efficacy of heterologous and homologous heat shock protein 70s as protective agents to *Artemia franciscana* challenged with *Vibrio campbellii*. *Fish Shellfish Immun*. 2010;29:733-739.
73. Yue X, Liu BZ, Sun L, Tang BJ. Cloning and characterization of a hsp70 gene from Asiatic hard clam *Meretrix meretrix* which is involved in the immune response against bacterial infection. *Fish Shellfish Immun*. 2011;30:791-799.
74. Delisle L, Pauletto M, Vidal-Dupiol J, et al. High temperature induces transcriptomic changes in *Crassostrea gigas* that hinders progress of *Ostreid herpesvirus* (OsHV-1) and promotes survival. *J Exp Biol*. 2020; 223(20). doi:10.1242/jeb.226233
75. Lin YR, Hung HC, Leu JH, Wang HC, Kou GH, Lo CF. The role of aldehyde dehydrogenase and hsp70 in suppression of white spot syndrome virus replication at high temperature. *J Virol*. 2011;85: 3517-3525.
76. Vabulas RM, Ahmad-Nejad P, Ghose S, Kirschning CJ, Issels RD, Wagner H. HSP70 as endogenous stimulus of the toll/interleukin-1 receptor signal pathway. *J Biol Chem*. 2002;277:15107-15112.
77. Plant KP, LaPatra SE, Cain KD. Vaccination of rainbow trout, *Oncorhynchus mykiss* (Walbaum), with recombinant and DNA vaccines produced to *Flavobacterium psychrophilum* heat shock proteins 60 and 70. *J Fish Dis*. 2009;32:521-534.
78. Prigot-Maurice C, Beltran-Bech S, Braquart-Varnier C. Why and how do protective symbionts impact immune priming with pathogens in invertebrates? *Dev Comp Immunol*. 2022;126:104245.
79. Coustau C, Kurtz J, Moret Y. A novel mechanism of immune memory unveiled at the invertebrate-parasite interface. *Trends Parasitol*. 2016;32:353-355.
80. Divangahi M, Aaby P, Khader SA, et al. Trained immunity, tolerance, priming and differentiation: distinct immunological processes. *Nat Immunol*. 2021;22:2-6.
81. Boraschi D, Italiani P. Innate immune memory: time for adopting a correct terminology. *Front Immunol*. 2018;9:799.
82. Serrato-Salas J, Izquierdo-Sánchez J, Argüello M, et al. *Aedes aegypti* antiviral adaptive response against DENV-2. *Dev Comp Immunol*. 2018;84:28-36.
83. Contreras-Garduno J, Rodríguez MC, Hernández-Martínez S, et al. *Plasmodium berghei* induced priming in *Anopheles albimanus* independently of bacterial co-infection. *Dev Comp Immunol*. 2015;52: 172-181.
84. Greenwood JM, Milutinović B, Peuß R, et al. Oral immune priming with *Bacillus thuringiensis* induces a shift in the gene expression of *Tribolium castaneum* larvae. *BMC Genomics*. 2017; 18:329.
85. Pinaud S, Portela J, Duval D, et al. A shift from cellular to humoral responses contributes to innate immune memory in the vector snail *Biomphalaria glabrata*. *PLoS Pathog*. 2016;12:e1005361.
86. Johnston PR, Makarova O, Roff J. Inducible defenses stay up late: temporal patterns of immune gene expression in *Tenebrio molitor*. *G3 (Bethesda)*. 2013;4:947-955.
87. Mikonranta L, Mappes J, Kaukoniitty M, Freitak D. Insect immunity: oral exposure to a bacterial pathogen elicits free radical response and protects from a recurring infection. *Front Zool*. 2014;11:23.
88. Lafont M, Vergnes A, Vidal-Dupiol J, et al. A sustained immune response supports long-term antiviral immune priming in the Pacific oyster, *Crassostrea gigas*. *mBio*. 2020;11:e02777-19.
89. Prigot-Maurice C, de Cerqueira De A, Araujo S, Beltran-Bech CB-V. Immune priming depends on age, sex and *Wolbachia* in the interaction between *Armadillidium vulgare* and *Salmonella*. *J Evol Biol*. 2021; 34:256-269.
90. Eleftherianos I, Marokhazi J, Millichap PJ, et al. Prior infection of *Manduca sexta* with non-pathogenic *Escherichia coli* elicits immunity to pathogenic *Photographus luminescens*: roles of immune-related proteins shown by RNA interference. *Insect Biochem Mol Biol*. 2006; 36:517-525.
91. Barshis DJ, Ladner JT, Oliver TA, et al. Genomic basis for coral resilience to climate change. *Proc Natl Acad Sci U S A*. 2013;110:1387-1392.
92. Gurr SJ, Trigg SA, Vadopalas B, Roberts SB, Putnam HM. Acclimatory gene expression of primed clams enhances robustness to elevated pCO₂. *Mol Ecol*. 2022;31:5005-5023.
93. Jeria E, Oyanedel D, Rojas R, et al. Resistance of *Argopecten purpuratus* scallop larvae to vibriosis is associated with the front-loading of immune genes and enhanced antimicrobial response. *Front Immunol*. 2023;14:1150280.
94. Vidal-Dupiol J, Harscouet E, Shefy D, et al. Frontloading of stress response genes enhances robustness to environmental change in chimeric corals. *BMC Biol*. 2022;20:167.
95. Collins M, Clark MS, Spicer JI, Truebano M. Transcriptional frontloading contributes to cross-tolerance between stressors. *Evol Appl*. 2021;14:577-587.
96. Brenner-Raffalli K, Vidal-Dupiol J, Adjeroud M, et al. Gene expression plasticity and frontloading promote thermotolerance in Pocilloporid corals. 2018. bioRxiv. doi:10.1101/398602
97. Vilcinskas A. The role of epigenetics in host-parasite coevolution: lessons from the model host insects *Galleria mellonella* and *Tribolium castaneum*. *Zoology (Jena)*. 2016;119:273-280.

98. Laubach ZM, Perng W, Dolinoy DC, et al. Epigenetics and the maintenance of developmental plasticity: extending the signalling theory framework. *Biol Rev Camb Philos Soc.* 2018;93:1323-1338.
99. Champagne FA. Epigenetics and developmental plasticity across species. *Dev Psychobiol.* 2013;55:33-41.
100. Arts RJ, Joosten LA, Netea MG. Immunometabolic circuits in trained immunity. *Semin Immunol.* 2016;28:425-430.
101. Penkov S, Mitroulis I, Hajishengallis G, Chavakis T. Immunometabolic crosstalk: an ancestral principle of trained immunity? *Trends Immunol.* 2019;40:1-11.
102. Dominguez-Andres J, Netea MG. Long-term reprogramming of the innate immune system. *J Leukoc Biol.* 2019;105:329-338.
103. Fallet M, Luquet E, David P, Cosseau C. Epigenetic inheritance and intergenerational effects in mollusks. *Gene.* 2020;729:144166.
104. McCoy KD, Burkhard R, Geuking MB. The microbiome and immune memory formation. *Immunol Cell Biol.* 2019;97:625-635.
105. Barno AR, Vilella HDM, Aranda M, Thomas T, Peixoto RS. Host under epigenetic control: a novel perspective on the interaction between microorganisms and corals. *Bioessays.* 2021;43:e2100068.
106. Eirin-Lopez JM, Putnam HM. Marine environmental epigenetics. *Ann Rev Mar Sci.* 2019;11:335-368.
107. Fallet M, Blanc M, Di Criscio M, et al. Present and future challenges for the investigation of transgenerational epigenetic inheritance. *Environ Int.* 2023;172:107776.
108. Fuse N, Okamori C, Okaji R, Tang C, Hirai K, Kurata S. Transcriptome features of innate immune memory in *Drosophila*. *PLoS Genet.* 2022;18:e1010005.
109. Gegner J, Baudach A, Mukherjee K, Halitschke R, Vogel H, Vilcinskas A. Epigenetic mechanisms are involved in sex-specific trans-generational immune priming in the lepidopteran model host *Manduca sexta*. *Front Physiol.* 2019;10:137.
110. Vilcinskas A. Mechanisms of transgenerational immune priming in insects. *Dev Comp Immunol.* 2021;124:104205.
111. Norouzitallab P, Baruah K, Biswas P, Vanrompay D, Bossier P. Probing the phenomenon of trained immunity in invertebrates during a transgenerational study, using brine shrimp *Artemia* as a model system. *Sci Rep.* 2016;6:21166.
112. Geckin B, Konstantin Fohse F, Dominguez-Andres J, Netea MG. Trained immunity: implications for vaccination. *Curr Opin Immunol.* 2022;77:102190.
113. Ciarlo E, Heinonen T, Théroude C, et al. Trained immunity confers broad-spectrum protection against bacterial infections. *J Infect Dis.* 2020;222:1869-1881.
114. Pham LN, Dionne MS, Shirasu-Hiza M, Schneider DS. A specific primed immune response in *Drosophila* is dependent on phagocytes. *PLoS Pathog.* 2007;3:e26.
115. Powell A, Pope EC, Eddy FE, et al. Enhanced immune defences in Pacific white shrimp (*Litopenaeus vannamei*) post-exposure to a vibrio vaccine. *J Invertebr Pathol.* 2011;107:95-99.
116. Johnson KN, van Hulten MC, Barnes AC. "Vaccination" of shrimp against viral pathogens: phenomenology and underlying mechanisms. *Vaccine.* 2008;26:4885-4892.
117. Moret Y. Explaining variable costs of the immune response: selection for specific versus non-specific immunity and facultative life history change. *Oikos.* 2003;102:213-216.
118. Schulenburg H, Boehnisch C, Michiels NK. How do invertebrates generate a highly specific innate immune response? *Mol Immunol.* 2007;44:3338-3344.
119. Li W. Dscam in arthropod immune priming: what is known and what remains unknown. *Dev Comp Immunol.* 2021;125:104231.
120. Roth O, Kurtz J. Phagocytosis mediates specificity in the immune defence of an invertebrate, the woodlouse *Porcellio scaber* (Crustacea: isopoda). *Dev Comp Immunol.* 2009;33:1151-1155.
121. Sheehan G, Farrell G, Kavanagh K. Immune priming: the secret weapon of the insect world. *Virulence.* 2020;11:238-246.
122. Moret Y. "Trans-generational immune priming": specific enhancement of the antimicrobial immune response in the mealworm beetle, *Tenebrio molitor*. *Proc Biol Sci.* 2006;273:1399-1405.
123. Baruah K, Norouzitallab P, Li SH, Sorgeloos P, Bossier P. Feeding truncated heat shock protein 70s protect *Artemia franciscana* against virulent *Vibrio campbellii* challenge. *Fish Shellfish Immunol.* 2013;34:183-191.
124. Bergin D, Murphy L, Keenan J, Clynes M, Kavanagh K. Pre-exposure to yeast protects larvae of *Galleria mellonella* from a subsequent lethal infection by *Candida albicans* and is mediated by the increased expression of antimicrobial peptides. *Microbes Infect.* 2006;8:2105-2112.
125. Tassetto M, Kunitomi M, Andino R. Circulating immune cells mediate a systemic RNAi-based adaptive antiviral response in *Drosophila*. *Cell.* 2017;169:314-325 e313.
126. Sterken MG, Snoek LB, Bosman KJ, et al. A heritable antiviral RNAi response limits Orsay virus infection in *Caenorhabditis elegans* N2. *PLoS One.* 2014;9:e89760.
127. Sadd BM, Kleinlogel Y, Schmid-Hempel R, Schmid-Hempel P. Trans-generational immune priming in a social insect. *Biol Lett.* 2005;1:386-388.
128. Sadd BM, Schmid-Hempel P. Facultative but persistent trans-generational immunity via the mother's eggs in bumblebees. *Curr Biol.* 2007;17:R1046-R1047.
129. FAO, Cultured Aquatic Species Information Programme. *Crassostrea gigas*. Cultured Aquatic Species Information Programme. FAO Fisheries and Aquaculture Department; 2016. http://www.fao.org/fishery/culturedspecies/Crassostrea_gigas/en
130. Dang VT, Benkendorff K, Speck P. In vitro antiviral activity against herpes simplex virus in the abalone *Haliotis laevigata*. *J Gen Virol.* 2011;92:627-637.
131. Nicolas JL, Basuyaux O, Mazurie J, Thebault A. *Vibrio carchariae*, a pathogen of the abalone *Haliotis tuberculata*. *Dis Aquat Organ.* 2002;50:35-43.
132. Travers MA, Le Goic N, Huchette S, Koken M, Paillard C. Summer immune depression associated with increased susceptibility of the European abalone, *Haliotis tuberculata* to *Vibrio harveyi* infection. *Fish Shellfish Immunol.* 2008;25:800-808.
133. Dubief B, Nunes FLD, Basuyaux O, Paillard C. Immune priming and portal of entry effectors improve response to vibrio infection in a resistant population of the European abalone. *Fish Shellfish Immunol.* 2017;60:255-264.
134. Grandiosa R, Young T, Van Nguyen T, Merien F, Alfaro AC. Immune response in probiotic-fed New Zealand black-footed abalone (*Haliotis iris*) under *Vibrio splendidus* challenge. *Fish Shellfish Immunol.* 2020;104:633-639.
135. Hadi JA, Gutierrez N, Alfaro AD, Roberts RD. Use of probiotic bacteria to improve growth and survivability of farmed New Zealand abalone (*Haliotis iris*). *N Z J Mar Freshw Res.* 2014;48:405-415.
136. Grandiosa R, Mérien F, Young T, et al. Multi-strain probiotics enhance immune responsiveness and alters metabolic profiles in the New Zealand black-footed abalone (*Haliotis iris*). *Fish Shellfish Immunol.* 2018;82:330-338.
137. Yao T, Lu J, Bai C, Xie Z, Ye L. The enhanced immune protection in small abalone *Haliotis diversicolor* against a secondary infection with *Vibrio harveyi*. *Front Immunol.* 2021;12:685896.
138. Zhang X, Guo M, Sun Y, Wang Y, Zhang Z. Transcriptomic analysis and discovery of genes involving in enhanced immune protection of Pacific abalone (*Haliotis discus hannai*) in response to the re-infection of *Vibrio parahaemolyticus*. *Fish Shellfish Immunol.* 2022;125:128-140.
139. Cong M, Song L, Qiu L, et al. The expression of peptidoglycan recognition protein-S1 gene in the scallop *Chlamys farreri* was enhanced after a second challenge by *Listonella anguillarum*. *J Invertebr Pathol.* 2009;100:120-122.

140. Wang J, Wang L, Yang C, et al. The response of mRNA expression upon secondary challenge with *Vibrio anguillarum* suggests the involvement of C-lectins in the immune priming of scallop *Chlamys farreri*. *Dev Comp Immunol*. 2013;40:142-147.
141. Yue F, Zhou Z, Wang L, et al. Maternal transfer of immunity in scallop *Chlamys farreri* and its trans-generational immune protection to offspring against bacterial challenge. *Dev Comp Immunol*. 2013;41:569-577.
142. Cong M, Song L, Wang L, et al. The enhanced immune protection of Zhikong scallop *Chlamys farreri* on the secondary encounter with *Listonella anguillarum*. *Comp Biochem Physiol B Biochem Mol Biol*. 2008;151:191-196.
143. Rey-Campos M, Moreira R, Gerdol M, Pallavicini A, Novoa B, Figueras A. Immune tolerance in *Mytilus galloprovincialis* hemocytes after repeated contact with *Vibrio splendidus*. *Front Immunol*. 2019;10:1894.
144. Detree C, Gallardo-Escarate C. Single and repetitive microplastics exposures induce immune system modulation and homeostasis alteration in the edible mussel *Mytilus galloprovincialis*. *Fish Shellfish Immunol*. 2018;83:52-60.
145. Romero A, del Mar Costa M, Forn-Cuni G, et al. Occurrence, seasonality and infectivity of vibrio strains in natural populations of mussels *Mytilus galloprovincialis*. *Dis Aquat Organ*. 2014;108:149-163.
146. Balbi T, Fabbri R, Cortese K, et al. Interactions between *Mytilus galloprovincialis* hemocytes and the bivalve pathogens *Vibrio aestuarianus* 01/032 and *Vibrio splendidus* LGP32. *Fish Shellfish Immunol*. 2013;35:1906-1915.
147. Canesi L, Grande C, Pezzati E, Balbi T, Vezzulli L, Pruzzo C. Killing of *Vibrio cholerae* and *Escherichia coli* strains carrying D-mannose-sensitive ligands by *Mytilus* Hemocytes is promoted by a multifunctional *Hemolymph serum* protein. *Microb Ecol*. 2016;72:759-762.
148. Pezzati E, Canesi L, Damonte G, et al. Susceptibility of *Vibrio aestuarianus* 01/032 to the antibacterial activity of *Mytilus haemolymph*: identification of a serum opsonin involved in mannose-sensitive interactions. *Environ Microbiol*. 2015;17:4271-4279.
149. Gerdol M, Moreira R, Cruz F, et al. Massive gene presence-absence variation shapes an open pan-genome in the *Mediterranean mussel*. *Genome Biol*. 2020;21:275.
150. Gerdol M, Venier P. An updated molecular basis for mussel immunity. *Fish Shellfish Immunol*. 2015;46(1):17-38. doi:10.1016/j.fsi.2015.02.013
151. Aleng NA, Sung YY, MacRae TH, Abd Wahid ME. Non-lethal heat shock of the Asian Green Mussel, *Perna viridis*, promotes Hsp70 synthesis, induces Thermotolerance and protects against vibrio infection. *PLoS One*. 2015;10:e0135603.
152. Auguste M, Balbi T, Ciacci C, et al. Shift in immune parameters after repeated exposure to nanoplastics in the marine bivalve *Mytilus*. *Front Immunol*. 2020;11:426.
153. Zhang L, Li L, Zhu Y, Zhang G, Guo X. Transcriptome analysis reveals a rich gene set related to innate immunity in the eastern oyster (*Crassostrea virginica*). *Mar Biotechnol (NY)*. 2014;16:17-33.
154. Green TJ, Vergnes A, Montagnani C, de Lorgeril J. Distinct immune responses of juvenile and adult oysters (*Crassostrea gigas*) to viral and bacterial infections. *Vet Res*. 2016;47:72.
155. Zhang T, Qiu L, Sun Z, et al. The specifically enhanced cellular immune responses in Pacific oyster (*Crassostrea gigas*) against secondary challenge with *Vibrio splendidus*. *Dev Comp Immunol*. 2014;45:141-150.
156. Li Y, Song X, Wang W, et al. The hematopoiesis in gill and its role in the immune response of Pacific oyster *Crassostrea gigas* against secondary challenge with *Vibrio splendidus*. *Dev Comp Immunol*. 2017;71:59-69.
157. Liu C, Zhang T, Wang L, et al. The modulation of extracellular superoxide dismutase in the specifically enhanced cellular immune response against secondary challenge of *Vibrio splendidus* in Pacific oyster (*Crassostrea gigas*). *Dev Comp Immunol*. 2016;63:163-170.
158. Wang W, Wang L, Liu Z, et al. The involvement of TLR signaling and anti-bacterial effectors in enhanced immune protection of oysters after *Vibrio splendidus* pre-exposure. *Dev Comp Immunol*. 2020;103:103498.
159. Lian X, Li Y, Wang W, et al. The modification of H3K4me3 enhanced the expression of CgTLR3 in Hemocytes to increase CgIL17-1 production in the immune priming of *Crassostrea gigas*. *Int J Mol Sci*. 2024;25:1036.
160. Wang H, Yang B, Li Q, Liu S. Low-dose of formalin-inactivated *Vibrio alginolyticus* protects *Crassostrea gigas* from secondary infection and confers broad-spectrum *Vibrio* resistance on offspring. *Dev Comp Immunol*. 2024;152:105122.
161. Lafont M, Goncalves P, Guo X, Montagnani C, Raftos D, Green T. Transgenerational plasticity and antiviral immunity in the Pacific oyster (*Crassostrea gigas*) against Ostreid herpesvirus 1 (OsHV-1). *Dev Comp Immunol*. 2019;91:17-25.
162. Lafont M, Petton B, Vergnes A, et al. Long-lasting antiviral innate immune priming in the Lophotrochozoan Pacific oyster, *Crassostrea gigas*. *Sci Rep*. 2017;7:13143.
163. Martins NE. Towards a mechanism for poly(I:C) antiviral priming in oysters. *MBio*. 2020;11:e00407-20.
164. Green TJ, Benkendorff K, Robinson N, Raftos D, Speck P. Anti-viral gene induction is absent upon secondary challenge with double-stranded RNA in the Pacific oyster, *Crassostrea gigas*. *Fish Shellfish Immunol*. 2014;39:492-497.
165. Green TJ, Helbig K, Speck P, Raftos DA. Primed for success: oyster parents treated with poly(I:C) produce offspring with enhanced protection against Ostreid herpesvirus type I infection. *Mol Immunol*. 2016;78:113-120.
166. Green TJ, Montagnani C. Poly I:C induces a protective antiviral immune response in the Pacific oyster (*Crassostrea gigas*) against subsequent challenge with Ostreid herpesvirus (OsHV-1 muvar). *Fish Shellfish Immunol*. 2013;35:382-388.
167. Green TJ, Speck P. Antiviral defense and innate immune memory in the oyster. *Viruses*. 2018;10:133.
168. Robinson AN, Green TJ. Fitness costs associated with maternal immune priming in the oyster. *Fish Shellfish Immunol*. 2020;103:32-36.
169. Pualetto M, Segarra A, Montagnani C, et al. Long dsRNAs promote an anti-viral response in Pacific oyster hampering ostreid herpesvirus 1 replication. *J Exp Biol*. 2017;220:3671-3685.
170. Mauch-Mani B, Baccelli I, Luna E, Flors V. Defense priming: an adaptive part of induced resistance. *Annu Rev Plant Biol*. 2017;68:485-512.
171. Dupont S, Lokmer A, Corre E, et al. Oyster hemolymph is a complex and dynamic ecosystem hosting bacteria, protists and viruses. *Anim Microbiome*. 2020;2:12.
172. Abt MC, Osborne LC, Monticelli LA, et al. Commensal bacteria calibrate the activation threshold of innate antiviral immunity. *Immunity*. 2012;37:158-170.
173. Sansone CL, Cohen J, Yasunaga A, et al. Microbiota-dependent priming of antiviral intestinal immunity in *Drosophila*. *Cell Host Microbe*. 2015;18:571-581.
174. Ichinohe T, Pang IK, Kumamoto Y, et al. Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proc Natl Acad Sci U S A*. 2011;108:5354-5359.
175. Carlson JS, Short SM, Anglero-Rodriguez YI, Dimopoulos G. Larval exposure to bacteria modulates arbovirus infection and immune gene expression in adult *Aedes aegypti*. *Dev Comp Immunol*. 2020;104:103540.
176. Galindo-Villegas J, Garcia-Moreno D, de Oliveira S, Meseguer J, Mulero V. Regulation of immunity and disease resistance by commensal microbes and chromatin modifications during zebrafish development. *Proc Natl Acad Sci U S A*. 2012;109:E2605-E2614.

177. Hoang KL, King KC. Symbiont-mediated immune priming in animals through an evolutionary lens. *Microbiology (Reading)*. 2022;168(4). <https://doi.org/10.1099/mic.0.001181>
178. Fallet M, Montagnani C, Petton B, et al. Early life microbial exposures shape the *Crassostrea gigas* immune system for life-long and intergenerational disease protection. *Microbiome*. 2022; 10:85.
179. Erttmann SF, Swacha P, Aung KM, et al. The gut microbiota prime systemic antiviral immunity via the cGAS-STING-IFN-I axis. *Immunity*. 2022;55:847-861.e810.
180. Liu OM, Hick PM, Whittington RJ. The resistance to lethal challenge with Ostreid herpesvirus-1 of Pacific oysters (*Crassostrea gigas*) previously exposed to this virus. *Viruses*. 2023;15:1706.
181. Delisle L, Pauletto M, Vidal-Dupiol J, et al. High temperature induces transcriptomic changes in *Crassostrea gigas* that hinder progress of ostreid herpesvirus (OsHV-1) and promote survival. *J Exp Biol*. 2020; 223:jeb226233.
182. Delisle L, Petton B, Burguin JF, Morga B, Corporeau C, Pernet F. Temperature modulate disease susceptibility of the Pacific oyster *Crassostrea gigas* and virulence of the Ostreid herpesvirus type 1. *Fish Shellfish Immun*. 2018;80:71-79.
183. Corporeau C, Petton S, Vilaça R, et al. Harsh intertidal environment enhances metabolism and immunity in oyster (*Crassostrea gigas*) spat. *Mar Environ Res*. 2022;180:105709.
184. Yang W, Petton S, Vilaça R, et al. Immune priming in shellfish: a review and an updating mechanistic insight focused on cellular and humoral responses. *Aquaculture*. 2021;530:735831.
185. Arch M, Vidal M, Koiffman R, Melkie ST, Cardona PJ. *Drosophila melanogaster* as a model to study innate immune memory. *Front Microbiol*. 2022;13:991678.
186. Cooper D, Eleftherianos I. Memory and specificity in the insect immune system: current perspectives and future challenges. *Front Immunol*. 2017;8:539.
187. Yi Y, Xu H, Li M, Wu G. RNA-seq profiles of putative genes involved in specific immune priming in *Bombyx mori* haemocytes. *Infect Genet Evol*. 2019;74:103921.
188. Wu G, Li M, Liu Y, Ding Y, Yi Y. The specificity of immune priming in silkworm, *Bombyx mori*, is mediated by the phagocytic ability of granular cells. *J Insect Physiol*. 2015;81:60-68.
189. de Melo ES, Brayner FA, Junior NCP, Franca IRS, Alves LC. Investigation of defense response and immune priming in *Biomphalaria glabrata* and *Biomphalaria straminea*, two species with different susceptibility to *Schistosoma mansoni*. *Parasitol Res*. 2020;119: 189-201.
190. Dhinaut J, Chogne M, Moret Y. Immune priming specificity within and across generations reveals the range of pathogens affecting evolution of immunity in an insect. *J Anim Ecol*. 2018; 87:448-463.
191. Melillo D, Marino R, Italiani P, Boraschi D. Innate immune memory in invertebrate metazoans: a critical appraisal. *Front Immunol*. 2018;9:1915.
192. Khan I, Prakash A, Agashe D. Divergent immune priming responses across flour beetle life stages and populations. *Ecol Evol*. 2016;6: 7847-7855.
193. Gensollen T, Iyer SS, Kasper DL, Blumberg RS. How colonization by microbiota in early life shapes the immune system. *Science*. 2016; 352:539-544.
194. Weiss BL, Wang J, Aksoy S. Tsetse immune system maturation requires the presence of obligate symbionts in larvae. *PLoS Biol*. 2011;9:e1000619.
195. Arrieta MC, Stiemsma LT, Amenogbe N, Brown EM, Finlay B. The intestinal microbiome in early life: health and disease. *Front Immunol*. 2014;5:427.
196. MacGillivray DM, Kollmann TR. The role of environmental factors in modulating immune responses in early life. *Front Immunol*. 2014; 5:434.
197. Delisle L, Rolton A, Vignier J. Inactivated ostreid herpesvirus-1 induces an innate immune response in the Pacific oyster, *Crassostrea gigas*, hemocytes. *Front Immunol*. 2023;14:1161145.
198. Mone Y, Gourbal B, Duval D, Du Pasquier L, Kieffer-Jaquinod S, Mitta G. A large repertoire of parasite epitopes matched by a large repertoire of host immune receptors in an invertebrate host/parasite model. *PLoS Negl Trop Dis*. 2010;4:e813.
199. Gorbushin AM, Iakovleva NV. Dscam receptors from mollusca: multiple isoforms are lacking. *Fish Shellfish Immun*. 2013;34:1653-1654.
200. Contreras-Garduno J, Méndez-López TT, Patiño-Morales A, et al. The costs of the immune memory within generations. *Naturwissenschaften*. 2019;106:59.
201. Tate AT. The interaction of immune priming with different modes of disease transmission. *Front Microbiol*. 2016;7:1102.
202. Luu H, Tate AT. Recovery and immune priming modulate the evolutionary trajectory of infection-induced reproductive strategies. *J Evol Biol*. 2017;30:1748-1762.
203. Tidbury HJ, Best A, Boots M. The epidemiological consequences of immune priming. *Proc Biol Sci*. 2012;279:4505-4512.
204. Tate AT. A general model for the influence of immune priming on disease prevalence. *Oikos*. 2017;126:350-360.
205. Akrich M, Callon M, Latour B. *Sociologie de la Traduction: Textes Fondateurs [Sociology of Translation: Founding Texts]*. Presses de l'Ecole des Mines; 2006:303.
206. Chevassus-au-Louis B. L'appropriation du vivant: de la biologie au débat social [the appropriation of living things: from biology to social debate]. *Le Courrier de l'Environnement de l'INRA*. 2000;40:5-21.
207. Zingg A, Siegrist M. Lay people's and experts' risk perception and acceptance of vaccination and culling strategies to fight animal epidemics. *J Risk Res*. 2012;15:53-66.
208. Kevles DJ. The advent of animal patents: innovation and controversy in the engineering and ownership of life, chap. 2. In: Newman MFRAS, ed. *Intellectual Property Rights in Animal Breeding and Genetics*. CABI publishing; 2002:17-30. doi:10.1079/9780851996417.0017
209. Le Déaut J-Y, Proccaccia C. Les enjeux économiques, environnementaux, sanitaires et éthiques des biotechnologies à la lumière des nouvelles pistes de recherche [the economic, environmental, health and ethical issues of biotechnologies in the light of new avenues of research] in Rapport d'information n° 507 (2016-2017). 2017 (Office parlementaire d'évaluation des choix scientifiques et technologiques), p. 367.
210. Rivaud D, Cazals C. Pour une vision élargie des performances de la filière ostréicole à partir d'une approche institutionnaliste en termes de patrimoine [for a broader vision of the performances of the oyster farming industry from an approach in terms of heritage]. *Développement Durable et Territoires*. 2012;3:1-27.
211. Dachary-Bernard J, Rivaud A. Evaluation des préférences des touristes en matière d'aménagement des zones côtières: regard sur et par l'ostréiculture [Evaluation of tourists' preferences for the development of coastal areas: a look at and through oyster farming]. Working Papers-Art Dev Unit 2014-10, 1-25 2014.
212. Callon M. Some elements of a sociology of translation: domestication of the scallops and the fishermen of St Brieuc Bay. *Sociol Rev*. 1984;32:196-233.
213. Menez F. La Parole de la Palourde: ontogénèse d'un Attachement Inter-spécifique Dans la Lagune de Venise: Ethnographie de Son récit Biographique [the Parable of the Clam: Ontogenesis of Inter-specific Attachment in the Lagoon of Venice: Ethnography of its Biographical Narrative]. *EHESS; Università Ca' Foscari (Venice, Italy)*. Università Ca' Foscari; 2015:500.
214. Degremont L, Lamy JB, Pepin JF, Travers MA, Renault T. New insight for the genetic evaluation of resistance to Ostreid herpesvirus infection, a worldwide disease, in *Crassostrea gigas*. *PLoS One*. 2015;10:e0127917.

215. Anonymous. First bee vaccine. *Nat Biotechnol.* 2023;41:163.
216. Chang YH, Kumar R, Ng TH, Wang HC. What vaccination studies tell us about immunological memory within the innate immune system of cultured shrimp and crayfish. *Dev Comp Immunol.* 2018;80:53-66. doi:[10.1016/j.dci.2017.03.003](https://doi.org/10.1016/j.dci.2017.03.003)
217. Davison A, Neiman M. Mobilizing molluscan models and genomes in biology. *Philos Trans R Soc Lond B Biol Sci.* 2021;376:20200163.

How to cite this article: Montagnani C, Morgia B, Novoa B, et al. Trained immunity: Perspectives for disease control strategy in marine mollusc aquaculture. *Rev Aquac.* 2024;1-27. doi:[10.1111/raq.12906](https://doi.org/10.1111/raq.12906)