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Infectious diseases of marine molluscs and host responses as revealed by genomic tools

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More and more infectious diseases affect marine molluscs. Some diseases have impacted commercial species including MSX and Dermo of the eastern oyster, QPX of hard clams, withering syndrome of abalone and ostreid herpesvirus 1 (OsHV-1) infections of many molluscs. Although the exact transmission mechanisms are not well understood, human activities and associated environmental changes often correlate with increased disease prevalence. For instance, hatcheries and large-scale aquaculture create high host densities, which, along with increasing ocean temperature, might have contributed to OsHV-1 epizootics in scallops and oysters. A key to understanding linkages between the environment and disease is to understand how the environment affects the host immune system. Although we might be tempted to downplay the role of immunity in invertebrates, recent advances in genomics have provided insights into host and parasite genomes and revealed surprisingly sophisticated innate immune systems in molluscs. All major innate immune pathways are found in molluscs with many immune receptors, regulators and effectors expanded. The expanded gene families provide great diversity and complexity in innate immune response, which may be key to mollusc's defence against diverse pathogens in the absence of adaptive immunity. Further advances in host and parasite genomics should improve our understanding of genetic variation in parasite virulence and host disease resistance.

1. Introduction

Molluscs are a major group of marine animals that play key roles in marine ecosystems. Most molluscs are calcifiers and many are filter-feeders, providing essential ecological services such as habitat for other organisms and water clarification. Molluscs such as oysters, clams, scallops, mussels, abalone and squid, support significant fishery and aquaculture industries around the world [1]. In molluscs, infectious diseases have devastated wild populations, fisheries and aquaculture industries [2]. There are indications that as the oceans are changing, marine diseases are becoming more frequent or severe, and these changes might be associated with climate change and human activities [3,4]. Our ability to predict how climate change and human activities may accelerate the emergence of marine diseases depends on our knowledge of the infection processes and the influence of environmental factors. Fundamental to the infection process is the complex interaction between the host and parasite, the outcome of which depends partly on the host's immune system.

All living organisms including single-celled algae and bacteria have parasites, and host–parasite interactions are one of the most fundamental processes in biology that drives evolution. The race between the host and its parasites as best characterized by the Red Queen hypothesis is intense and everlasting [5]. As a result, host organisms have evolved sophisticated host-defence mechanisms against parasites that constantly invent new ways to infect. Thus, the genotypic or molecular interactions between the host and parasites are complex and present a challenge to our understanding of infection dynamics.

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Most biologists are familiar with the adaptive immune system in vertebrates, but invertebrates also have effective innate immune responses to infectious agents. As filter-feeders living in microorganism-rich environments, bivalve molluscs may have evolved advanced host-defence mechanisms against pathogens [6]. Recent advances in molecular genetics and genomics have provided insights into the complex molecular machineries involved in host–parasite interactions. In this paper, we will review major infectious diseases of marine molluscs, their transmission history and the molecular components of the molluscan immune system, as revealed by genomic methodology, that are essential to our understanding of the infection processes and transmission dynamics of marine pathogens.

2. Major infectious diseases of marine molluscs

Although mass mortalities of commercially important molluscs were noted in the early twentieth century and presumed to have an infectious agent aetiology [7–9], it was not until mid-century that specific disease agents were clearly observed, by microscopy and culture methods, just before and during mortality outbreaks. In this section, we review some of these diseases and their agents, with emphasis on the role, if any, posed by human activities in epizootics—specifically culture and commercial practices that trigger or aggravate disease outbreaks. Very few studies have explicitly addressed the role of climate change on molluscan diseases; therefore, we note, where data are available, the association of temperature with epizootics that might suggest the potential for climate warming to exacerbate disease outbreaks. We include climate change as an anthropogenic impact [10]. We then explore in greater detail disease outbreaks associated with ostreid herpesvirus 1 (OsHV-1) that infects a wide range of molluscs. We argue that OsHV-1 outbreaks in oysters in Europe and scallops in China may be caused by increasing ocean temperature as well as culture practices that both increase the likelihood of transmission and stress molluscs. We are not able to cover all the diseases that affect marine molluscs in the following overview, much less all of the parasites that have been reported in them; however, we summarize, in table 1, important points about other pathogens and diseases not covered in the text.

(a) Dermo

In the mid-1940s, eastern oyster (*Crassostrea virginica*) mortalities in the Gulf of Mexico off the coast of Louisiana were blamed on the oil industry, which spent heavily on research that eventually found the cause—a protozoan now known as *Perkinsus marinus* and the disease it causes, as Dermo disease [11]. It was noted that the mortalities coincided with warm, dry periods—and that similar mortalities had been evident in earlier decades [65], suggesting that *P. marinus* had been present for many years. Examination of oysters in other locations along the US coastline in the late 1940s found the pathogen as far north on the eastern seaboard as the lower Chesapeake Bay [66,67], but epizootics were restricted to this range until the mid-1980s [12]. Although *P. marinus* is transmitted directly among oysters [66], transmission is not necessarily density-dependent and uninfected oysters can become infected even when the nearest known infected stocks are several kilometres away [68]. Infection acquisition and development are temperature-dependent and follow a distinct seasonal cycle in which infections are acquired and develop at temperatures greater than or equal to 20°C and mortalities predominate at temperatures greater than 25°C [69,70]. Seed oysters, likely infected by this parasite, had been shipped over many years to northern locations to replenish overfished beds, but temperatures were too low to launch epizootics or even sustain detectable parasite populations [71] until a warming trend, which began in the mid-1980s and accelerated in the early1990s, was associated with disease and mortality outbreaks over a 500 km range north [12]. It should be noted that many other species of *Perkinsus* have been found to infect a variety of non-oyster species in many parts of the world. Many show evidence of a negative effect on their hosts; however, the impact is not always clear or well documented (see http://www.dfo-mpo.gc.ca/science/aah-saa/diseases-maladies/perkincc-eng.html).

(b) MSX

Massive mortalities of *C. virginica* due to MSX (multinucleated sphere unknown) disease were first noted in Delaware Bay and subsequently in Chesapeake Bay on the mid-Atlantic coast of the USA, between 1957 and 1959 [72]. An estimated 90–95% of affected oysters died within 2 years [73,74]. The aetiological agent, *Haplosporidium nelsoni*, is an introduced parasite of the Pacific oyster, *C. gigas* in Asia, where it exists at low prevalence and without causing mortality [75]. Like others in the same genus, *H. nelsoni* has never been transmitted experimentally and an intermediate or alternative host is suspected [76]. Oyster density is not a factor and infections can be acquired in the absence of nearby infected oysters. Infections are acquired during the late spring and summer, and parasites proliferate most rapidly at temperatures greater than 20°C. Mathematical modelling suggested that climate warming could shift the parasite northward, and indeed epizootics were recorded in Maine (1995 and 2010) and eastern Canada (2002) as the water temperatures have increased [77–79]. Warm temperature, itself, however, is not a sufficient trigger, as epizootics caused by *H. nelsoni* have not been reported in the southeastern USA where the parasite is present [80,81] and temperatures are high.

(c) Marteiliosis

In 1968, not long after the first MSX disease outbreak in the USA, two protozoans both in the genus *Marteilia*: *M. refringens* and *M. sydneyi*, were identified as the causes of disease and heavy mortalities in the flat oyster, *Ostrea edulis*, in France and the Sydney Rock oyster, *Saccostrea glomerata*, in Australia, respectively [17,22]. Since the initial outbreaks, the reports of the parasite have become widespread. *Marteilia refringens* is now found in Europe from Sweden and Britain in the north to Greece and Croatia in the Mediterranean [18] and in Australia, *M. sydneyi* is now found over 900 km south of where it was first reported [23]. The spread is thought to have occurred via the commercial movements of stocks among growers in different regions; however, neither pathogen is directly transmitted among oysters and potential alternative hosts have been described [19,24], although a complete life cycle has not yet been elucidated. Thus, an alternative host would have had to be already present in the new locations or moved along with the oysters. The genotype of the parasite that is predominantly in mussels is

Table 1. Listing of major marine molluscan diseases and disease agents of current importance with information pertinent to climate (temperature) change and culture practices. Note that the symbol '>' does not necessarily imply a trail of introductions, but simply notes reports following the initial one.

disease (agent)	host[a] (mortalities[b])	first outbreak; known distribution	transmission	temperature association[c]	culture association, including commercial transport of molluscs	references
protists						
Dermo (*Perkinsus marinus*)	oyster *Crassostrea virginica* (60%)	Gulf of Mexico (approx. 1946) > east coast of USA; east and west coasts of Mexico	direct	infection proliferation and mortality at $t \geq 25^{\circ}$C; northward spread of epizootics associated with winter warming trend	documented movement northward in transport of infected oysters	[11–13]
MSX (*Haplosporidium nelsoni*)	oyster *C. virginica* (70–90%)	Delaware Bay, USA (1957) > Atlantic coast North America: Nova Scotia, Canada to Florida, USA	probable intermediative or alternative host necessary	infection and proliferation greatest at $t \geq 15^{\circ}$C; outbreaks in some northern locations associated with higher temperatures, but no outbreaks in southern USA	possible introduction to USA east coast in *C. gigas* (natural host?) being tested as possible aquaculture species	[14–16]
Aber (*M. refringens*)	oyster *Ostrea edulis* (90%) mussel *Mytilus galloprovincialis* (25–30%)	northern Brittany, France (1968) > northern France through Mediterranean	possible intermediate hosts described (zooplankton)	infection and proliferation at warm temperatures; 17°C triggers proliferation and sporulation	highest prevalence appears to be in enclosed farming areas. Thought to have been transmitted in transfers of oysters around Europe	[17–21]
QX (*M. sydneyi*)	oyster *Saccostrea glomerata* (90–95%)	Moreton Bay, Queensland (1968) > Queensland and New South Wales, Australia, a range of approximately 950 km	possible intermediate host described (polychaete)	infection and proliferation favoured by warm temperatures	known movement of oysters among farms. Agricultural practices lead to sedimentation, which favours a potential intermediate host polychaete, which inhabits muddy sediments	[22–24]

(*Continued*.)

Table 1. (*Continued.*)

disease (agent)	host[a] (mortalities[b])	first outbreak; known distribution	transmission	temperature association[c]	culture association, including commercial transport of molluscs	references
marteiliosis (*M. cochillia*)	cockle *Cerastoderma edule* (up to 100%, but associated with a virus)	Ebro Delta, Spain (2008) > Mediterranean and Atlantic coast of Spain; possibly Atlantic France (<1975)	probable intermediative or alternative host necessary	initial outbreak associated with high temperature (29°C). Proliferation in early spring ($t > 15$°C) to 100% prevalence	frequent imports of molluscs to the area of mortality	[25–27]
bonamiosis (*Bonamia ostreae*)	oyster *O. edulis* (60–95%)	California, USA (1966) > western Brittany, France (1979) > Atlantic coast of western Europe; west coast and Maine, USA	direct	prevalence usually highest during warm months, but no strong seasonality. Infection and mortality occur all year	density and handling stress appear to enhance infections; slower development in wild stocks than cultured. Clear trail of outbreaks following importation of oyster host	[28–31]
bonamiosis (*B. exitiosa*)	oyster *O. chilensis* (60% in 1964 outbreak)	Foveaux Strait, New Zealand (1964) > Europe and mid-Atlantic, USA	direct	rapid proliferation in summer; association with gametogenesis	pathogen found in many locations, but a trail of introductions has not been documented	[32–36]
Denman Island (*Mikrocytos mackini*)	oyster *C. gigas. O. lurida* (30% in Canada; none in USA)	British Columbia, Canada (1960) > Washington State, USA	direct	temperatures less than 10°C favour disease; disappears at approx. 18°C	none reported	[37–40]
QPX (unnamed thraustochytrid)	clam *M. mercenaria* (90%)	New Brunswick, Canada (late 1950s) > lower Chesapeake Bay, USA	direct	experimental infection prevalence, intensity and mortality higher at 13°C compared to 21°C or 27°C	outbreaks associated with use of susceptible, southern seed clam, high density and/or probable poor husbandry	[41–44]
bacteria						
ROD (*Roseovarius crassostreae*)	oyster *C. virginica (juveniles)* (90–95%)	Maine, USA (1988; maybe as early as 1984) > Maine to New York, USA	direct	mortalities begin when temperature rises above 20°C	occurs in hatchery-produced juvenile oysters. Exacerbated by crowding and poor water circulation in containers	[45–48]

(*Continued.*)

Table 1. (*Continued.*)

disease (agent)	host[a] (mortalities[b])	first outbreak; known distribution	transmission	temperature association[c]	culture association, including commercial transport of molluscs	references
BRD (*Vibrio tapetis*)	clam *Venerupes philippinarum* (approx. 20% over 2 years estimated from model simulations)	northern Brittany, France > western Europe, including British Isles, to the Mediterranean	direct	experimental infection produced more infections at 8°C and 14°C than at 21°C	first outbreak in culture park, although density may not be a factor. Likely an introduced host susceptible to resident bacterium	[49–51]
withering syndrome (*Candidatus* Xenohaliotis californiensis)	abalone *Haliotis* spp. esp. *H. cracherodii*	southern California (1985) > California coast to Baja California, Mexico	direct	elevated temperatures (18–20°C) enhance disease and mortality. High seawater temperatures during El Niño have been associated with increased mortality	some evidence that agent may have spread from plantings of hatchery-reared stocks	[52–54]
vibriosis (*Vibrio aestuarianus* and *V. splendidus*)	oyster (adults) *C. gigas*	France (approx. 2001); associated with Summer Mortalities; usually isolated from moribund animals	direct	outbreaks at summer temperatures, generally approximately 19–23°C	high density and proximity to sediment enhance mortality	[55–58]
viruses						
OsHV-1 (oyster herpes virus)	oyster (spat) *C. gigas* (up to 90%) scallop *Chlamys farreri* (up to 90%)	France—hatcheries (1991) Shandong, China (1990s) California, USA (2002)[d] Normandy, France (2008, -µ.var)	direct	high temperatures favour transmission, viral replication and mortality	movement of infected seed along with high densities in culture parks likely facilitates transmission. Large-scale and high-density culture preceeded outbreaks in Chinese scallop	[59–64]; this paper

[a]Aetiological agent may well infect other species; only the principal ones causing, or associated, with important mortalities are listed here.
[b]Mortalities are the maximum reported for susceptible stocks in experimental deployments, or cultured or natural beds. Time course not always provided, but typically over the course of a growing season.
[c]Temperatures are from field studies or experimental challenge.
[d]Likely linked to mortalities since 1993.

somewhat different from that found mostly in oysters, but both forms can parasitize both hosts leading to the possibility that mussels, which experience relatively low disease levels, might act as an alternative/reservoir host for the oyster parasite in areas where both are cultured [82]. Both *M. refringens* and *M. sydneyi* are acquired, proliferate and cause oyster mortality during the warm months of the year.

(d) Bonamiosis

By 1979, the French flat oyster industry, already hit by *M. refringens*, was devastated by another protozoan, *Bonamia ostreae*. Like most members of the genus, *B. ostreae* can be transmitted directly among oysters; thus movements of seed or adult oysters, and even larvae [83], from enzootic regions are potential sources of infection. The well-documented history of transfers of flat oysters, and likely the parasite, too, from California to France and Spain, and thence to other regions of Europe, has been previously described [28]. *Bonamia ostreae* prevalence is greatest during warm months, but infections show no strong seasonality [83]. In fact, *in vitro* experiments showed greater survival of the parasite at 4–15°C than at 25°C [84]. Density and handling stress appear to enhance infections, which develop more rapidly in cultured than in wild stocks [29].

In 1986, the New Zealand dredge oyster, *Tiostrea (Ostrea) chilensis*, suffered a loss of at least 60% in the area of Foveaux Strait. By 1992, only 9% of the stock that was present in 1975 remained [85]. The causative agent was identified as *B. exitiosa* [32], which was subsequently identified in oyster samples collected in 1964 [33]. Since then, the same pathogen has been detected in several species of oysters in North and South America and Europe as well as Australia/New Zealand [86], although whether it was introduced into any of these regions is not known.

(e) QPX

Most known bivalve diseases have affected oyster species, although a recently described disease, caused by an unnamed thraustochytrid parasite called QPX (quahog parasite unknown) has caused mortality in the hard clam (=quahog), *Mercenaria mercenaria*, in the northeastern USA [41,87]. In contrast to the previously described obligate pathogens of oysters, QPX is considered to be a facultative parasite, which, like others in the group, can live on macroalgae and detritus [88]. In fact, QPX DNA can be found widely distributed in environments where QPX-associated clam mortalities are documented [89]. The parasite appears to be favoured by colder temperatures [42] and is not found south of Chesapeake Bay. QPX can be transmitted directly and high density tends to increase prevalence [43,90], but there is no evidence that it is associated with hatchery production of clam seed [91]. On the other hand, QPX has been linked to clam culture practices, including the use, in northern climes, of southern seed clams, which grow fast, but are more susceptible to infection [92].

(f) Bacterial diseases of clams and oysters

Two bacterial diseases, one of the Manila clam, *Venerupes* (=*Ruditapes*) *philippinarum* in Brittany, France, and the other of the oyster, *C. virginica*, in New England, USA were first observed in the late 1980s. Both are diagnosed by similar 'external' signs—an anomalous ring of conchiolin around the inner edge of the valves, formed in response to the bacteria in the extrapallial space. They are called brown ring disease (BRD) and juvenile oyster disease (JOD), respectively, but are caused by quite different bacterial species. The aetiological agent of BRD is a marine *Vibrio*: *V. tapetis* [93]; that of JOD is an α-proteobacterium, *Roseovarious crassostreae* [45]. The latter, which has been renamed roseovarious oyster disease (ROD), affects juvenile oysters and can result in mortalities of up to 90% within a week of first detection [46]. BRD affects both juveniles and adults, killing up to 100% of clams if the bacterium is able to penetrate soft tissues [94]. Both diseases appeared first and have had the most detrimental effect, in culture settings, although BRD signs have also been found at very low levels in naturalized Manila clams [47,51].

ROD outbreaks are triggered by temperatures rising above about 21°C [47]. In contrast, BRD has been considered a 'cold water' disease because experimental infections were more successful at 14°C than at 21°C; however, the first outbreaks were in spring and summer [93] and a modelling exercise that included elements of the clam defence system as well as bacterial temperature tolerances, suggested that a temperature increase would increase the risk of disease [51]. Whereas culture conditions, including high densities and handling stress are known to exacerbate both diseases [46,49], another element is at play in the case of BRD. *Venerupes philippinarum* is an introduced species, brought, in the early 1970s, to France where it initially adapted well to culture [95]. *Vibrio tapetis* is a native and does not cause disease in the native *V. decussatus* [94], arguing that BRD is the result of an introduced host being susceptible to disease caused by a resident bacterium.

(g) OsHV-1

Herpes-like viruses were first found by Farley [96] in 1970 in *C. virginica* that died in cages under elevated water temperature (28–30°C) in Maine, USA. They were found in intranuclear inclusions of aggregated cells in haemolymph sinuses. The viruses were hexagonal, 70–90 nm in diameter and had a single coat, characteristic of herpes-like viruses. They were enzootic under natural temperature conditions in Marsh and Piscataqua Rivers of Maine but did not cause mortalities except under elevated temperatures.

Herpes-like viruses were not observed or reported to cause oyster mortalities for about 20 years after Farley's initial report. Then in summer of 1991, herpes-like viruses were found in moribund larvae of *C. gigas* that experienced abnormal mortalities in hatcheries in northern France [97]. In the same year, herpesviruses associated with mortalities of hatchery-reared Pacific oyster larvae were reported in New Zealand [98]. In July 1993, herpesviruses were observed in *C. gigas* spat experiencing mortalities in France [99]. The mortalities up to 80–90% occurred at the beginning of July in both wild and hatchery-produced spat at four locations and were over within a week. It was suggested that warm temperature and overcrowding might play a role in disease development. Mature viral particles were found in Pacific oyster larvae cultured at 25–26°C but not in those cultured at 22–23°C [64]. Although no biochemical diagnosis was made in the early reports, the morphology, size and cellular location of the viruses observed by electron microscopy strongly resembled those of herpes-like viruses.

Summer mortalities of *C. gigas* have been reported for many decades, although in most cases the causative agent

was unknown. Summer mortalities were observed as early as 1945 in Japan [100] and in 1950 in the USA [101]. In France, summer mortalities began in the mid-1970s and intensified over the next three decades. They were associated with a complex of interactions between host, environment and opportunistic pathogens [55]. Since 2008, an extreme mortality has been associated with a new and highly virulent oyster herpes variant, OsHV-1 µVar [59]. The mortality is associated with warm water temperatures and generally not observed below 16°C [102]. In the USA, severe summer mortalities of spat have been observed annually in Tomales Bay, California, since 1993, probably due to OsHV-1 infections [103,104]. In Australia, a herpes-like virus has been reported in haemocytes of adult flat oyster *Ostrea angasi* [105]. The first confirmed cases of OsHV-1, causing heavy mortality of wild Pacific oysters, occurred in 2010 and 2011 [106].

OsHV-1 has a broad host range. In addition to hosts mentioned above, herpes-like viruses have been observed in European flat oyster *O. edulis*, dredge oyster *T. chilensis*, clam *V. decussatus*, hard clam *M. mercenaria*, Manila clam *V. philippinarum* and scallops [107–110]. OsHV-1 has been detected in *Crassostrea sikamea*, *C. virginica*, *O. edulis*, *Mytilus galloprovincialis* and *V. phillipinarum* in California [111]. It is also detected in diverse bivalve molluscs in China, including *C. gigas*, *Crassostrea hongkongensis*, *Chlamys farreri*, *Patinopecten yessoensis*, *Meretrix meretrix*, *V. philippinarum* and *Scapharca broughtonii* [112].

Starting in the early 1990s, mortalities began to occur in Chinese scallops *C. farreri* cultured in lantern nets in Shandong, China. The mortalities occur in early August when the water temperature exceeds 28°C and last for about 20 days, decreasing with decreasing temperatures [1]. Cumulative mortality can reach as high as 90% in about two to three weeks [60]. At Rizhao in southern Shandong, mortalities were first observed in 1994 and continued thereafter. Mortalities worsened in 1997 and 1998, reaching 80% at many sites [1]. For many years, the cause of the scallop mortality in China was unknown, and suspected causes included stress caused by high temperature, overcrowding, starvation and parasites. Later, it was demonstrated that the mortality is caused by a variant of OsHV-1 virus [113].

Temperature might have played a role in the development of OsHV-1 infections in scallops as well as in oysters. The worsening of scallop mortality in 1997 and 1998 correlates with rising ocean temperature. The 11-month running mean sea surface temperature in Yellow Sea that covers Rizhao and most of the Chinese scallop culture sites increased from about 15°C in 1995 to 19°C in 1998 [114]. But other changes happened along with increasing temperature. The period between 1994 and 1997 was also the time when aquaculture production of Chinese scallops reached its peak (figure 1*a*). Field observations noted that scallop farmers often overstocked their lantern nets by two to three times their capacity [1]. The overcrowding was not limited to the cage. Bays and coastal areas were densely populated with scallop longlines and cages, which might have exceeded the carrying capacity of the culturing environment [1]. The high density of cultured scallops at cage level could have stressed scallops, while a high density of cages might have facilitated easy transmission of the virus and large-scale epizootics. The availability of hosts in high numbers might also promote the proliferation and mutation of the virus. Similarly, in France, OsHV-1 outbreaks in *C. gigas* also followed a period of rapid expansion of the oyster aquaculture industry (figure 1*b*). Thus, it is possible that large-scale aquaculture at high densities, or ocean warming, or both could have contributed to the large-scale epizootics of OsHV-1 in scallops and oysters.

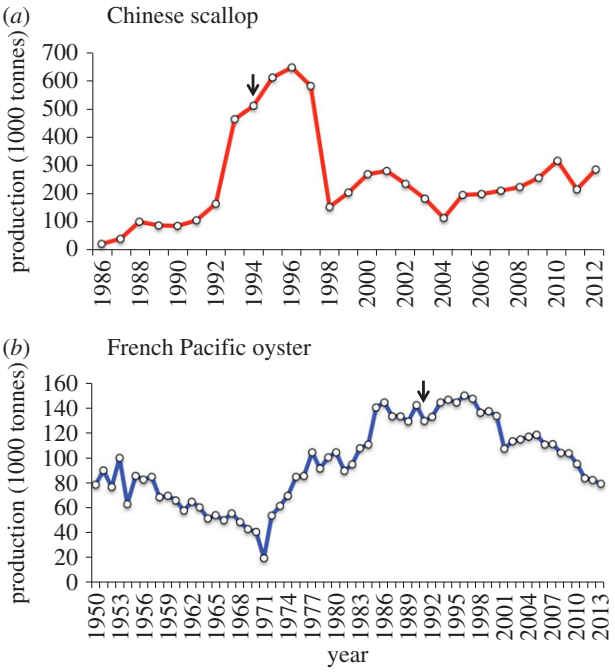

Figure 1. Large-scale aquaculture production precedes outbreaks of OsHV-1 in Chinese scallop *C. farreri* in China (*a*) and in Pacific *C. gigas* cultured in France (*b*). Arrows point to first reports of OsHV-1 caused mortalities. Production statistics for Chinese scallop are estimated based on total scallop production data from Ministry of Agriculture of China and personal knowledge of regional production of Chinese scallop. Production of *C. gigas* in France is extracted from United Nations Food and Agriculture Organization's database.

3. Genomes of molluscs and their parasites

Disease and mortality are the outcome of host–parasite interactions, which are complex and poorly understood in molluscs. Genomic tools and resources are helpful for studying host–parasite interactions. Whole genome or transcriptome sequencing can generate a complete inventory of genes that participate in host–parasite interactions. Recent advances in next-generation sequencing technologies have greatly reduced the cost of transcriptome and whole-genome sequencing. The whole genomes of three molluscs have been sequenced: the Pacific oyster *C. gigas* [115], the owl limpet *Lottia gigantea* [116], and the octopus *Octopus bimaculoides* [117]. These three molluscan genomes give unprecedented insights into the genome architecture of molluscs as well as a rich repertoire of immune-related genes for studying host–parasite interactions. In *C. gigas*, many gene families related to immune responses are expanded [115,118]. The expansion has resulted in great diversity in sequence, structure and function of immune and stress response genes, which may be central to oysters' remarkable resilience against highly stressful and pathogen-rich environments [6]. In *O. bimaculoides*, expanded gene families included C2H2 zinc-finger proteins, interleukin-17-like genes, and G-protein-coupled receptors, which may be involved in regulating the immune system [117]. A survey sequence of the pearl oyster has also been published [119], and genomes of *C. virginica* [120], *Pinctada martensii* and *P. yessoensis* are being sequenced.

Transcriptomes have been sequenced in many molluscs and identified many genes related to the immune response [6,121,122]. Transcriptomes are often sequenced after challenge with pathogens, and transcriptomic comparison between control and challenged molluscs can suggest the possible involvement of candidate genes in immune responses.

Several molluscan pathogen genomes have also been sequenced [122]. They include OsHV-1 [123], *P. marinus* (PRJNA46451) and several *Vibrio* species [124,125], and comparative analysis of the last has identified antibiotic genes. Clearly, the number of pathogens that have been sequenced is small, and further genome and transcriptome sequencing of molluscan parasites are needed. Genome sequencing of some molluscan pathogens is limited by the inability to culture them *in vitro*, although metagenomics and metatranscriptomics can be used to sequence and identify potential pathogens in the environment and the host [126,127].

4. Host immune responses

Host–parasite interactions are extremely complex, and recent genomic studies have led to the identification of thousands of immune-related genes in molluscs. Infection of the Pacific oyster by OsHV-1 alone induces the upregulation of 1942 genes [128]. The large numbers of candidate genes and their possible roles in molluscan immunity have been the subjects of several recent reviews [6,129–131]. Below is a brief summary of the basic components of mollusc's immune response to some important pathogens.

Recognition by the host of parasites as non-self triggers immune signal transduction and host immune responses. The recognition of parasites or foreign agents is accomplished by immune receptors. In vertebrates, highly diverse and specific antibodies are produced as part of the adaptive immune system and used to recognize specific pathogens based on immune memory. Innate immunity uses relatively few receptors that can recognize broad pathogen-associated molecular patterns (PAMPs). Invertebrates such as molluscs have no adaptive immunity and rely solely on innate or non-specific immunity for host defence. However, molluscs and some other invertebrates have greatly expanded sets of innate immune receptors, which may provide great specificity in immune recognition in the absence of adaptive immunity, and without the autoimmune costs of adaptive immunity.

(a) Toll-like receptors

Toll-like receptors (TLRs) are an ancient family of pattern recognition receptors (PRRs) that are involved in pathogen recognition and immune modulation in diverse organisms [132]. TLRs have been identified and implicated in immune responses in all molluscs studied so far, including *C. farreri* [133], *C. virginica* [121], *V. philippinarum* [134], *M. galloprovincialis* [135] and *C. gigas* [118,128]. The *C. gigas* genome encodes an expanded set of 83 TLRs, compared with nine in the fruit fly, 10 in humans and 12 in mice, and the expanded oyster TLRs have shown great diversity in structure and function [118,128]. The expansion and diversification of TLRs may be essential in the oyster's adaptation to diverse pathogens under dynamic environmental conditions [6].

Upon binding to PAMPs, TRLs activate downstream pathways through either the myeloid differentiation factor 88 (MyD88) or an MyD88-independent pathway (figure 2). Downstream factors of the TLR pathway include TNF (tissue necrosis factor) receptor-associated factor 6 (TRAF6), transforming growth factor-beta-activated kinase 1 (TAK1), nuclear factor kappa-B (NF-κB), inhibitor of NF-κB (IκB), IκB kinase (IKK), c-Jun N-terminal kinase (JNK), activator protein 1 (AP-1), Fas-associated protein with death domain (FADD), caspases, TRAF3 and interferon regulatory factors (IRFs) [132]. NF-κB and AP-1 are transcription factors that activate the transcription of other immune regulators and effector genes, while caspases participate in apoptosis (figure 2). TLRs may also activate TRAF3 through an MyD88-independent pathway, leading to the induction of IRFs.

TLRs and TLR pathways are activated by infections in molluscs [129,130]. In the Pacific oyster, four TLR-like and four MyD88-like genes along with several genes for downstream factors are highly upregulated by OsHV-1 infections [128]. Some upregulated TLR-like and MyD88-like genes lack essential domains and may play an antagonistic role in immune regulation.

(b) RIG-1 receptors and signalling

Retinoic acid-inducible gene (RIG) 1 like receptors (RLRs) are a group of PRRs that recognize viral nucleic acids and trigger antiviral inflammatory responses. Upon activation, RIG-1 receptors send signals through TRAF3, TRAF6 and FADD to activate terminal transcription factors and apoptosis (figure 2). Several RLRs and downstream factors such as NFκB, AP-1, IRFs and IF44 L, are highly upregulated in the Pacific oysters infected by OsHV-1 [128,136]. Genes involved in RNA destruction or editing such as ribonucleases, RNA-specific adenosine deaminase and Dicer-like proteins are also highly upregulated, and ribonuclease inhibitors are downregulated, suggesting that RIG-1 receptors and viral RNA destruction may be critical to oyster's antiviral response against OsHV-1 [128]. Oyster's immune response to poly I : C, a mimic of dsRNA, has been demonstrated in the Pacific oyster [137].

(c) Lectins and other carbohydrate pattern recognition receptors

Lectins and other carbohydrate PRRs recognize characteristic carbohydrates and glycoproteins located on cell walls of bacteria and other microbes as PAMPs. They bind, agglutinate and opsonize microbes to promote phagocytosis and destruction. C-type (calcium-dependent) lectin is the most common lectin found in molluscs. The *C. gigas* genome encodes 266 and the *M. galloprovincialis* transcriptome contains 154 C-type lectin genes, compared to 34 found in *Drosophila melanogaster* and 81 in *Homo sapiens* [115,129]. Molluscan C-type lectins agglutinate bacterial cells and are upregulated by bacterial challenges in several molluscs including *C. farreri* and *C. virginica* [130,138]. *Crassostrea virginica* galectins, a family of lectins that bind to β-galactosides, selectively recognize *P. marinus* that may use galectin to gain entry to host cells [139,140]. The down-regulation of galectin after *P. marinus* infection [141] may represent an attempt of the host to limit infections.

Other sugar-binding PRRs that may participate in immune responses include fibrinogen-related proteins (FREPs), peptidoglycan recognition proteins (PGRPs) and Gram-negative binding proteins (GNBPs). The freshwater snail *Biomphalaria glabrata* has highly diverse FREPs with immunoglobulin-like

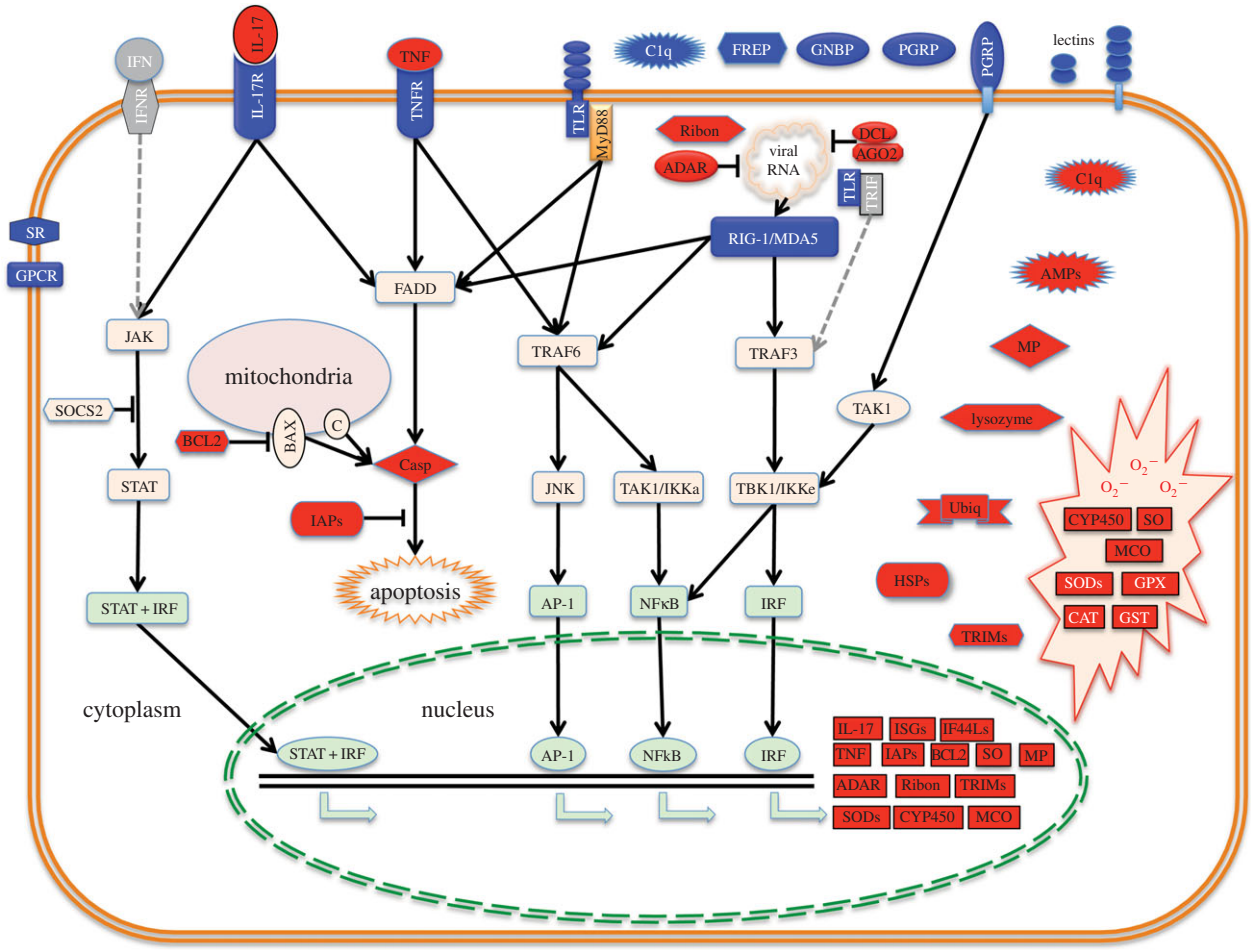

Figure 2. Hypothetical immune pathways in molluscs based on genes identified and their functions in model organisms. Genes shared in blue are receptors, genes in red are immune effectors, genes in green are transcriptional factors and genes in grey are suspected but have not been identified or confirmed in molluscs. IFN, interferon; IFNR, IFN receptor; IL-17, interleukin 17; IL-17R, IL-17 receptor; TNF, tissue necrosis factor; TNFR, TNF receptor; TLR, Toll-like receptor; MyD88, myeloid differentiation primary response gene 88; TRIF, TIR-domain-containing adapter-inducing interferon-β; ADAR, double-stranded RNA-specific adenosine deaminase; RIG-1, retinoic acid-inducible gene 1; MDA5, melanoma differentiation-associated protein 5; DCL, Dicer-like; AGO2, argonaute 2; Ribon, ribonucleases; PGRP, peptidoglycan recognition protein; GNBP, Gram-negative binding protein; FREP, fibrinogen-related protein; GPCR, G-protein-coupled receptor; SR, scavenger receptor; JAK, Janus kinase; STAT, signal transducer and activator of transcription; SOCS2, suppressor of cytokine signalling 2; IRF, interferon regulatory factor; BCL2, B-cell lymphoma 2; BAX, Bcl-2-associated X; C, cytochrome *c*; Casp, caspase; IAPs, inhibitors of apoptosis; FADD, Fas-associated protein with death domain; TRAF, TNF receptor-associated factors; JNK, c-Jun N-terminal kinases; NFκB, nuclear factor kappa B; IκB kinase (IKK); IKK, IκB kinase; TBK1, TANK-binding kinase 1; TAK1, transforming growth factor-beta-activated kinase 1; AP-1, activator protein 1; HSPs, heat shock proteins; CYP450, cytochrome P450; C1q, globular head C1q domain containing protein; IF44 L, interferon-induced protein 44-like; MCO, multi-copper oxidase; SO, spermidine oxidase; MP, metalloproteinase; SOD, superoxide dismutase; GPX, glutathione peroxidase; GST, glutathione *S*-transferase; CAT, catalase; AMP, antimicrobial peptide; TRIM, tripartite motif family; and ISGs, IFN-stimulated genes. (Adapted from [6].)

domains that arise from somatic variation similar to receptors of adaptive immunity of vertebrates [142]. Large numbers of FREPs are also found in other molluscs: 190 in the *C. gigas* genome [118] and 150 in the *M. galloprovincialis* transcriptome. The *C. gigas* genome encodes nine PGRPs [118]. One *C. gigas* PGRP is upregulated by *Marinococcus halophilus* and *Vibrio tubiashii* exposures [143]. Also, GNBP or *β*-1,3-glucan binding protein is involved in responding to *Vibrio* infections in *C. farreri* [144] and *Perna viridis* [145]. Many canonical immune receptors and response genes including PGRPs and GNBPs show the highest levels of expression in the digestive gland of *C. gigas*, suggesting that the gut may be a primary site of host–parasite interactions [115].

(d) The complement system

The complement system is an important part of the immune system. It functions through classical, alternative and lectin pathways, leading to the agglutination, opsonization, chemotaxis and lysis of foreign cells. The classical pathway requires the activation of C1q by antigen : antibody complexes. Although molluscs do not produce antibodies, many C1q domain containing proteins (C1qDCs) have been found: 321 in *C. gigas* genome, 1274 in *M. galloprovincialis* genome and 187 in *C. virginica* transcriptome, compared with four in sea urchin and 31 in human [115,121,129]. The globular C1q domain recognizes a broad range of ligands, and its expansion in bivalve molluscs may provide great binding specificity to diverse pathogens. The expanded C1qDCs show diverse expression profiles with some responding to bacterial and viral pathogens and others to abiotic stresses [118,128,136]. Although incomplete, other components of the complement pathways such as C3, factor B, membrane attack complex and perforin domain containing proteins have been identified [129,146], pointing to the presence of an ancient but complex complement system in bivalve molluscs.

(e) Cytokine receptors and apoptosis

Cytokines such as TNFs and interleukin 17 (IL-17) and their receptors are important regulators of immunity, inflammation and apoptosis [147]. Several TNF, TNFR and downstream TRAF genes of *C. gigas* are upregulated by bacterial and/or OsHV-1 challenges [118,128]. TNFs promote apoptosis, but the activation of TNF pathway by OsHV-1 in *C. gigas* coincides with significant upregulation of several inhibitors of apoptosis [128,148,149]. The complex regulation of apoptosis may reflect dynamic competition for control between the host and pathogens [150]. Several IL-17 and downstream genes are highly upregulated by OsHV-1 infection in *C. gigas* [128]. A putative IFN-like gene (CGI_10023118) has been identified in *C. gigas* [151]. However, this gene did not respond to OsHV-1 challenge, while several downstream genes such as JAK, STAT, IRFs and IF44Ls are highly upregulated [128]. It is still uncertain if IFN exists in molluscs or if its role is assumed by IL-17 as the two cytokines share downstream pathways (figure 2).

(f) Scavenger receptors

Scavenger receptors (SR) are PRRs that recognize modified low-density lipoproteins and participate in the scavenging or removal of foreign macromolecules [152]. Like sea urchins, bivalve molluscs have many SR-like genes: 71 in *C. gigas* genome and 62 in *C. virginica* transcriptome, compared with 16 in humans [115,118,121]. Several *C. virginica* SR transcripts are upregulated by *R. crassostreae* [153]. One of *C. gigas* SRs is upregulated by OsHV-1 infection, but two are downregulated [128].

(g) G-protein-coupled receptors

G-protein-coupled receptors (GPCRs) are a large family of seven-transmembrane domain receptors that regulate diverse cellular processes by sensing molecular cues outside the cells including light-sensitive compounds, odours, hormones and neurotransmitters. OsHV-1 infection of *C. gigas* induces upregulation of a large number of GPCR genes, including those coding for prostaglandin E2 receptors, FMRFamide (Phe-Met-Arg-Phe) receptors, cholecystokinin receptors, melatonin receptors, prolactin-releasing peptide receptors, adenosine receptors and dopamine D2-like receptors [128]. These results suggest GPCRs play an important role in the oyster's immune response. In scallops, several neuroendocrine signalling systems may be involved in immune response or regulation [130].

(h) Immune effectors

The recognition of pathogens by receptors triggers immune responses and the production of effectors that directly act on pathogens and foreign substances for their destruction and removal. A large number of immune effectors have been identified in molluscs including diverse antimicrobial peptides or proteins (AMPs), lysozymes, proteases, ribonucleases and oxidases [6,129–131].

Diverse cysteine-rich AMPs have been reported in mussels including defensins, big defensins, mytilins, myticins, mytimacins, mytimycins, myticusins and mytiCRPs [129]. Defensins [154,155], big defensins [156], proline-rich peptides (Prp) [157] and bactericidal/permeability-increasing proteins (BPI) [158,159] have been identified in oysters [131]. Most of these AMPs have shown antimicrobial activities *in vitro*. Oyster AMPs are highly diverse and variable in copy number [160], with different AMPs differing in antimicrobial potency [157].

Lysozymes degrade bacterial cell walls by hydrolysing glycosidic bonds in peptidoglycans. Several lysozymes have been identified and implicated in antibacterial defence in *M. galloprovincialis* [129]. Recombinant lysozymes from *C. gigas* and *C. farreri* inhibit bacterial growth [161,162].

Proteases may play an important role in the degradation of foreign proteins as part of the immune system. In *C. gigas* infected by OsHV-1, several metalloproteinase genes are upregulated, and several genes encoding tissue inhibitors of metalloproteinases and serine protease inhibitors are downregulated [128], suggesting proteinase activity is heightened, probably for the destruction of viral proteins or damaged host proteins. Parasites may use proteases during the infection process. Two serine protease inhibitors of *C. virginica*, named cvSI-1 and cvSI-2, have strong affinity and inhibitory effects on proteases of *P. marinus* [163–165].

Several ribonuclease genes are upregulated and ribonuclease inhibitor genes downregulated in *C. gigas* infected by OsHV-1, probably in a concerted effort to destroy viral RNAs [128], which together with the high upregulation of RNA sensing RIG-1/MDA5 receptors supports the idea of the destruction of viral RNAs as a major antiviral response in *C. gigas*.

Oxidative burst is an important component of immune response where reactive oxygen species such as superoxide anion (O_2^-), nitric oxide (NO) and hydrogen peroxide (H_2O_2), are created by oxidases and act to degrade invading cells and macromolecules. In *C. gigas* infected by OsHV-1, several oxidase genes including *cytochrome P450*, *multi-copper* and *spermine oxidases* are highly upregulated, while antioxidant genes such as *extracellular superoxide dismutase*, *glutathione peroxidase* and *glutathione S-transferase* are downregulated, suggesting a concerted effort in creating an oxidative burst for host defence [128].

5. Genetics of disease resistance

Host immune response is complex and involves many genes, and genetic variation in these genes can affect the effectiveness of immune response or disease resistance. Understanding the genetic variation in disease resistance is important for modelling the dynamics of disease transmission [166]. Because many marine molluscs are important aquaculture species, studies on disease resistance, especially the identification of disease-resistance markers, can contribute to the development of disease-resistant stocks through marker-assisted selection [167].

Genetic variation in disease resistance has been demonstrated through selective breeding of disease-resistant oysters [6]. Selective breeding in oysters has improved resistance against several diseases including MSX, Dermo and ROD in *C. virginica* [167–170], QX disease in Sydney rock oyster [171] and OsHV-1 infections in *C. gigas* [172]. The speed of improvement varies, depending on host species and diseases, possibly due to differences in infection mechanisms, host responses and effective selection pressure.

Advances in molecular genetics and genomics have enhanced our ability to identify disease-resistance genes and understand genetics of disease resistance. Valuable resources such as large numbers of genetic markers, genetic maps, transcriptomes and whole-genome sequences have been developed in several marine molluscs [6,122]. Quantitative trait loci

for OsHV-1 and Dermo disease resistance have been identified and mapped [173,174]. Studies on host immune response genes have led to the identification of genetic variations affecting disease resistance. In *C. virginica*, variation in serine protease inhibitor 1 (CvSI-1), which inhibits proteases from *P. marinus* [163,164], is associated with *P. marinus* resistance [175,176]. Variations in lysozyme and Cu/Zn superoxide dismutase genes are associated with resistance to *V. anguillarum* in scallops [177,178]. Variation in phenoloxidase is associated with QX resistance in Sydney rock oysters [179]. Polymorphism and variation in expression of AMPs may be associated with summer mortality resistance in the Pacific oyster [180].

With the increasing availability of genomic resources, advanced approaches such as genome-wide association studies may facilitate rapid identification of disease-resistance genes. The high polymorphism and diversity in immune-related genes of molluscs provide a good opportunity for studying immune gene function through association studies as well as for understanding the genetics of disease resistance.

6. Conclusion and perspectives

An increasing number of infectious diseases have been reported affecting marine molluscs. Some of the diseases have caused mass mortalities in commercially important molluscs. Although the route and mechanism of transmission are not completely understood, essentially most diseases of molluscs, and especially of cultured species, can be linked to human activities that either introduced new pathogens to naive populations or made environmental conditions conducive to disease outbreaks (table 1). Human activities have led to the introduction of pathogens such as *H. nelsoni* and *Bonamia* spp. into new regions. Climate warming due to anthropogenic activities is associated with the range extension of *P. marinus*-caused epizootics, and elevated temperature promotes the spread of many other pathogens including OsHV-1. Hatchery production and intensive farming place molluscan larvae, juveniles and adults at extreme densities and create incubators for bacterial and viral pathogens, producing stress and amplifying what is found naturally in the environment. High-density conditions at both local and ecosystem levels in large-scale aquaculture along with ocean warming may have contributed to the outbreaks of OsHV-1 in Chinese scallop. The possibility that large-scale aquaculture facilitates OsHV-1 outbreaks deserves attention as it has important implications in future aquaculture developments and the management of marine diseases [181].

Disease and mortalities are the outcome of complex host–parasite interactions influenced by the environment. Our ability to understand transmission dynamics and predict future disease outbreaks depends on knowledge of host–parasite interactions. Recent advances in genomics have provided insights into host and parasite genomes, and revealed surprisingly sophisticated innate immune systems in molluscs. All major innate immune pathways are found in molluscs, and many immune receptors, regulators and effectors are greatly expanded, providing remarkable diversity in immune response genes. The great diversity in sequence, structure and functions of immune response genes may be key to molluscs' defence against diverse pathogens in the absence of adaptive immunity. Genetic variation associated with disease resistance has been identified and used for modelling disease processes [6,166].

Despite recent advances, our understanding of molluscan immune systems and their interaction with parasites remains limited. Further efforts are needed to answer some of the key questions on the emergence of molluscan diseases and mortalities, such as: (i) Where in Asia did the MSX pathogen, *H. nelsoni* originate, how was it introduced into the USA, and what is/are its alternative host(s)? (ii) Are OsHV-1 outbreaks in oysters and scallops due to mutations in the virus or changes in environmental conditions? (iii) How do environmental factors such as temperature, salinity, ocean acidification and stress affect host–parasite interaction and disease? and (iv) How fast can a host develop resistance to a particular disease? Genomic and phylogenomic analyses of parasites are needed to develop diagnostic tools for epidemiological studies [182], determine possible transmission routes and identify virulence factors. Also needed are in-depth studies on genetic variations in both parasite and host that determine the outcome of disease and permit better predictions on emerging epizootics [183].

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