



# The Pacific Oyster Mortality Syndrome, a Polymicrobial and Multifactorial Disease: State of Knowledge and Future Directions

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The Pacific oyster (*Crassostrea gigas*) has been introduced from Asia to numerous countries around the world during the 20th century. *C. gigas* is the main oyster species farmed worldwide and represents more than 98% of oyster production. The severity of disease outbreaks that affect *C. gigas*, which primarily impact juvenile oysters, has increased dramatically since 2008. The most prevalent disease, Pacific oyster mortality syndrome (POMS), has become panzootic and represents a threat to the oyster industry. Recently, major steps towards understanding POMS have been achieved through integrative molecular approaches. These studies demonstrated that infection by Ostreid herpesvirus type 1  $\mu$ Var (OsHV-1  $\mu$ var) is the first critical step in the infectious process and leads to an immunocompromised state by altering hemocyte physiology. This is followed by dysbiosis of the microbiota, which leads to a secondary colonization by opportunistic bacterial pathogens, which in turn results in oyster death. Host and environmental factors (e.g. oyster genetics and age, temperature, food availability, and microbiota) have been shown to influence POMS permissiveness. However, we still do not understand the mechanisms by which these different factors control disease expression. The present review discusses current knowledge of this polymicrobial and multifactorial disease process and explores the research avenues that must be investigated to fully elucidate the complexity of POMS. These discoveries will help in decision-making and will facilitate the development of tools and applied innovations for the sustainable and integrated management of oyster aquaculture.

**Keywords:** Pacific oyster mortality syndrome, polymicrobial disease, multifactorial disease, *Crassostrea gigas*, OsHV-1, opportunistic bacterial pathogens

## INTRODUCTION

Aquaculture is one of the fastest-growing food industries, representing more than 50% of worldwide seafood production (1). Mollusk aquaculture, in which oysters are the most important taxonomic group (by volume), has become one of the largest animal food-producing industries.

The Pacific oyster *Crassostrea gigas* was introduced from Asia to numerous countries throughout the world (e.g. Canada, the USA, Brazil, Australia, New Zealand, Chile, Mexico, Argentina, South Africa, Namibia, and numerous European countries including France) during the 20th century (2). Worldwide production of *C. gigas* is estimated at 4.7 million tons per year (3). China is, by far, the leading producer, followed by South Korea, Japan, and France.

For decades, *C. gigas* has suffered from mortality due to disease (4), but the severity of these outbreaks has increased dramatically since 2008. These outbreaks affect the juvenile stages of the oyster, killing more than 35% of the cultivated and natural oysters in France every year (5, 6). The corresponding syndrome, referred to as Pacific oyster mortality syndrome (POMS) (7), has become panzootic; it is observed in all the coastal regions of France, and in numerous other countries worldwide (8). The ecological and economic consequences of POMS can be dramatic (9), and POMS represents a threat to the worldwide oyster industry.

POMS is a disease with complex etiology. Research efforts have revealed a series of factors that contribute to the disease, including interactions between infectious agents and seawater temperature, oyster genetics, food availability, and oyster growth (9–17).

In this review, we present the research performed in the past decade that has contributed to a better understanding of POMS. These studies have deciphered the polymicrobial nature of the disease. Although this is a first and important step, POMS remains an incompletely understood multifactorial disease that is controlled by a series of host and environmental factors. It is crucial that POMS be fully deciphered by the determination of which factors control disease expression, how these factors control disease expression, the weight of these different factors, and their putative synergistic and antagonistic effects. Only once this work is complete will we completely understand POMS and be able to propose solutions to ensure the durability of the oyster industry.

## POMS IS A POLYMICROBIAL DISEASE

Dramatic increases in oyster mortality (observed since 2008) have been found to coincide with the recurrent detection of *Ostreid herpesvirus* (OsHV-1) variants in moribund oysters in France (17–19) and worldwide (9, 20–23). Because of this, research efforts have historically focused on the viral etiology of POMS and a series of diagnostic assays such as PCR, real-time PCR, and *in situ* hybridization have been developed to detect OsHV-1 (18, 19, 24–26). Nevertheless, the involvement of other

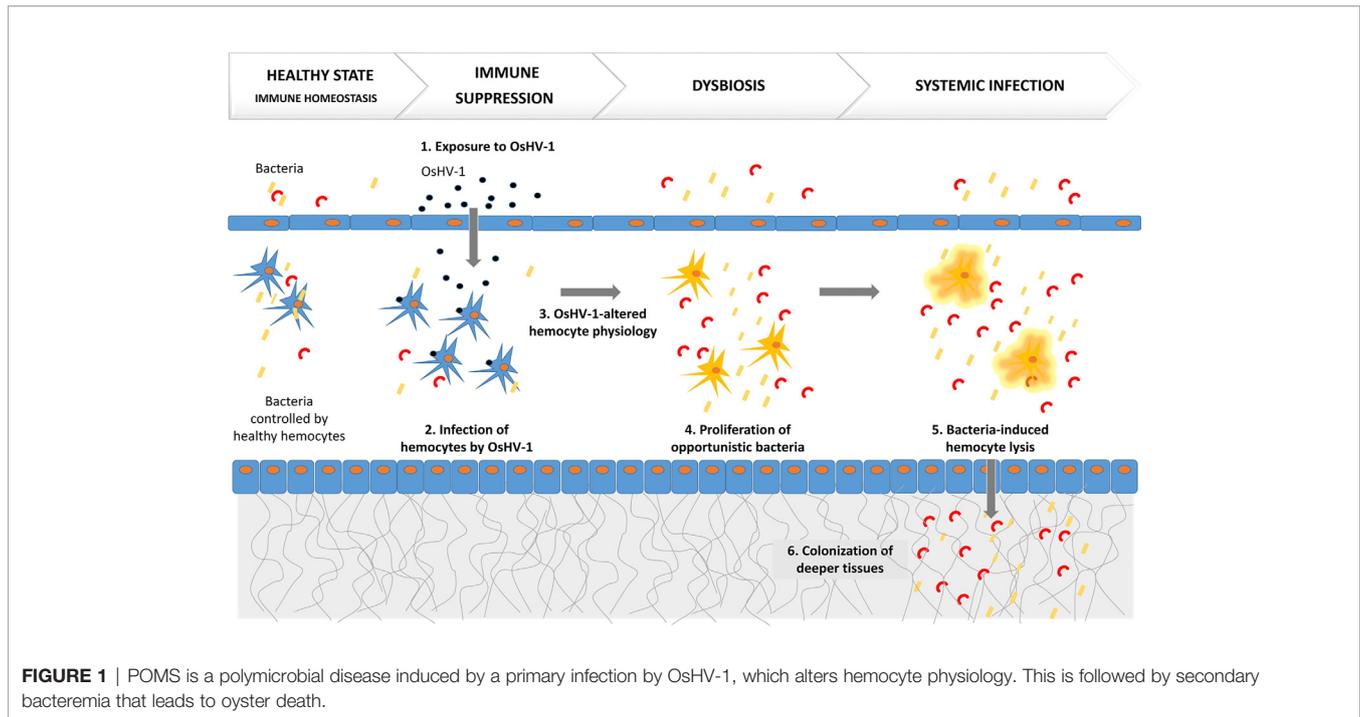
etiological agents was suspected (15). In particular, bacterial strains of the genus *Vibrio* had been shown to be associated with POMS (27, 28). However, the role of these different putative pathogens has been tested in isolation, using experimental systems (bacterial or viral filtrate injections) that did not reproduce the natural route of infection. Consequently, the complex POMS infectious process remained misunderstood.

Several breakthroughs have advanced our understanding of the complexity of POMS. The development of an ecologically realistic model of infection was the first major progress (16, 29). Briefly, this method of infection uses pathogen-free oysters that are reared in bio-secured conditions from birth to 3 months of age. Some of these pathogen-free oysters are then naturally infected in the field to become “donors,” while the remaining oysters are maintained in bio-secured conditions as “recipients.” Recipients are subsequently exposed to donor oysters through cohabitation. This method retains the complexity of the infectious environment (OsHV-1 and populations of virulent bacteria) and mimics the natural route of infection (16, 29). This method also allows simultaneous triggering of disease in all recipients and parallels the dynamics of the disease through time on oysters that are phased according to the infectious process in which they are engaged. A second important breakthrough was the use of oyster biparental families selected for higher disease resistance (10). By reducing genetic diversity, which is particularly high in oysters, the disease dynamics can be monitored in oysters that have contrasting phenotypes (susceptible or resistant) during pathogen challenge. A final breakthrough was the use of integrative molecular approaches that allowed the observation of the dynamics of the host response and the microbiota, including putative pathogens, in the same experimental framework.

One recent study that combined an ecologically realistic model of infection, the use of susceptible and resistant oyster families, and integrative molecular approaches (dual RNAseq, 16S rDNA metabarcoding, and histology) deciphered the mechanism of POMS (30). This study demonstrated that POMS is a polymicrobial disease (**Figure 1**). Early gill damages, infection of hemocytes by OsHV-1, and bacterial colonization both inside and outside the gill tissues were evidenced in susceptible oysters only (30). This study revealed that infection by OsHV-1 is the first critical step in POMS and leads to an immunocompromised state by infecting and altering hemocyte physiology (30). This immunosuppression subsequently evolves towards bacteremia, which involves a series of opportunistic bacteria (30). This bacteremia leads to oyster death. Indeed, POMS requires both OsHV-1 and opportunistic bacteria: preventing either viral replication or bacterial proliferation with poly-IC injection or antibiotics, respectively, blocks the infectious process and prevents mortality (30).

## Primary Viral Infection

Infection by OsHV-1  $\mu$ Var is the first event in the infectious process of POMS, and intense viral replication is a prerequisite for the development of the disease. The inability of susceptible oysters to control viral replication during POMS is associated



with a strong, but late, antiviral response (30). Concomitant with the intense replication of the virus, oysters intensively express several genes that encode endogenous Inhibitors of Apoptosis (IAPs) (30). Although the pathway by which OsHV-1 could control endogenous IAP expression is unknown, such a mechanism has been described in a human Gammaherpesvirus, Epstein-Barr virus, which is able to increase the expression of IAP-2 to inhibit apoptosis (31). Remarkably, intense OsHV-1 replication is also associated with high expression of exogenous IAPs of viral origin (30). Such viral proteins, which are of the BIR family, are known to have anti-apoptotic activities that favor viral replication (32). These results suggest that both endogenous and exogenous anti-apoptotic processes, which are strongly activated in susceptible oysters, play a key role in the success of OsHV-1 infection (30).

Oyster immune cells, called hemocytes, are targeted by OsHV-1 (30, 33). Infection of hemocytes by OsHV-1 impacts hemocyte physiology and impairs the expression of antimicrobial peptides (30), either directly (through transcriptional regulation) or indirectly (through the induction of cell death or lysis processes) (33).

Since the description of the first OsHV-1  $\mu$ Var genotype in 2010 (17), increasing NGS sequencing data have revealed the diversity of OsHV-1  $\mu$ Var genotypes (17, 22, 23, 34, 35). This observation, similar to observations made in a number of RNA and DNA viruses (36–39), raises the question of what impact this genetic diversity has on the fitness of the virus and the consequences of the disease. Many viruses produce diverse genetically linked variants that can be defined as viral populations. These populations are maintained by mutation-selection equilibrium (40, 41), and have the potential to generate

beneficial interactions and cooperation which increase viral fitness and adaptability to the host (42–45).

A recent study investigated these possibilities in OsHV-1 (46). Different biparental families of oysters were confronted with two different infectious environments. Because susceptibility to POMS can differ not only between families within the same environment, but also within the same family between the two environments, viral diversity was analyzed between families and environments (46). This analysis revealed distinct viral populations in the two infectious environments (46). Moreover, the different oyster families were infected by distinct viral populations within the same infectious environment (46). These results suggest that there are co-evolutionary processes at play between OsHV-1  $\mu$ Var, and that oyster populations have selected for a diversity of viral populations that could, in turn, facilitate viral adaptation to various environments and various host genotypes.

## Secondary Bacterial Infection

Until recently, the bacterial component of POMS has mainly been studied using culture-based approaches. These approaches revealed a clear association between some *Vibrio* species and POMS, which has led to extensive characterization of the roles and contributions of *Vibrio* bacteria to oyster mortality.

Using pathogen-free oyster spats and field-based approaches, Le Roux and collaborators characterized the population structure of *Vibrio* species found in naturally infected oysters during POMS episodes on the French Atlantic coast. Members of the *Splendidus* clade (e.g. *V. tasmaniensis*, *V. splendidus*, *V. cyclitrophicus*, *V. harveyi*, *V. aestuarianus*, and *V. crassostreae*) have been systematically isolated from diseased juvenile oysters,

as have (to a lesser extent) *V. harveyi* and *V. aestuarianus*, which fall outside the clade (17, 27, 47). Bruto et al. demonstrated that the *Vibrio* population structure is seasonal and varies in oysters affected by POMS (27). Notably, *Vibrio* of the *Splendidus* clade are present in healthy oysters when no mortalities occur (27), but they only express low to moderate pathogenic potential in these circumstances (48). *V. crassostreae* is predominant during mortalities and is almost exclusively associated with oyster tissues (27). *V. crassostreae* has been shown to replace the resident *Vibrio* community during a POMS episode (49).

Some factors that contribute to *Vibrio* virulence in oysters have been discovered in species that exhibit pathogenic potential in experimental infections (e.g. bacteria injected in the adductor muscle). These factors have mostly been described in *V. crassostreae* and *V. tasmaniensis* [a facultative intracellular pathogen of oyster hemocytes (50)] isolated from the Atlantic during POMS episodes (27, 28, 49–52). Bruto et al. (28) found that the r5.7 gene is required for virulence and is ancestral in the *Splendidus* clade. The R5.7 protein itself is not cytotoxic (28); however, to mediate cytotoxicity, R5.7-expressing *V. crassostreae* requires physical contact with hemocytes (51). Interestingly, upon the loss of the ancestral r5.7 gene, *V. tasmaniensis* has acquired a type 6 secretion system (T6SS) on chromosome 1. This T6SS intracellularly delivers cytotoxic effectors to oyster hemocytes (51). These findings show that *Vibrio* cytotoxicity is a key determinant of oyster colonization that allows *Vibrio* to escape from potent cellular defenses and cause systemic infection (51). Furthermore, these findings show that distinct molecular determinants can confer similar dampening of host immune defenses in *Vibrio* species associated with POMS.

To date, *Vibrio* is the only bacterial group that has been studied in detail as an etiological agent of POMS, in part because *Vibrio* species are readily cultured and amenable to functional studies. Other bacterial groups (e.g. *Arcobacter* and *Shewanella*) have been associated with oyster mortality (30, 53, 54). However, their particular contributions to, and potential cooperation with other bacterial communities that participate in, fatal dysbiosis remain unknown, largely due to technical limitations.

Recently, we studied the structure of bacterial communities (as determined by 16S metabarcoding) and the functions expressed by different bacterial genera (as determined by metatranscriptomics) in different oyster biparental families submitted to different infectious environment (55). Five bacterial genera (*Arcobacter*, *Marinobacterium*, *Marinomonas*, *Vibrio*, and *Pseudoalteromonas*) that colonize oysters during POMS were remarkably consistent between families and environments. These genera are referred to as the POMS pathobiota.

The core functions identified by metatranscriptomics in POMS pathobiota reveal the importance of general metabolism and adaptation to stress (imposed by host defenses) in the success of colonization (55). Beyond general metabolism, detoxifying enzymes (such as alkylhydroperoxydases) involved in the resistance to or tolerance of host immune defenses are expressed by all successful colonizers. Most of the successful genera also express enzymes required for tolerance/resistance to oxidative stress. Reactive Oxygen Species (ROS) and

Reactive Nitrogen Species (RNS) are major players at the oyster-microbiota interface (56). Thus, it is not surprising that successful colonizers express ROS/RNS detoxifying enzymes during oyster colonization.

A number of genus-specific functions that likely confer cross-benefits at the community level are also expressed during pathogenesis (55). These include genes related to metal homeostasis (siderophore production), iron uptake, and virulence (e.g. T6SS), all of which contribute to suppression of oyster cellular defenses (51, 57). The expression of these genes is likely beneficial to the entire bacterial community. From a metabolic point of view, cross-benefits are best exemplified by sulfur metabolism, a pathway which is non-redundantly encoded by five different genera. This suggests that sulfur cycling is a core property of the colonizing microbiota as a whole, rather than of a single genus. Such metabolic interdependence between, and cooperation of, microbial communities is likely a key determinant of the structure of the POMS pathobiota, and may dictate its conservation across distinct environments and oyster genetic backgrounds.

Many questions remain to be studied, including how *Vibrio* and/or other bacterial genera collaborate with OsHV-1 to kill oysters. It is known that the molecular manipulation of hemocyte molecular responses by OsHV-1 (i.e. the inhibition of antimicrobial peptide expression) is a key determinant of bacterial colonization, without which *Vibrio* species fail to efficiently colonize oysters and express their pathogenic potential (30). It is also known that the dampening of oyster cellular defenses by *Vibrio* (through active hemocyte lysis), or possibly by other pathogenic bacteria, is key in the development of systemic infection (51). Because hemocytes are composed of diverse populations of cells that play different roles in infection control, better identification of the cell populations targeted by each POMS-associated pathogen is required to understand the polymicrobial nature of POMS at the cellular and molecular scales.

## POMS IS A MULTIFACTORIAL DISEASE

The risk of POMS outbreaks has been linked to subtle interactions between not only host and pathogens, but also environmental factors. Thus, POMS is a not only a polymicrobial disease, but also a multifactorial disease. In this review, we focus on the five main environmental and host factors associated with disease expression: i) temperature, ii) oyster genetics, iii) oyster age, iv) oyster energetic status, and v) microbiota.

### Temperature

Temperature increases linked to climate warming are a major driver of disease outbreaks (58). In different invertebrate-pathogen interactions, temperature has been found to affect the outcome of the interaction by influencing host and/or pathogen physiology (59–63). A permissive temperature range for POMS has been clearly identified, specifically, 16°C to 24°C (5, 6, 64).

The same trend was observed in mesocosm experiments in which recipient oysters were confronted to disease donors at different temperature [Figure 2A, (16)].

Although higher temperatures decrease oyster susceptibility to POMS, viral infectivity is quite similar to infectivity at permissive temperatures (66). This reinforces the hypothesis that other factors (e.g. bacterial virulence or oyster physiology) could be influenced by high temperatures and thereby affect POMS permissiveness. Although the effect of high temperatures on bacterial virulence remains to be investigated, a recent work demonstrated that oyster immunity is modulated, and apoptotic processes induced, by high temperatures, which could explain the observed decrease of permissiveness (11).

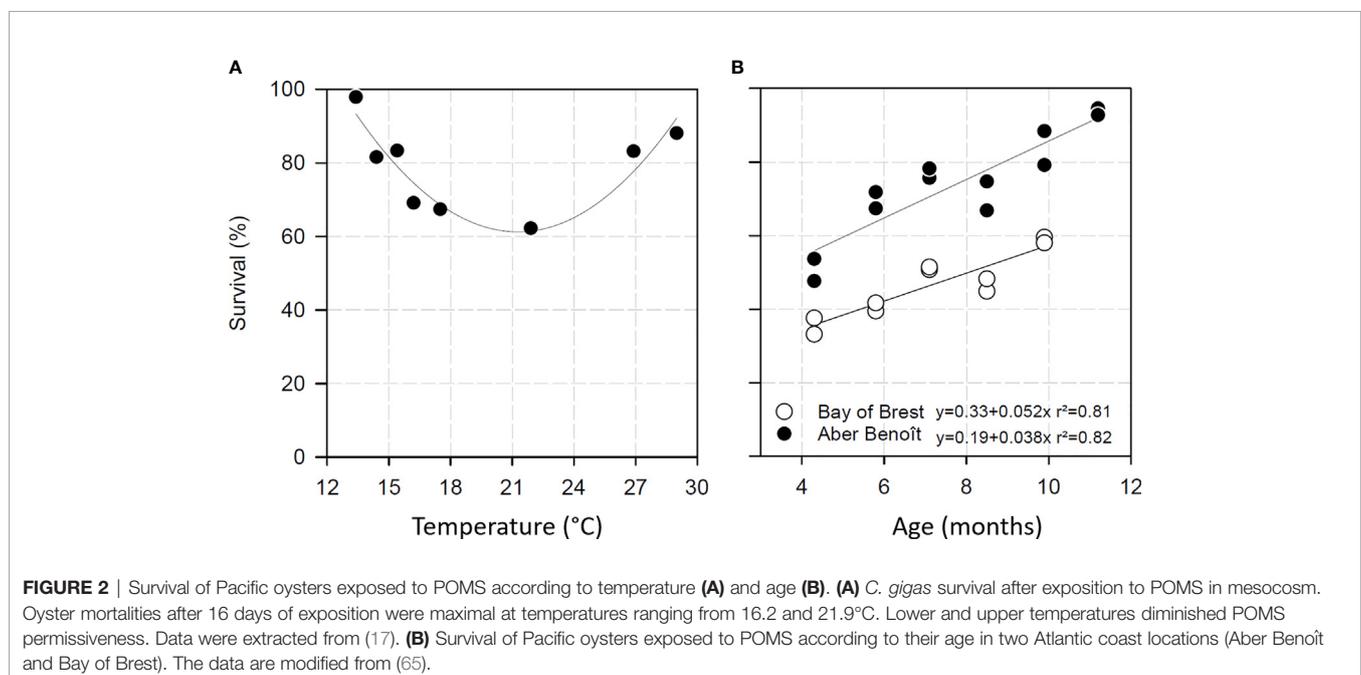
With respect to lower temperatures, diseased oysters exposed to low temperature (13°C) over the course of 40 days exhibited no mortality, were negative for OsHV-1, and did not transmit the disease to healthy oysters (16). The mechanisms by which low temperatures affect POMS permissiveness remain to be investigated.

## Oysters Genetics

Some oysters, particularly those able to limit infection by OsHV-1, are resistant to POMS. Such resistant oysters were first described in field challenges (67), and subsequently in laboratory challenges, as exhibiting a 3 log of decrease in viral DNA copies (30, 68, 69). Genetic studies of oyster resistance have revealed a significant additive genetic component of survival during OsHV-1 infection (10, 70–72). Over the past decade, many oyster genomic tools [including a reference genome (73) and SNP arrays (74)] have been developed to expand the study of the genetic underpinnings of *C. gigas* resistance to OsHV-1 infection.

The results of genome-wide association studies in juvenile oysters experimentally challenged with OsHV-1 and genotyped using a high-density linkage map constructed for the Pacific oyster (75) suggest that OsHV-1 resistance is polygenic in nature. These studies also highlighted that region of linkage group 6 contains a significant QTL which affects host resistance (75). Several SNPs, associated with survival and/or viral load, were located in several genes that encode RAN Binding Protein 9, a Coronin and Myo10 (an actin motor protein). However, the specific roles of these genes in the resistance process remain to be elucidated.

A recent transcriptomic study on several oyster biparental families that exhibited different susceptibilities to POMS revealed that the early induction of genes involved in antiviral defense is a hallmark of resistant oyster families (30). However, the specific genetic components responsible for this early induction remain unidentified. To identify putative transcriptomic determinants associated with POMS resistance, basal transcriptomes (no disease challenge) of three resistant oyster families were compared to the basal transcriptomes of three susceptible families (76). POMS resistant oysters showed constitutive differences in the expression of genes involved in stress responses, protein modification, maintenance of DNA integrity and DNA repair, and immune and antiviral pathways. Similarities and differences between molecular pathways were observed among the resistant families. For example, several genes related to the TLR-NF- $\kappa$ B, JAK-STAT, and STING-RLR pathways were identified (76). Among them, only one transcript was overrepresented in the three resistant families (76). This transcript corresponded to an endosomal Toll-like Receptor that displays similarities to TLR 13, which can act as a sensor of viral and bacterial RNA in the TLR-NF- $\kappa$ B signaling



pathway (77, 78). This gene is particularly interesting because its function could explain how these resistant oyster families detect viral infection early, which allows them to mount a more rapid and efficient antiviral response (30). Given this putative antiviral role, TLR 13 represents a good candidate for future study.

Overall, the results of these studies suggest that the POMS resistance process is polygenic and varies somewhat with oyster genotype.

## Oyster Age

A series of studies have reported that POMS-induced mortality rates are lower in adult oysters than in spat and juvenile oysters (13, 20, 79). The experimental design of these studies, however, did not allow researchers to disentangle the effect of selection on resistance and age. In fact, the adult oysters analyzed in these studies had survived one or more POMS events, and thus might have been selected for resistance to POMS over time.

To further investigate the effect of oyster age on mortality, healthy oysters at different developmental stages (juveniles and adults) were exposed to the Marennes-Oléron Bay (France) infectious environment (80). In this study, oyster age was found to be inversely correlated with mortality. This result was confirmed both in the same infectious environment (10) and in another infectious environment (Brest Bay, France) (65). In the Brest Bay study, healthy oysters of different ages were simultaneously confronted with the same infectious environment. Most oysters appear to become resistant to POMS with age (Figure 2B), and resistance appears to be acquired after 24 months (10, 65). It is expected that the maturation of the immune system underlies the acquisition of resistance; however, the underlying molecular mechanisms remain to be investigated.

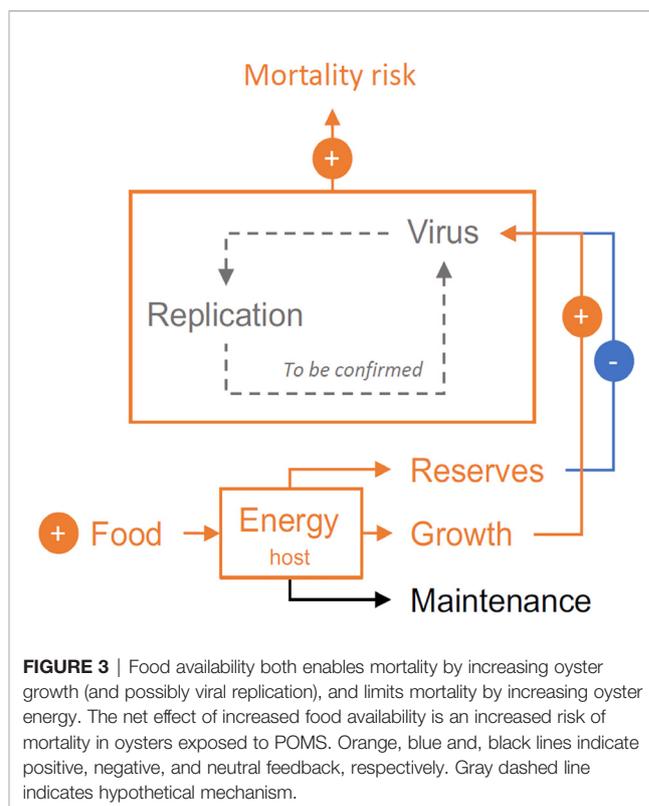
## Food Availability, Growth, and Energy Reserves

Food availability is known to influence disease risk and outcome in two ways. First, food availability improves the physiological condition of the host and lowers their susceptibility to infectious disease, which reflects a tradeoff between immunity and other functions [e.g. (81, 82)]. Second, food scarcity limits the resources available to the pathogen, because it slows the growth and metabolism of the host, which the pathogen depends on in order to proliferate (83–87). Therefore, food availability can have both positive and negative effects on the severity of infectious diseases.

Previous studies have demonstrated that fed oysters are at a higher risk of death than starved oysters (88, 89). Paradoxically, the energy reserves of oysters, which reflect food availability, are associated with increased resistance or tolerance to OsHV-1 (13, 64, 90). In light of these results, Pernet et al. (14) investigated how food availability, growth rate, and energy reserves drive the outcome of POMS. Briefly, the authors selected fast- and slow-growing oysters and exposed these oysters to high and low food rations. The oysters were evaluated for energy reserves, challenged with POMS, and monitored for survival. The study found that although higher food levels and oyster growth are associated with an increased mortality risk, higher energy

reserves are associated with decreased mortality risk (14). Thus, food availability both enables mortality by increasing oyster growth and limits mortality by increasing energy reserves (Figure 3). This study clarifies the impact of food resources on disease susceptibility and suggests how host physiological condition could mitigate epidemics (14).

The specific mechanisms by which food availability, growth rate, and energy reserves modulate oyster susceptibility to infection remain to be elucidated. We hypothesize that the positive association between food level and mortality risk reflects increased host growth and metabolic rate, which in turn amplifies pathogen replication. Like other viruses, OsHV-1 uses host cellular machinery to replicate (91, 92), thus stimulation of host cellular growth could amplify viral gene expression and replication. However, there is currently no evidence that demonstrates increased viral replication in response to increased food availability and host growth rate. Furthermore, we cannot exclude the possibility that food availability, growth rate, and energy reserves act on susceptibility to secondary bacterial infection. Finally, the lower mortality risk observed at low food levels may also reflect induced autophagy, an evolutionarily conserved cell recycling process that is activated in response to stress (including starvation) (93). Autophagy also controls microbial infections, both through direct destruction of the pathogen, and indirectly as a key mediating factor in host innate and adaptive immunity (93). The autophagy pathway is functional in oysters and could explain why starvation reduces mortality during OsHV-1 infection (89). Further investigations are needed to assess



**FIGURE 3** | Food availability both enables mortality by increasing oyster growth (and possibly viral replication), and limits mortality by increasing oyster energy. The net effect of increased food availability is an increased risk of mortality in oysters exposed to POMS. Orange, blue and, black lines indicate positive, negative, and neutral feedback, respectively. Gray dashed line indicates hypothetical mechanism.

whether and how food levels, growth, and energy reserves control viral proliferation, bacteremia, and/or autophagy.

## Microbiota

Several independent studies used 16S metabarcoding to investigate uncultivable bacterial microbiota. These studies identified shifts in the composition of the microbiota community (dysbiosis) associated with POMS. In a large-scale analysis of the microbiota of diseased oysters from three different sites in Europe, Lasa and colleagues identified dysbiosis in OsHV-1 positive oysters. This dysbiosis was characterized by the emergence of a pathobiota composed of opportunistic pathogens (including *Vibrio* and *Arcobacter* species) (54). In the most integrative study of POMS to date, ecologically realistic infection demonstrated that bacterial dysbiosis is subsequent to viral infection, that viral infection leads to antibacterial defense impairment, and that this impairment allows opportunistic pathogens to colonize oysters (30).

In addition, oyster genotype-specific microbial associations have been identified between genetically differentiated oyster beds especially for the rare phylotype assembling (94). This association disappeared under environmental stress. Constitutive differences in microbiota have also been identified in 35 healthy oyster breeds displaying different levels of resistance to POMS (95). Operational Taxonomic Units (OTUs) of the *Photobacterium*, *Vibrio*, *Aliivibrio*, *Streptococcus*, and *Roseovarius* genera were significantly associated with the most susceptible oyster families, as was a higher background load of rare *Vibrio* OTUs in the healthy state. This suggests that these susceptible families exhibit a decreased immune response to these pathogens (95).

In another study, the Mycoplasmataceae, Rhodospirillaceae, and Vibrionaceae bacterial families, as well as the *Photobacterium* genus, were associated with susceptible oyster families (96). The proportion of specific taxa, including Cyanobacteriaceae, Colwelliaceae, and Rhodobacteraceae, was significantly higher in resistant oyster families that survived POMS after transplant into the field during an infectious period. Resistant oysters also displayed a higher evenness of the bacterial colonizers, suggesting that a decrease in microbial diversity may be associated with a loss of microbiota function, which could allow colonization by opportunistic pathogens (96).

This research raises questions about the role of microbiota composition and stability in oyster health and susceptibility to POMS. Whether differences in bacterial community composition and dynamics are responsible for different disease resistance levels, or are only related to the host-genotype specificity of the microbiota, remains to be elucidated. Future studies are needed to identify the relative contributions of the oyster microbiota to disease outcome, including studies of direct interactions with pathobiota and possible immune stimulation of the host.

## CONCLUSIONS AND PERSPECTIVES

Determination of the mechanism of POMS pathogenesis and identification of host, pathogen, and environmental factors that

influence POMS are important achievements. However, these are only initial steps in the development of new approaches to counter POMS and its devastating effects on the oyster industry.

The research discussed above, along with future studies, will help identify the main factors involved in permissiveness to POMS. It will also be necessary to evaluate the relative weights of, and interactions between, these factors during pathogenesis. This can be achieved through the study of different markers (including oyster genes, viral and bacterial load and genes, *etc.*) identified in real farming conditions with oysters of different ages and genetic backgrounds in different environmental conditions and under different physiological conditions. Such studies are necessary for the implementation of predictive models of the epidemiological risk of POMS.

Theoretical approaches to the study of bivalve infections are still in their infancy (97). Moreover, only a few epidemiological models have been developed for multiple infections, and these models usually oversimplify within-host dynamics (98). Consequently, an additional research objective could be the development of models that synthetically represent POMS under the influence of relevant host, pathogen, and environmental factors. Importantly, these models will help quantify the relative benefits and risks of changes in oyster farming practices (e.g. provision of food resources, age of the oysters at the time of transfer from the bio-secured hatcheries to the natural farming environment).

To conclude, we cannot exclude that other factors could influence the disease. As an example, we can mention field studies indicating that POMS could be influenced by the presence of particles (e.g. plankton) carrying OsHV-1 (90, 99–101). Future studies will focus on these factors and their weight in POMS expression, and they can be included in the proposed modeling approaches to get a comprehensive view of POMS and predict as faithfully as possible the epidemiological risk.

## AUTHOR CONTRIBUTIONS

DD-G, BP, and FP contributed equally to the content of the review and figures. JD, ET, and LD contributed to the content of the review. GM supervised, edited, and added references in the review. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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