1 Parasite transmission through suspension feeding

2 Tal Ben-Horin^{1*}, Gorka Bidegain², Lauren Huey¹, Diego A. Narvaez³, and David Bushek¹

3

4 ¹Haskin Shellfish Research Laboratory, Department of Marine and Coastal Sciences, Rutgers
5 University, 6959 Miller Ave, Port Norris, NJ 08349

6 ²Gulf Coast Research Laboratory, University of Southern Mississippi, 703 East Beach Dr, Ocean
7 Springs, MS 39564

8 ³Department of Aquatic Systems, Faculty of Environmental Sciences, Center EULA-Chile,
9 Universidad de Concepción, Concepción, Chile

10

11

12 Corresponding author: Tal Ben-Horin, Haskin Shellfish Laboratory, Department of
13 Marine and Coastal Science, Rutgers University

14

15 ^{*}Present address: College of the Environment and Life Science, University of Rhode Island,
16 Kingston, RI 02881

17 ABSTRACT

18
19 Suspension-feeding bivalve molluscs are confronted with a wide range of materials in
20 the benthic marine environment. These materials include various sized plankton and the
21 organic material derived from it, macroalgae, detritus and a diversity of microbial
22 parasites that have adapted life stages to survive in the water column. For bivalve
23 parasites to infect hosts though, they must first survive and remain infectious in the water
24 column to make initial contact with hosts, and once in contact, enter and overcome
25 elaborate pathways for particle sorting and selection. Even past these defenses, bivalve
26 parasites are challenged with efficient systems of mechanical and chemical digestion and
27 highly evolved systems of innate immunity. Here we review how bivalve parasites evade
28 these hurdles to complete their life cycles and establish within bivalve hosts. We broadly
29 cover significant viral, bacterial, and protozoan parasites of marine bivalve molluscs, and
30 illustrate the emergent properties of these host-parasite systems where parasite
31 transmission occurs through suspension feeding.

32
33 Keywords: bivalve, disease, life cycle, parasite, suspension feeding, transmission

34	Contents
35	
36	1. Introduction
37	2. Parasites of bivalve molluscs
38	3. Parasite interactions in the environment
39	4. Suspension feeding and portals of entry
40	5. Parasite life cycles
41	6. Concluding remarks: an ecological perspective
42	Acknowledgments
43	References

1. Introduction

A typical liter of seawater collected from the surface waters of coastal seas can contain millions of algal cells, billions of bacteria and microbial eukaryotes, and up to 100 billion viruses (Pedros-Alio 2006; Suttle 2007; Amend et al. 2012; Breitbart 2012). This abundance of microbial life suspended in the water column has created a niche for suspension feeding, a trophic strategy that only rarely occurs on land. Suspension feeders have adapted numerous mechanisms for sorting and capturing food particles that are often aggregated with other microbes, mineral grains, detritus, and various other incidental particles (Rubenstein and Koehl 1977; Shimeta and Jumars 1991; Kach and Ward 2008). The importance of this feeding strategy is apparent in the bivalve molluscs, which have evolved distinct morphological structures capable of efficiently exploiting suspended particles as a source of food (Jorgensen 1996; Ward 1996). Owing to this efficiency, bivalves simultaneously sort, select, process, reject, ingest and transport food items (Shumway 1985; Riisgard et al. 1995; Ward and Shumway 2004). These bulk-feeding activities contribute significantly the flux of organic material and nutrients from the water column to the benthos and back (Dame et al. 1984; Prins et al. 1998; Chauvand et al. 2000; Ward and Shumway 2004), and also confront these mostly sedentary and sessile generalist consumers with a diverse range of materials, including the many pathogenic microparasites that have adapted free-living stages capable of surviving adrift in the water column (Thompson et al. 2005).

Parasites with free-living stages often function as prey for generalist consumers (Johnson et al. 2010; Thieltges et al. 2013) such as bivalve molluscs (Thieltges et al.

2008). The consumption of parasites is neither rare nor limited to bivalves. Lafferty et al. (2006), for example, estimated that almost half of the trophic links in an estuarine food web involved predation on parasites. Although consumption by non-focal hosts is common (Mouritsen and Poulin 2002; Lafferty et al. 2006; Thieltges et al. 2008; Johnson and Thieltges 2010), the ubiquity of free-living stages of viruses, bacteria, and protozoans in coastal waters (Poulin and Morand 2000; Thompson et al. 2005) suggests that consumption is a major pathway for parasite transmission in marine bivalves. The question of how bivalve molluscs survive in parasite-rich environments without adaptive immunity has been of fundamental interest to physiologists and immunologists (Martin et al. 2006), and a considerable body of work has identified sophisticated systems of innate immunity in bivalves (Kiss 2010; Zhang et al. 2014; Zhang et al. 2015) including highly developed processes for cellular internalization of parasites (Hine 1999; Canesi et al. 2002; de Freitas Rebelo et al. 2013). Despite progress understanding the processes that allow bivalve parasites to avoid these cellular and molecular defense factors and proliferate in host tissues (Hervio et al., 1991; Wright et al. 2002; Ahmed et al. 2003, Brown and Reece 2003; Munoz et al. 2003; Schott and Vasta 2003, Kim et al. 2008, Flye-Sainte-Marie et al. 2009; Soudant et al. 2013), our understanding of parasite transmission remains limited by our knowledge of 1) the fate of free-living parasite stages in the environment, 2) processes of initial dissemination in host tissues, and 3) parasite life cycles, including interactions with secondary or intermediate hosts.

Here, we review mechanisms of parasite transmission and spread in suspension feeding marine bivalves. We first begin with a brief review of parasites of marine and estuarine bivalve molluscs, focusing on microparasites that are encountered through

suspension feeding processes. We review the present understanding of their transmissive stages in the water column and dispersal from hosts, and then focus on the selection of particulate matter by bivalve molluscs, emphasizing behaviors of bivalve parasites to evade digestion and host defenses to successfully establish within host tissues. We conclude by integrating interactions between parasites and bivalve hosts to describe the life and reproductive cycles of bivalve parasites. Our intention is not to exhaustively describe all parasites of marine bivalves. Rather, we aim to direct new perspectives for integrative research by highlighting and connecting emergent properties from systems where bivalve hosts encounter parasites through feeding,

2. Parasites of bivalve molluscs

Bivalve molluscs, including oysters, clams, quahogs, scallops, cockles, and mussels, play host to a wide range of parasites (Table 1). These can include many agents of human disease, such as polio (Lees 2000), Norwalk (Morse et al. 1986; Baker et al. 2011) and hepatitis (Mele et al. 1989) viruses, *Listeria* (de Simon et al. 1991), *Salmonella* (Greenwood et al. 1998), *Shigella* (Iwamoto et al. 2010; Oliveira et al. 2011) and *Vibrio* (Iwamoto et al. 2010, Audemard et al. 2011; Powell et al. 2013; Thongchan et al. 2013) species of bacteria, and protozoan parasites such as *Cryptosporidium spp.*, *Giardia duodenalis* and *Toxoplasma gondii* (Fayer et al. 2004; Robertson 2007) that are free-living in coastal waters and incidentally concentrate in bivalve tissues through particle-feeding processes (Meyers 1984; Rippey 1994). The role of bivalves as transient reservoirs of parasites of humans and other vertebrates is well documented (Potasman et

al. 2002; Daniels 2011; Sandifer and Sutton-Grier 2014). Generally, these disease agents cause little to no ill effects in bivalve hosts but are transmitted to their definitive vertebrate hosts through the consumption of raw or undercooked shellfish (Rippey 1994; Bellou et al. 2013).

There are numerous examples of parasites targeting bivalve molluscs as definitive hosts (Sindermann and Rosenfield 1967; Sindermann 1990; Bower et al. 1994; Elston 1997; Paillard et al. 2004; Renault and Novoa 2004; Soudant et al. 2013; Table 1), and disease outbreaks are increasingly recognized as a significant constraint to aquaculture production and the sustainability of wild shellfisheries (Harvell et al. 1999; Mann and Powell 2007; Mann et al. 2009). Marine protists, among the most abundant eukaryotes on Earth (Baldouf 2003; Massana et al. 2006; Tsui 2009; Raghukumar and Damare 2011), are also among the most commonly reported agents of bivalve diseases (Fig. 1). Among the most destructive are the intracellular parasites of the genera *Bonamia*, *Haplosporidium*, *Marteilia*, and *Perkinsus*, including *Bonamia exitiosa*, *Bonamia ostreae*, *Marteilia refringens*, *Perkinsus marinus*, and *Perkinsus olseni* (Sinderman and Rosenfield 1967; Grizel et al. 1998; Ragone Calvo et al. 2003; Carnegie and Cochenne-Laureau 2004; Villalba et al. 2011; Fernandez-Robledo et al. 2014; Soudant et al. 2014). These protozoan parasites are under surveillance by the World Organization for Animal Health (OIE; <http://www.oie.int/>; Aquatic Animal Health Code, Section 11: Diseases of Molluscs) due to their cosmopolitan distribution and severe impacts on harvests of wild and farmed bivalves. Groundbreaking progress on methods of parasite purification and cell culture, parasite virulence factors, and molecular tools have advanced both mechanistic studies of transmission and reporting of these and other protozoan parasites.

This wealth of information has elevated protozoan parasites as models for basic and translational research on the emergence and spread of marine diseases (Grizel et al. 1988; Carnegie and Burrenson 2011; Burge et al. 2014; Fernandez-Robledo et al. 2014).

Viruses are by far the most abundant and genetically diverse life form in the oceans (Cochlan et al. 1993; Maranger and Bird 1995; Wommack and Colwell 2000; Suttle 2005; Suttle 2007; Breitbart 2012) (Fig. 1). The sheer abundance and narrow host range of most viruses suggests that viral infections place significant controls on the abundance and composition of planktonic communities (Wommack and Colwell 2000).

Methodological constraints, such as the lack of continuous bivalve cell lines, have impeded the discovery, detection, and classification of most viral infections in marine bivalves (Elston 1997; Renault and Novoa 2004), and bivalve virology remains an emerging research frontier. Viruses are likely responsible for many of the cryptic mortality events observed both in bivalve hatcheries and wild stocks (Renault and Novoa 2004) and virus-like infections have been identified and associated with mortalities of wild and cultured bivalves since the late 1960s (Farley 1972; Elston 1997; Renault and Novoa 2004). Many proliferative diseases of commercially important marine bivalves, such as disseminated neoplasia, are of likely but unconfirmed viral etiology (Elston et al. 1992; Barber 2004; Romalde et al. 2007). The initial classification of bivalve viruses is most often based on observations of morphology determined from histopathology and electron microscopy, making the definitive identification of viral taxonomy tenuous. However, viral infections have been interpreted as putative members from a diversity of viral taxa, including DNA viruses such as Herpesviridae (Farley et al. 1972; Comps and Cochenec; Hine and Thorne 1997; Hine et al. 1998; Arzul et al. 2001; Renault et al.

159 2001; Renault and Arzul 2001; Friedman et al. 2005; Burge et al. 2006) and
 160 Papovaviridae (Choi et al. 2004), RNA viruses such as Reoviridae (Meyers 1979),
 161 Iridoviridae (Elston and Wilkinson 1985), and Picornaviridae (Rasmussen 1986; Jones et
 162 al. 1996; Novoa and Figueras 2000; Carballal et al. 2003), and retroviruses (Oprandy et
 163 al. 1981). As with many viruses infecting vertebrate hosts, most bivalve viruses are host-
 164 specific (Davidson et al. 2005; Suttle 2007), although this is not always the case (Arzul et
 165 al. 2001b; Arzul et al. 2001c). Herpes-like viruses infecting oysters have been classified
 166 as a member of the Herpesviridae family under the name Ostreid herpesvirus-1 (OsHV-1;
 167 Davidson et al. 2005; Savin et al. 2010; Jouaux et al. 2013). The apparent lack of host
 168 specificity of OsHV-1 is unique among the Herpesviridae, and this herpesvirus has been
 169 found to infect at least seven bivalve species across four genera (Arzul et al. 2001b; Arzul
 170 et al. 2001c) where putative hosts include *Crassostrea gigas*, *Crassostrea angulata*,
 171 *Crassostrea ariakensis*, *Ostrea edulis*, *Ruditapes philippinarum*, *Ruditapes decussatus*,
 172 and *Pecten maximus* (Renault et al. 2000; Arzul et al. 2001a; Arzul et al. 2001b; Lynch et
 173 al. 2012). In the case of OsHV-1, it is suspected that the virus uses common, conserved
 174 systems among bivalves to enter and establish within this diverse host community,
 175 having emerged as a consequence of selection arising from intensive aquaculture of
 176 multiple bivalve species (Arzul et al. 2001b; Arzul et al. 2001c). Further studies seem to
 177 indicate that OsHV-1 has the capacity to evolve rapidly, and an increase in mortalities of
 178 *Crassostrea gigas* linked with OsHV-1 infection along French coasts since 2008 has been
 179 associated with the detection of a virulent OsHV-1 variant, designated OsHV-1 μ var
 180 (Segarra et al. 2010; Martenot et al. 2011). Continued observations of additional, new

forms reinforce the possibility of the emergence of different and potentially virulent OsHV-1 variants (Martenot et al. 2011; Martenot et al. 2012)

Bacterial parasites, although only rarely reported to be the primary cause of disease in adult bivalves (Bower et al. 1994; Lane and Birkbeck 2000), are commonly described in bivalve larval stages and often associated with high mortalities in shellfish hatcheries (Sindermann 1990; Paillard et al. 2004; Sainz-Hernandez and Maeda-Martinez 2005). For many bivalve species, bacteria can provide some component of carbon and nitrogen requirements (Birkbeck and McHenry 1982; Langdon and Newell 1990; Paillard 2004). At the same time, bivalves provide habitat for diverse communities of commensal bacterial microbiota, including various members of *Vibrio*, *Pseudomonas*, *Acinetobacter*, *Photobacterium*, *Moraxella*, *Aeromonas*, *Micrococcus*, and *Bacillus* (Pujalte et al. 1999; Romero et al. 2002; Beaz-Hidalgo et al. 2010). Nevertheless, major disease outbreaks in cultured larval and juvenile bivalves have been attributed to a number of bacterial parasites, particularly when environmental parameters such as water temperature and salinity influence bacterial diversity and the physiological state of bivalve hosts (Beaz-Hidalgo et al. 2010). These parasites include *Roseovarius crassostreae*, the etiological agent of Roseovarius Oyster Disease in *Crassostrea virginica* (Ford and Borrero 2001; Boettcher et al. 2005; Maloy et al. 2007), *Nocardia crassostreae*, the causal agent of Pacific Oyster Nocardiosis (Friedman et al. 1991; Friedman et al. 1998; Bower et al. 2005), and *Vibrio pectenicida* and *Vibrio tubiashii*, significant parasites of *Pecten maximus* and *Crassostrea gigas* larvae in hatcheries (Hada et al. 1984; Lambert et al. 1998; Lambert et al. 2001). Brown Ring Disease, caused by *Vibrio tapetis* (Borrego et al. 1996), is a noteworthy exception to the observation that bacterial parasites principally

target larval bivalves. This disease occurs in both wild and cultured *Ruditapes philippinarum* in France, England, Ireland, Spain and Italy (Allam et al. 2000; Paillard 2004b). Signs of the characteristic brown deposit on the inner surface of the valve caused by colonization of *Vibrio tapetis* have also been detected in a number of bivalves, including *Ruditapes decussatus*, *Venerupis aurea*, *Dosinia exoleta* and *Pecten maximus* (Paillard 2004), although resistance to infection and signs of disease vary significantly by species (Allam and Ford 2006). In the case of Brown Ring Disease, *Vibrio tapetis* acts initially as an external microparasite and does not directly enter host tissues (Allam et al. 2001; Allam et al. 2002). Rather, this bacterial parasite adheres to the surface of the host's mantle edge, which provides the substrate for initial colonization and subsequent spread throughout host tissues (Allam and Ford 2006).

3. Parasite interactions in the environment

There is much to learn about the biological, morphological, and behavioral diversity of parasites in the sea and its consequences on modes of parasite transmission. Viruses are the most abundant members of marine microbial communities. In the water column, free-living but non-replicating viral particles exist as a protein coat or capsid, enclosing the DNA or RNA that code for the virus elements. Viral capsids can themselves be enclosed within a membrane, and many viruses, including the Herpesviridae and Retroviridae, have a supplementary phospholipid and protein bilayer, known as a viral envelope, covering their protective protein capsids (Brum et al. 2013). The viral envelope interacts with receptors on target cells to mediate virus entry, fusing viral and host cell

membranes while evading host defenses (Wyatt and Sodroski 1998; Poranen et al. 2002). This latter property is suggested to be associated with persistent infections (Forns et al. 2000). The phospholipid bilayer of the viral envelope also makes these viral particles sensitive to both desiccation and sunlight, suggesting that these viruses have limited survival outside host environments (Poranen et al. 2002).

Most of the virus inactivation in clear oceanic waters exposed to full sunlight can be attributed to solar radiation (Heldal and Bratbak 1991; Suttle and Chen 1992). However, the most mutagenic and lethal effects of solar radiation, in particular DNA photoproducts such as pyrimidine dimers, are often repaired by host-cell mechanisms such as photoreactivation and the majority of viral particles in the water column are thought to remain infective despite high rates of DNA damage (Wilhelm et al. 1998; Wommack and Colwell 2000; Suttle 2005). In addition, the lethal effects of solar radiation diminish at high latitude and in productive coastal waters, particularly at depth. In these regions, the most prevalent finding among studies of natural virus inactivation is that the presence of natural bacterial communities is critical to the rate of viral inactivation and decomposition (Wommack and Colwell 2000; Simon et al. 2002; Weinbauer et al. 2009; Breitbart 2012). This conclusion is supported by laboratory manipulations, where removal of bacterioplankton by filtration or autoclaving greatly improves virus survival (Wommack and Colwell 2000). Temperature is an important interacting factor as well, as observations of increased viral inactivation at higher incubation temperatures can often be attributed to temperature-mediated enhancement of bacterial activity in seawater (Mathias et al 1995; Garza and Suttle 1998). The inactivating effects of bacterioplankton may be offset by the protective effect of viral adsorption to particulates suspended in the

water column (Bitton 1975). However, suspended particulates are more likely to further enhance viral decomposition. Suspended marine aggregates, sometimes referred to as marine snow, are heavily colonized by bacteria and other heterotrophic microbes (Lyons et al. 2010) and have been identified as hotspots of microbial decomposition of organic material. The abundance of heterotrophic bacterial production suggests that marine aggregates are more likely to serve as a sink rather than a source of suspended viral particles (Simon et al. 2002). The relative importance of aggregates on the decay and decomposition of suspended parasites is therefore likely to be variable (Kramer et al. 2013), ultimately depending on specific characteristics of the parasites themselves. For example, bacterial parasites such as *Vibrio spp.* and protozoan parasites such as QPX have been observed on marine aggregates (Lyons et al. 2005; Lyons et al. 2007). Whittington et al. (2015a) observed that mortality of *Crassostrea gigas* associated with the microvariant genotype of OsHV-1 was prevented when seawater pumped through an upwelling nursery system holding oysters was filtered to 5 μm . Since individual viral particles can certainly pass through a filter of this size, this observation supports the hypothesis that OsHV-1 is also associated with marine aggregates. It remains unknown whether suspended aggregates serve as a refuge or even abiotic reservoir for viral, bacterial and protozoan parasites though. Concentrations of aggregate-associated parasites may simply reflect ambient concentrations of these parasites in coastal marine ecosystems.

Many bacterial parasites of bivalves, such as *Vibrio spp.*, *Nocardia crassostreae*, and *Roseovarius crassostreae*, are common, though inconspicuous, members of coastal marine ecosystems (Gonzalez and Moran 1997; Paillard et al. 2004; Wagner-Dobler and

Biebl 2006; Beaz-Hidalgo et al. 2010). Though these parasites cause disease and mortalities in bivalves, most are not strictly intracellular and have adapted to alternate between growth within hosts and prolonged free-living survival and reproduction in the environment (Gonzalez and Moran 1997; Yildiz and Visick 2009). This distinction is important, as bivalves most often serve only as incidental hosts for these facultative parasites. Many of the *Vibrio* species play an additional, larger role in nearshore marine ecosystems through the decomposition of organic material and cycling of nutrients (Benitez-Nelson 2000; Fischer et al. 2014). Many bacterial parasites have also been identified in association with marine aggregates (Lyons et al. 2005; Lyons et al. 2007), and a key factor for their survival in the environment, in particular for the *Vibrio* species, is their ability to form biofilms on various surfaces, including benthic sediments and hard substrate in addition to the constituent particles of marine aggregates (Nyholm et al. 2000; Yildiz and Visick 2009). The biofilm forming capacity of vibrios enhances their growth and survival in the environment by providing these bacteria both access to nutrients and protection from predators and antimicrobial compounds (Costerton 1999; Donlan and Costerton 2002), and may even be linked with the pathogenicity of some species within hosts (Rodrigues et al. 2014). As a consequence, studies concerning the transmission and decay of bacterial parasites in coastal waters have focused on identifying the timing of, and environmental conditions associated with, periods of their abundance in the water column (Paillard et al. 2004; Elston et al. 2008; Beaz-Hidalgo et al. 2010; Paillard et al. 2014), rather than the effects of these variables on the inactivation and infectivity of free-living bacteria.

Protozoan parasites differ from many bacterial parasites in that most, but not all, require primary and sometimes secondary hosts to complete their life cycles. Although most protozoan parasites cannot reproduce in the water column, experimental studies have demonstrated that infective stages for many of these parasites, including *Perkinsus marinus*, *P. olsenii*, *Marteilia sydneyi*, and *Bonamia ostreae* can survive for days, weeks, and even months in the water column, and remain infective when they enter a new host (Auzoux-Bordenave et al. 1995; Chu 1996; Wesche et al. 1999; Chu and Lund 2006; Arzul et al. 2009). Some parasite stages, such as hypnospores of *Perkinsis spp.*, formed in decaying tissues of moribund hosts, can survive for months under environmental conditions that prevent further proliferation (i.e. zoosporulation) (Auzoux-Bordenave et al. 1995; Casas et al. 2002). The free-living and metabolically active meront stage of *P. marinus* has demonstrated the ability to survive for at least two weeks in seawater (Chu et al. 2002; Chu and Lund 2006). The longevity of meront stages in seawater has been partially attributed to the ability of cells to synthesize a range of saturated and unsaturated fatty acids, including the essential fatty acid, arachidonic acid (AA), while maintained in seawater (Soudant and Chu 2001; Lund et al. 2004; Chu and Lund 2006). This capability is novel, as no other protozoan parasites have been reported to demonstrate such a capability, instead relying entirely on their hosts for essential lipids. Lipids play a vital role in both the long-term survival and the completion of the life cycle of all endogenous parasites (Soudant and Chu 2001). In the case of *P. marinus*, lipids may facilitate disease transmission processes by serving as energy reserves for growth and survival during times of limited or nonexistent nutrient supply (Lund et al. 2004; Chu and Lund 2006).

The capability of *P. marinus* to synthesize essential fatty acids for growth is unique to this parasite, but other protozoan parasites may be capable of long-term survival and growth outside hosts. QPX is a member of the phylum Labyrinthulomycota (Kleinschuster et al. 1998; Ragan et al. 2000; Stokes et al. 2002). Members of this phylum are ubiquitous epibionts of marine vascular plants, macroalgae, and detritus (Raghukumar and Damare 2011). A distinguishing feature of the labyrinthulomycetes is their ectoplasmic net, an external cytoplasmic network that attaches the cell to its substrate and secretes digestive enzymes for absorptive nutrition (Tsui et al. 2009). This structure enables these microbes to live as mutualists, commensals, saprobes, and less frequently parasites (Muehlstein et al. 1988; Raghukumar and Damare 2011). Laboratory experiments and field sampling have demonstrated that, although QPX does not appear to grow isolated in seawater, the parasite is capable of long-term survival outside quahog hosts when associated with marine aggregates. QPX can even grow outside its host if provided live or decaying macroalgae homogenates as a source of nutrients (Lyons et al. 2005; Bugge and Allam 2007; Gast et al. 2008). The species of macroalgae is important however, as QPX growth can be limited and even inhibited depending on the taxonomy and transience of macroalgal media (Bugge and Allam 2007). The specificity of QPX growth is most likely a consequence of species-specific plant antimicrobials present (Raghukumar and Damare 2011). Consequently, QPX appears to be an opportunist, adapting to parasitism when conditions are favorable (Ford et al 2002; Dahl and Allam 2007; Dahl et al. 2008). This apparently facultative parasite can be present throughout the year, and environmental sampling has confirmed that QPX is regularly present in samples of seawater, marine aggregates, detritus, sediments, live macroalgae and marine vascular

plants, and various invertebrates (Lyons et al. 2005; Bugge and Allam 2007; Gast et al. 2008).

Even in the absence of adequate sources of nutrition, free-living stages of protozoan parasites of the genera *Perkinsus*, *Bonamia*, and *Marteilia* have demonstrated the ability to survive for weeks or more in the water column (Grizel et al. 1988; Auzoux-Bordenave et al. 1995; Wesche et al. 1999; Casas et al. 2002; Chu and Lund 2006; Arzul et al. 2009).

A number of physical factors can influence the decay and inactivation of free-living stages of protozoan parasites, and extensive research has identified temperature and salinity as the most important environmental factors affecting the rate of cell inactivation outside hosts (La Peyre et al. 2006; La Peyre et al. 2008; Arzul et al. 2009; La Peyre et al. 2010; Perrigault et al. 2010). A fairly precise range of optimal environmental conditions has been defined for the *in vitro* and *in vivo* growth and proliferation of many of these parasites. For example, optimal temperatures supporting the proliferation of *P. marinus* range from 28 – 32°C and optimal salinity ranges from 24 – 36 ppt (Burreson et al. 1994; Dungan and Hamilton 1995; Gauthier and Vasta 1995; La Peyre et al. 2010). Cellular and humoral parameters measured from quahogs challenged with QPX have demonstrated little difference compared to uninoculated control groups at 27°C, whereas different responses have been observed at 13°C and 21°C (Perrigault et al. 2011). This result supports observations of QPX disease occurring only in the cooler waters characterizing the region from Maritime Canada through New England to Virginia. Zoosporulation of *P. olseni* in seawater is abruptly reduced at salinities lower than 25 ppt, and zoospores have demonstrated the ability to survive for up to 14 and 22 days at 10°C and 28°C respectively (Casas et al. 2002). Contrary to observations of increased survival with

increasing temperature in *P. olseni*, *B. ostreae* has demonstrated significantly lower survival at 25°C, compared to 4°C and 15°C (Arzul et al. 2009). The viability of *Marteilia sydneyi* spores also appears to increase with decreasing temperature (Wesche et al. 1999). In addition, the survival of purified *B. ostreae* has been observed to increase at salinities greater than 35 ppt (Arzul et al. 2009). The survival of QPX in seawater is also higher at 15°C, relative to 20°C and 23°C, but in contrast to the observed survival of *B. ostreae*, increases with decreasing salinity (Perrigault et al. 2010). These studies suggest that the environmental conditions favoring the survival of free-living and metabolically active parasite stages are species-specific. In the cases of *B. ostreae* and QPX, relatively cool temperatures could decrease the metabolic rates of free-living parasites, delaying the onset of mortality and augmenting the parasites' ability to disperse throughout nearshore marine ecosystems.

Dispersal to new hosts begins with the release of parasite stages into the water column. Given the small size and limited swimming ability of most viral, bacterial, and protozoan parasites of bivalves, it is assumed that dispersal is passive, and driven by physical processes such as winds, tides, bathymetry, and river runoff (MacCready and Geyer 2010). The relative contribution of these mechanisms varies, and ultimately defines the scale of parasite dispersal. For example, the short dispersal lengths of oyster larvae generally seen in the lower Delaware Bay, a semi-enclosed embayment characterized by high particle retention time, have been associated with an extensive recirculation that is maintained by the area's topography (Narváez et al. 2012). Suspended parasites are often retained by this recirculation, reducing their transport to other areas of Delaware Bay. Broad transport of particles throughout Delaware Bay has

386 been demonstrated to occur though, often in association with high river runoff (Narváez
387 et al. 2012). Below average flows, on the other hand, allow particles to move further into
388 upstream regions (Narváez et al. 2012). Short term changes in river flows can therefore
389 be crucial for the transmission of parasites that remain infective in the water column often
390 for weeks or more.

391 Other physical mechanisms, such as tides and winds, are equally important.

392 Dispersion by tides is dependent on the size of the estuary relative to the tidal excursion
393 length. In small bays and estuaries, or areas with abrupt changes in topography, the
394 consequences of tidal dispersion can be significant (Geyer and Signell 1992). In mid-
395 latitude regions, ebb and flood tide velocities can range between 0.5 and 1 m s⁻¹
396 potentially transporting particles up to 4 km per hour (Valle Levinson et al. 2000). This
397 estimate is supported somewhat by observations of new infections with *P. marinus* in
398 disease-free oyster cohorts as early as ten days after placement in disease-enzootic waters
399 of Chesapeake Bay. These new *P. marinus* infections occurred despite approximately 5
400 km of separation from naturally infected oyster reefs (McCollough et al. 2007). Wind
401 patterns can also exert significant controls on estuary circulation, having both local and
402 remote effects (Wong and Garvine 1984). Local winds have an immediate effect on local
403 circulation, but remote winds can also result in an elevation or depression of regional sea
404 level, with cascading consequences on local circulation in estuaries (Wong 1994).
405 Furthermore, vertical mixing by winds can be important in the redistribution of particles
406 in the water column, exposing displaced particles to different and often opposing
407 currents. The consequences of these interacting physical mechanisms are difficult to

interpret, imposing complexity to the prediction of parasite dispersal in nearshore marine systems.

4. Suspension feeding and portals of entry

Suspension feeding bivalve molluscs are confronted with a wide range of materials in the seston and benthic environment, including various sized plankton and the organic material derived from it, benthic microalgae, detritus, microorganisms, fecal and pseudofecal pellets, and parasites that have adapted to survive in the water column.

Suspension feeding processes and the mechanisms of particle selection have become a major area of marine biological inquiry (Shiment and Jumars 1991; Chauvand et al. 2000; Ward and Shumway 2004). Combined with studies of nutrient assimilation and biodeposition (Newell 2004; Kellogg et al. 2013; Smyth et al. 2013), advances in research on the processes of suspension feeding have provided a robust mechanistic understanding of role of bivalves in the regulation of water column processes and the flux of organic material and nutrients from the water column to the benthos (reviewed by Dame 2011). It has also become clear that particle-feeding bivalves rarely just encounter and consume particulate matter. Rather, particles are rapidly sorted, ingested, and assimilated based on a number of chemical, physical, and biological factors (reviewed by Ward and Shumway 2004).

Bivalves have evolved morphological structures and behaviors to exploit a diet of dilute, heterogeneous particles contained within the seawater matrix. Suspended particles are captured by ciliated feeding structures that sweep selected material toward the labial

palps and mouth and into the stomach. Rejected material is diverted away from the mouth. Particle selection can occur either before capture or following capture but prior to ingestion (Shimanta and Jumars 1991; Ward and Shumway 2004). Post-capture selection occurs by the differential transport of material along feeding structures, the end result of which is the collection of materials that are periodically expelled as pseudofeces (Shumway 1985; Ward et al. 1998). In the stomach, selected and ingested cells and particulates are mechanically broken up and mixed with digestive enzymes. Bivalves may also postingestively discriminate among particles based on attributes such as size, density, and chemical properties through sorting in the gut lumen and preferential transport of selected particles to the digestive diverticula, the site of primary intracellular digestion (Brillant and MacDonald 2000; Brilliant and MacDonald 2003; Ward and Shumway 2004). Intracellular digestion also occurs in the gut lumen and digestive diverticula by circulating hemocytes, and phagocytized materials are then transported across the gut lining to other tissues for assimilation (Hine 1999; Canesi et al. 2002; Wooten et al. 2003). In addition to this digestive function, hemocytes encapsulate and phagocytize foreign materials, including any parasites present in the gut or within the hemolymph (Hine 1999). Elimination of microbes results from the coordinated action of these phagocytic processes with humoral defense factors such as agglutinins, lysosomal enzymes, toxic oxygen intermediates, and various antimicrobial peptides (Chu 1988; La Peyre et al. 1995; Hine 1999; Canesi et al. 2002; de Freitas Rebelo et al. 2013). Microbial defense is not restricted to the digestive tract either. Due to the open circulatory system of molluscs, hemocytes remain present in sinuses throughout all soft tissues (Hine 1999) and even outside the external epithelium in extrapallial fluids (Allam and Paillard 1998).

For bivalve parasites to enter and infect hosts, they must first enter and overcome these elaborate pathways for particle sorting and selection, and once within host tissues, are faced with highly diverse systems of innate immunity (Chu 1988; Bayne 1990; Hine 1999; Canesi et al. 2002; McDowell et al. 2014; Zhang et al. 2014; Zhang et al. 2015). How bivalve parasites evade these behavioral, physical, chemical, humoral, and molecular defenses to infect and establish within hosts is remarkable, and bivalve parasites have demonstrated numerous adaptive strategies to evade particle sorting and immune defense processes to establish and proliferate within hosts.

The entry of viruses into bivalve hosts, when compared to the entry of bacterial and protozoan parasites, is perhaps most associated with normal feeding activities. It remains open how small virus particles (<50 nm) enter bivalve hosts (Provost et al. 2011), as the ciliated structures of gills can often be spaced as far as 500 to 600 nm apart. It has been suggested that viral particles can both become electrostatically bound to sulfates on the mucopolysaccharides of shellfish mucus (DiGirolamo et al. 1977) and become entrapped in feeding structures when associated with larger marine aggregates, plankton, and organic and inorganic particles large enough to be captured by the gill (Gentry et al. 2009; Whittington et al. 2015a). Both of these mechanisms of entry may occur simultaneously, and both lead to the rapid entry of viral particles into digestive organs. In fact, many viruses, including those of human health concern such as hepatitis A, murine norovirus, and poliovirus, have been observed to readily bioaccumulate within the lumen and cells of digestive tract tissues (Le Guyader et al. 2006; McLeod et al. 2009; Richards et al. 2010). Once there, circulating hemocytes may encapsulate and traffic viral particles across the gut lining and throughout bivalve tissues (Provost et al. 2011). The extent to

which phagocytic and humoral processes can inactivate viral particles within bivalves remains open, as direct studies comparing the abilities of viruses to persist within hemocytes have been limited (Renault and Novoa 2004; Provost et al. 2011). Studies of the persistence of enteric viruses infecting humans contaminating bivalve tissues suggest that certain acid-tolerant viruses can survive and remain infectious within bivalve hemocytes for extended periods, while other viruses decline more rapidly (Ueki et al. 2007; McLeod et al. 2009; Provost et al. 2011). Viruses have demonstrated the capacity to be retained by bivalve hemocytes for significantly longer periods of time than bacterial indicators such as *Escherichia coli* and fecal coliforms (Power and Collins 1989; Power and Collins 1990).

Studies of human enteric viruses in bivalve tissues suggest that digestive organs and circulating hemocytes are the primary sites of virus entry and persistence (Richards et al. 2010; Provost et al. 2011). The direct and rapid penetration of viruses into digestive tissues and hemolytic systems, due to the open circulatory system of bivalves, suggests that these may be primary sites for virus replication as well. The targeting of hemocytes for virus entry and persistence is supported by the likely viral etiology of disseminated neoplasia, a progressive and lethal condition of bivalve hemolytic systems (Elston et al. 1992; Barber 2004; Romalde et al. 2007) as well as the diversity of observations of hemocyte-infecting viruses, including iridio-like viruses associated with widespread mortality outbreaks of *Crassostrea angulata* in France in the early 1970s (Elston 1997) and picornavirus-like particles interpreted as granulocytomas localized in hemolymph spaces in *M. edulis* from Denmark (Rasmussen 1986). More recent evidence from the dynamics of OsHV-1 infections within *Crassostrea gigas* suggests that hemocytes,

though not necessarily the site of viral replication, play an important role in the dissemination of virus particles to target tissues (Schikorski et al. 2011). OsHV-1 viral particles presumptively contact hosts through feeding activities, entering the digestive tract and the hemolymphatic system. Viruses are then transported by hemocytes to the target organs (Schikorski et al. 2011), thought to be the gills and mantle (Arzul et al. 2001b; Sauvage et al. 2009; Segarra et al. 2014b). Once there, viral particles begin intense replication and can spread rapidly throughout host tissues, explaining observations that only a short duration of exposure to OsHV-1 is sufficient for transmission in the field and laboratory (Arzul et al. 2001b; Burge and Friedman 2012; Schikorski et al. 2011; Segarra et al. 2014b; Segarra et al. 2014c). The mechanisms underlying interactions between viruses and components of hemolymphatic systems remain mostly unresolved, but observations of the kinetics of viral infections in bivalve hosts suggest that hemocytes play an important role in the replication and dissemination of viral particles within bivalve hosts.

Digestive processes appear to be a strong barrier against the initial colonization by non-viral parasites in healthy bivalve hosts. Bacteria are encountered by many bivalve species as a food source (Birkbeck and McHenry 1982; Langdon and Newell 1990), and can be assimilated efficiently into bivalve tissues through digestive and phagocytic processes. Although the relative contribution of bacteria to the energy and nutrient demands of most bivalves is likely small compared to the contribution of phytoplankton (Lucas et al. 1987), the utilization of bacteria varies by bivalve species (Silverman et al. 1989) and with respect to the relative abundance of bacteria in the water column. Nevertheless, bivalves have demonstrated the ability to efficiently clear natural

523 bacterioplankton assemblages in experimental systems (Lucas et al. 1987). *Mytilus*
524 *edulis*, for example, have demonstrated the capacity to degrade bacteria at rates up to 10^9
525 cells hr^{-1} (Birkbeck and McHenry 1982). Lysozymes, in particular, play a central role in
526 the digestion and defense against bacterial parasites due to the ability of these humoral
527 defense factors to break down bacterial cell walls (McHenry et al. 1986; Bachali et al.
528 2002). The susceptibility of bacterial parasites to elimination is a product of the
529 sensitivity of bacteria to humoral defense factors and the capability of hemocytes to bind
530 to and encapsulate different bacteria, reflecting both the ability for bacteria to evade
531 hemocytes as well as the sensitivity of bacteria to intracellular elimination (Chu 1988;
532 Allam et al. 2002; Bachali et al. 2002; Canesi et al. 2002). As a consequence, the
533 pathogenicity of bacterial parasites is often directly associated with the capacity for these
534 parasites to evade and avoid host defense factors (Allam et al. 2002; Canesi et al. 2002;
535 Pruzzo et al. 2002). For example, the persistence of pathogenic vibrios in hemolymph
536 depends largely on their sensitivity to the bactericidal activity of circulating hemocytes
537 and soluble humoral factors (reviewed by Pruzzo et al. 2002). The pathogenic effects of
538 *Vibrio splendidus*, the likely cause of summer mortalities of juvenile *C. gigas* in Brittany,
539 has shown to result from the capacity for some strains to interfere with the signaling
540 pathways involved in hemocyte function (Lacoste et al. 2001; Labreuche et al. 2006).
541 *Vibrio tapetis* avoids phagocytic encapsulation by impairing the adhesion properties of
542 hemocytes (Choquet et al. 2003). The physiological state and immunity of hosts is also an
543 important factor determining the capacity for parasites to evade defense factors and
544 establish within hosts (Lacoste et al. 2002). *Nocardia crassostreae* appears to enter oyster
545 hosts directly through the digestive tract (Bower et al. 2005). Infection seems to occur

primarily during periods of host physiological stress, presumably associated with periods of high metabolism and reproductive maturation when water temperatures rise (Paillard et al. 2004; Bower et al. 2005). Initial signs of brown ring disease also appear to coincide with the reproductive period of *Ruditapes philippinarum*, which corresponds to a drop in the clam condition index (Martinez-Manzanares et al. 1998; Paillard 2004).

Bacterial parasites have also demonstrated the capacity to evade digestion by behaving, at least initially, as external parasites, colonizing the pseudo-internal extrapallial spaces and peripheral compartments (Paillard and Maes 1994; Allam 1996; Paillard et al. 2004; Boettcher et al. 2000; Boardman et al. 2008). *Roseovarius crassostreae*, for example, colonizes the inner shell surfaces of *Crassostrea virginica*, stimulating the deposition of conchiolin around the outer edge of the mantle margin (Boettcher et al. 2000; Ford and Borrero 2001). The nature of conchiolin deposition in oysters colonized by *R. crassostreae* is similar to that observed in *R. philippinarum* colonized by *V. tapetis*, which uses a number of adherence factors, including pili, to adhere to the periostracal lamina of its clam host (Paillard et al. 1994; Paillard and Maes 1994; Allam 1996; Allam et al. 2000). Adherence to the periostracal lamina is a required step in the transmission cycle of *V. tapetis* (Martinez-Manzanares et al. 1998; Lopez-Cortes et al. 1999b), and the attachment of bacteria onto the shell secretion is structurally similar to biofilms formed by other vibrios, including *V. cholerae*, *V. fisheri*, and *V. parahaemolyticus* (Costerton 1999; Yildiz and Visick 2009; Rodrigues et al. 2014). The biofilm forming capacity of vibrios is directly linked with the pathogenicity of these bacteria (Costerton 1999; Nyholm et al. 2000; Yildiz and Visick 2009). Once formed along the growing edge of the clam shell, the *V. tapetis* biofilm can advance into internal

tissues when environmental conditions permit. Extensive bacterial proliferation ruptures the external epithelium of the mantle and disseminates this parasite throughout the extrapallial fluid and into and through the connective tissue of the mantle (Paillard et al. 1994; Lopez-Cortes et al. 1999b; Allam et al. 2000; Allam 2002; Paillard 2004).

Some protozoan parasites, such as *Bonamia exitiosa*, appear to enter hosts directly through the digestive tract, escaping digestion in the case of *B. exitiosa* by burrowing under the gut basement membrane (Hine 1991). Most protozoans though seem to evade direct feeding and digestion by initiating infections outside the digestive tract, most often in the pallial organs. Initial infections with QPX typically occur in the mantle, gill, and incurrent siphon (Smolowitz et al. 1998; Dahl et al. 2010). This also appears to be the case for infections with *Haplosporidium nelsoni* and *H. costale* in *Crassostrea virginica* and *Bonamia ostreae* in *Ostrea edulis*, as these parasites initially infect the gill epithelium before invading nearby connective tissues (Andrews 1982; Montes et al. 1994; Sunila et al. 2000; Bureson and Ford 2004). Oysters with Denman Island disease, caused by infection with *Mikrocytos mackini*, have been observed to first develop lesions in the mantle and labial palps (Farley et al. 1988; Carnegie et al. 2003). The initial infective stages of *Marteilia refringens* and *M. syneyi* also enter through the labial palps (Audemard et al. 2002; Kleeman et al. 2002), and all members of the genus *Perkinsus* appear to infect their bivalve hosts through the pallial organs (Azevedo et al. 1989; Rodriguez and Navas 1995; Villalba et al. 2004; Allam et al. 2013; Soudant et al. 2013). In some instances, such as with QPX and *P. marinus*, the initial source of infectious stages appears to be from the accumulated particles captured by the gills but not ingested that await rejection as pseudofeces along the outer edge of the mantle (Lyons et al. 2005;

Allam et al. 2013). Further studies indicate that the mucus covering oyster pallial organs, which provides an efficient protective barrier for these feeding organs, plays a significant role in the pathogenesis of *P. marinus*, enhancing parasite proliferation and infectivity (Pales Espinosa et al. 2013). Although the mechanisms remain unresolved, it appears that contact between *P. marinus* and pallial mucus causes significant changes to the parasite's metabolic processes, increasing the expression of virulence factors to facilitate infections on the pallial organs (Pales Espinosa et al. 2013). In addition, *P. marinus* has demonstrated greater infectivity when cells are incorporated within marine aggregates (Allam et al. 2013). These results implicate a role for marine aggregates, in addition to host factors such as pallial mucus, as abiotic vectors or mediators of parasite transmission through suspension feeding processes.

Once established within pallial organs, many protozoan parasites have demonstrated the ability to evade phagocytic and humoral factors (La Peyre et al. 1995; Volety and Chu 1995; Kang et al. 2006; Tasumi and Vasta 2007; La Peyre et al. 2010) and directly infect hemocytes to disseminate throughout host tissues via the open hemolymphatic system (Villalba et al. 2004; Soudant et al. 2013). Interactions between *P. marinus* and its oyster hosts have become a model for the identification of mechanisms of immunoinhibition and dissemination through host tissues. Previous work has shown that this protozoan uses a suite of intricate mechanisms to sequester cellular functions of its host's immune system to assure propagation (La Peyre et al. 1995; Garreis et al. 1996; Sunila and LaBanca 2003; Villalba et al. 2004; Hughes et al. 2010; Soudant et al. 2013). *Perkinsus marinus* can inhibit phagocytic processes (Anderson 1999; Schott and Vasta 2003), allowing this parasite to hijack circulating hemocytes for dissemination throughout host tissues by

615 diapedesis. This parasite can also suppress hemocyte apoptosis (Sunila and LaBanca
616 2003; Hughes et al. 2010). Apoptotic death of hemocytes infected with *P. marinus* would
617 likely restrict the spread of this parasite to initial infection foci. Suppressing apoptosis
618 can further help *P. marinus* survive and disseminate throughout host tissues, specifically
619 within host hemocytes. Targeting hemocytes for parasite replication and spread through
620 host tissues is not unique to *Perkinsus spp.* Most microcell haplosporidians, including
621 *Bonamia ostreae*, *B. exitiosa*, and *B. roughleyi* infect and proliferate in hemocytes (Farley
622 et al. 1988; Montes et al. 1994; Culloty et al. 1999; Cochenne-Laureau et al. 2003;
623 Carnegie and Cochenne-Laureau 2004). *Mikrocytos mackini* also occurs in hemocytes
624 but is more conspicuously associated with vesicular connective tissues of the pallial
625 organs and does not appear to proliferate in hemocytes (Farley et al. 1988; Carnegie and
626 Cochenne-Laureau 2004). These observations demonstrate that hemocytes, while
627 serving a primary role in non-specific immune defense, are often targets of parasitic
628 infection. Infection of hemocytes is directed by host phagocytosis, but even this may not
629 always be the case. Cochenne (2001) demonstrated that live *B. ostreae* are internalized
630 by hemocytes at a greater rate than heat-killed parasites, suggesting that the parasites
631 themselves initiate the infection of these microbial defense agents. This paradoxical role
632 of phagocytes in the pathogenesis of infectious diseases, known as the Trojan horse
633 effect, has been of widespread interest to investigators studying a diversity of host-
634 parasite systems, including *Bacillus anthracis* in livestock and humans, *Streptococcus*
635 *iniae* in fish, *Toxoplasma gondii* in mammals, and *Vibrio cholerae* in humans (Roy and
636 Wainberg 1988; Guidi-Rontani 2002; Zlotkin et al. 2003; Nguyen and Pieters 2005;
637 Lambert and Barragan 2010). The inherent migratory functions of circulating phagocytes

appear to make them also a very suitable target for parasites to mediate their dispersion throughout host tissues.

5. Parasite life cycles

The diverse mechanisms bivalve parasites use to infect and disperse throughout host tissues directly reflect the diversity of life and replication styles of bivalve parasites. Viruses, most often transmitted directly between bivalve hosts, can have several different replication cycles once established within host tissues. The two most dominant are the lytic, or virulent cycle, and the lysogenic, or latent cycle. Virulent viruses infect a cell or tissue, replicate, and are released throughout host tissues or into the environment by lysis of the host cell. Alternatively, latent viruses infect the host and the viral DNA stays within the host cell, often in the host genome. Here, the viral genome replicates along with the host genome until some factors, such as DNA damage, induce the lytic cycle. It has been suggested that lytic viruses dominate in productive ecosystems, where the abundance and density of target hosts is high which would facilitate host contacts (Wilcox and Fuhrman 1994; Suttle 2005; Breitbart 2012). Herpesviruses, such as OsHV-1 can have both virulent and latent cycles (Honest and Roizman 1974). In herpesviruses the virulent cycle has demonstrated to be induced by a range of host factors, including life stage and environmental stress, and involves a significant increase in viral replication, often leading to an increase in pathogenic effects on hosts (Wolf and Darlington, 1971; Eisenberg et al. 2012). Like human herpesviruses (Schmader 2001), OsHV-1 appears to be able to persist as latent infections in asymptomatic *Crassostrea gigas* (Arzul et al.

2002; Dégremont et al. 2013; Whittington et al. 2015b). Apparent infections have been observed to persist in asymptomatic oysters, particularly during the cooler winter months (Petton et al. 2015) or when OsHV-1 infected oysters are held within aquaria maintained at cooler temperatures ($<13^{\circ}\text{C}$; Pernet et al. 2015). OsHV-1 transmission and oyster mortalities occur seasonally, when seawater temperatures exceed 16°C (Pernet et al. 2012; Dégremont et al. 2013; Petton et al. 2013; Renault et al. 2014), and the highest rates of OsHV-1 transmission occur at seawater temperatures ranging from 16 to 24°C (Pernet et al. 2012; Petton et al. 2013). This temperature range corresponds to the temperature range that supports the highest clearance rates in *C. gigas* (Bougrier et al. 1995), lending support to direct filtration of OsHV-1 particles in the environment as the portal of entry for viral infection (Fig. 2).

The concentration of OsHV-1 particles in the environment can be substantial during herpesvirus epizootics, reaching 6 to 7×10^9 virions per liter of seawater (Vigneron et al. 2004). Such seawater concentrations likely reflect the high prevalence of infection and high rate of mortality during epizootics, and would lead to high concentrations of directly infectious OsHV-1 particles in the environment and a high rate of transmission to new susceptible hosts. Advection of seawater, and its consequences on the dilution of seawater concentrations of OsHV-1, appears to strongly influence the risk and severity of herpesvirus outbreaks. In experimental laboratory tanks holding oysters exposed to OsHV-1 in the field, Petton et al. (2015) manipulated the rate of water renewal demonstrating the risk of OsHV-1 mortality to decrease with increasing rates of water renewal. The observed importance of water renewal agrees with observations of oyster farming practices by Pernet et al. (2012), finding that oysters cemented onto ropes, where

water circulation around individual oysters is enhanced, suffered a lower rate of OsHV-1 mortality compared to oysters held in Australian baskets, where the circulation and flushing of water around individual oysters is comparably low. In addition, Pernet et al. (2014) observed that the OsHV-1 mortality risk decreased when oysters were deployed in the vicinity of mussel (*Mytilus galloprovincialis*) farms, suggesting that mussels, which are incompetent hosts for OsHV-1, can further dilute the water column abundance of viral particles through suspension feeding.

Complete life cycles have yet to be fully explored for many bacterial parasites of bivalves, but many of these parasites appear to be members of ambient marine microbial communities. It is well known that environmental factors, such as changes in water temperature and salinity, exert significant controls on the abundance and diversity of *Vibrio* species in the environment (Paillard 2004; Labreuche et al. 2006; Beaz-Hidalgo et al. 2010), many of which have been described as agents of disease in wild and cultured bivalve molluscs. Infections with *Nocardia crassostreae* have been associated with summer mortalities in wild populations of *Crassostrea gigas*, *Ostrea edulis*, and *Mytilus galloprovincialis*, particularly during periods of host physiological stress or in association with other pathogenic *Vibrio* spp. (Friedman et al. 1998; Engelsma et al. 2008; Carella et al. 2013). This parasite reproduces in host tissues by mycelial fragmentation and can be shed from hosts into the water column (Friedman et al. 1991). Since most terrestrial *Nocardia* are soil saprophytes, it is suggested that *Nocardia crassostreae* infection in wild oyster populations occurs through a parasite reservoir that is regularly present in benthic sediments (Friedman and Hedrick 1991). However, this parasite has not been demonstrated to transmit directly through sediments from enzootic areas, nor has

transmission been observed through cohabitation with infected oysters (Friedman et al. 1991). Experimental infection has been achieved by inoculation with a relatively high parasite dose (Friedman and Hedrick 1991), suggesting that, in conjunction with observations of mortality during periods of host physiological stress, *Nocardia crassostreae* is an opportunistic and facultative parasite that is regularly present in benthic marine sediments (Bower et al. 2005; Engelsma et al. 2008).

Although bacterial parasites appear to behave as members of ambient microbial communities little is known about the source and fate of these parasites outside bivalve hosts. *Roseovarius crassostreae* is a member of the marine Roseobacter clade, a diverse group of α -Proteobacteria comprising up to 25% of marine microbial communities in coastal oceans (Wagner-Dobler and Biebl 2006). Among the cultured members of the Roseobacter clade, only *Roseovarius crassostreae* is known to be pathogenic (Boardman et al. 2008). After colonizing the inner shell surface of *Crassostrea virginica*, this bacterium divides by budding where new cells either become available to further colonize available shell surfaces or can be transmitted directly to new hosts (Ford and Borrero 2001). Conchiolin deposition can serve to wall off colonizing bacteria, however, in advanced cases of disease conchiolin deposits eventually cover the entire soft body mass, forming a raised ring around the entire outer edge of the mantle margin (Ford and Borrero 2001). The inhibition of filter feeding observed in laboratory-challenged oysters suggests that inadequate nutrition is a consequence of advanced infection (Boettcher et al. 2000; Boardman et al. 2008), and smaller and physiologically stressed oysters, with comparatively limited metabolic resources, have a higher incidence of mortality from disease (Davis and Barber 1999; Ford and Borrero 2001). *Roseovarius crassostreae* can

spread rapidly through shellfish hatcheries and grow-out operations (Lewis et al. 1996), likely from aided host contacts due to dense culture conditions. Population genetic studies confirm that most epizootics result from site-specific acquisition of *Roseovarius crassostreae* (Maloy et al. 2007). This result, combined with repeated summer epizootics, suggests that *Roseovarius crassostreae* is present but not abundant in the environment. Once established within a bivalve culture facility or grow-out site *R. crassostreae* can be transmitted rapidly when environmental conditions are favorable.

Conchiolin deposition in oysters infected with *R. crassostreae* is similar to that observed in manila clams infected with *Vibrio tapetis*. After colonizing the periostracal lamina, *V. tapetis* begin reproducing along surfaces of the external epithelium, particularly during periods of physiological stress when host defenses may be compromised (Allam 1996; Lopez-Cortes et al. 1999; Paillard 2004). Parasite replication occurs through binary fission, and in favorable conditions, the bacterial biofilm associated with this conchiolin deposit can eventually rupture the external epithelium of the mantle, allowing *V. tapetis* to penetrate into and proliferate rapidly in soft tissues (Allam et al. 1996; Lopez-Cortes et al. 1999; Allam et al. 2002; Paillard 2004). Results from transmission experiments suggest that direct contact between infected and healthy clams enhances the transmission of *V. tapetis* and the development of brown ring disease (Martinez-Manzanares et al. 1998). *Vibrio tapetis* can also be transmitted through exposure to the feces of infected clams (Maes 1992). The severe tissue disruption that results from bacterial proliferation (Allam et al. 2002) also suggests that infective cells are released into the water column from decaying moribund hosts, and available to directly infect new susceptible hosts (Fig. 3). Like other vibrios, *V. tapetis* is likely to be

well adapted for survival in seawater (Yildiz and Visick 2009; Beaz-Hidalgo et al. 2010; Balboa et al. 2012). Related *V. tapetis* strains have also been detected in moribund Corkwing wrasse (Jensen et al. 2003) and Atlantic halibut (Reid et al. 2003) exhibiting signs of vibriosis. Although these observations of *V. tapetis* infection in finfish were outside the geographic range limits of reported detections of brown ring disease, the association of this parasite with other hosts suggests that *V. tapetis* is a widespread, though not necessarily numerically dominant member of marine microbial communities. Since transmission is likely to occur through direct contact with both water-borne *V. tapetis* and infected clams, the density of hosts, particularly under intensive culture conditions, likely plays an important role in the control of brown ring disease (Paillard 1994; Paillard 2004).

Bacterial parasites are not alone in their ability to reproduce outside their internal host environment. QPX can survive and grow on many available nutrient sources in the environment, including various species of macroalgae (Bugge and Allam 2007; Gast et al. 2008). This is not surprising for a labyrinthulomycete parasite, as these saprotrophic protists commonly occur on or in living marine vascular plants and macroalgae, or in association with decomposing plant material and detritus (Tsui 2009). Ectoplasmic nets, used by labyrinthulomycetes to solubilize nutrients in decomposing plant material, have not been identified in foci of QPX-like infections within quahog hosts (Smolowitz et al. 1997) or in association with QPX cells held in culture media (Kleinschuster et al. 1998). Instead, within hosts, QPX thalli and sporangia appear to produce a homogenous mucofilamentous material (Smolowitz et al. 1997), which is unusual for labyrinthulomycetes (Kleinschuster et al. 1998). QPX thalli, endospores, and zoospores

776 have demonstrated the ability to produce the ectoplasmic net however (Kleinschuster et
777 al. 1998). This occurs when QPX cells are suspended in seawater and adhere to substrate,
778 such as culture flasks (Kleinschuster et al. 1998). Vegetative reproduction occurs within
779 hosts and in culture media, where growth and maturation of thalli results in the formation
780 of sporangia containing many vegetative endospores. Endospores are released when
781 sporangia are ruptured, and the endospores form into thalli, repeating the life cycle
782 (Kleinschuster et al. 1998). In seawater, motile zoospores can also form from endospores
783 and thalli; however, zoospores have not been identified in infected clam tissue
784 (Smolomitz et al. 1997). Vegetative reproduction can commence when zoospores are
785 placed in culture media though (Kleinschuster et al. 1998). These observations suggest
786 that zoospores occur in seawater and can infect hosts but do not commonly occur in the
787 internal host environment. These observations of the QPX life cycle in infected hosts and
788 culture media suggest that QPX can alternate between vegetative reproduction within
789 clam hosts and survival and growth on substrates outside clam hosts, which is supported
790 by the detection of QPX in association with a wide range of environmental samples,
791 including seawater, marine aggregates (Lyons et al. 2005), marsh grasses, macroalgae,
792 gastropods, ascidians, amphibians, barnacles, polychaetes, annelids, and bivalves other
793 than quahogs (Gast et al. 2008). Detection of QPX in association with environmental
794 samples increases in regions where QPX disease in quahogs is prevalent, suggesting that
795 alternating between saprotrophy and parasitism may increase the distribution and spread
796 of this labyrinthulomycete. In the absence of quahog hosts, or when environmental
797 conditions do not favor infections within these hosts, QPX is likely to remain a common
798 though inconspicuous component of the coastal marine microbial community.

The capacity for QPX to alternate between parasitism and survival and growth on substrates outside hosts appears to be unique among protozoan parasites infecting bivalves. The majority of these parasites require suitable hosts to complete their life cycles. All protozoans of the genus *Perkinsus* are obligate parasites, but many have proven to be very successful, able to infect a diversity of bivalve hosts and maintain high prevalence and persistence in host populations (Bushek and Allen 1996; Goggin et al. 1989; Casas et al. 2002; Calvo et al. 2003; Villalba et al. 2004; Villalba et al. 2005; Bushek et al. 2012; Soudant et al. 2013). Their success is linked to qualities such as their capacity to survive outside hosts for prolonged periods (Chu et al. 2002; Chu and Lund 2006), a battery of intricate mechanisms to degrade and sequester host immune systems (La Peyre et al. 1995; Garreis et al. 1996; Sunila and LaBanca 2003; Hughes et al. 2010), and their ability to form chronic infections (Ford et al. 1999) while continuously dispersing from host tissues into the environment (Bushek et al. 2002). All *Perkinsus* species share a common life cycle. Infective trophozoites, or meronts, occur within host tissues as well as outside hosts in the water column, and undergo vegetative proliferation within hosts. After several internal cell divisions, four to 64 daughter cells become aggregated within the wall of schizont stages. Daughter cells are liberated through a tear in the schizont wall, and each daughter cell enlarges into an immature trophozoite, itself eventually dividing to repeat the vegetative reproduction cycle (Perkins 1988; Perkins 1996; Sunila et al. 2001). When host tissues infected by *Perkinsus* spp. are incubated in fluid thioglycollate medium (FTM), trophozoites enlarge and develop a thick cell wall, becoming a prezoosporangium (also referred to as a hypnospore). Zoosporulation begins when prezoosporangia are transferred to seawater or culture media (Perkins 1988; La

822 Peyre 1996), where numerous zoospores form by multiple fission within the
823 prezoosporangia cell wall (Sunila et al. 2001) and emerge through a single discharge tube
824 (Azevedo et al. 1990; Casas et al. 2002). Prezoosporangia have also been observed in
825 decaying tissues of moribund hosts, and begin zoosporulation when placed in seawater
826 (Perkins 1996; Casas et al. 2002). Zoosporulation following transfer to seawater or
827 culture media has been observed in all *Perkinsus* spp. except for *P. qugwadi*. In the case
828 of *P. qugwadi*, the development of *P. qugwadi* zoospores has been observed to occur
829 only within the interstitial spaces of scallops infected by this parasite (Blackbourn et al.
830 1998; Bower et al. 1998). All life stages of *Perkinsus* spp. have been shown
831 experimentally to cause infection in bivalves, and all species can be transmitted directly,
832 without intermediate or secondary hosts. Viable *Perkinsus* spp. trophozoites are released
833 into the water column from live infected bivalves through diapedesis and in feces
834 (Scanlon et al. 1996; Bushek et al. 2002; Casas et al. 2002). Infected hosts appear to
835 provide a continuous source of infectious stages to new hosts, which are exposed to these
836 parasites through feeding processes (Fig. 4). Maximum rates of transmission of *P.*
837 *marinus* in oysters appear to occur during periods of maximum mortality caused by this
838 parasite, most often in the late summer and early autumn when infection intensities peak
839 (Ragone Calvo et al. 2003). This observation suggests that prezoosporangia, forming in
840 moribund hosts and releasing numerous zoospores into the water column, contribute
841 substantially to *P. marinus* transmission. White et al. (1987) also demonstrated that *P.*
842 *marinus* could be transmitted through hemolymph meals taken by the ectoparasitic
843 gastropod *Boonea impressa* that had previously been fed *P. marinus*-infected oysters.

This vector is not essential for transmission though, since *P. marinus* transmission regularly occurs outside the geographic range of *B. impressa*.

Both the prevalence of infection with *Perkinsus* spp. and the transmission of these parasites are strongly tied to environmental conditions. Long-term field studies monitoring infection with *P. marinus* in its eastern oyster host along estuary salinity gradients, capturing annual variability in temperature and salinity, have identified these two factors as the key drivers of the incidence and expression of disease (Burreson and Ragone Calvo 1996; Ragone Calvo et al. 2003; Soniat 1996; Bushek 2012). In temperate regions that experience broad annual temperature fluctuations, such as the mid-Atlantic and New England coasts of the United States, this temperature-dependence leads to a strong annual cycle of *P. marinus* proliferation (Ford et al. 1999). The cycle begins with an initial increase in the intensities of overwintering infections, when water temperatures increase above approximately 20°C (Soniat 1996; Ford et al. 1999; Bushek 2012). Parasite proliferation and host mortality increase through the summer and early fall, as does the release of viable parasites through the feces of live hosts and decaying tissues of moribund hosts (Ford et al. 1999; Bushek et al. 2002; Bushek et al. 2012). Parasite proliferation within hosts and oyster mortality are followed by increases in the water column abundance of free *P. marinus* (Ragone Calvo et al. 2003; Audemard et al. 2006) and transmission to new hosts (Burreson and Ragone Calvo 1996; Ragone Calvo et al. 2003; Bushek 2012). Remission of infections in oyster populations occurs through the winter and early spring as water temperatures decrease (Ford et al. 1999). An early onset of spring, a delayed onset of winter, or both, allow for longer periods of parasite proliferation, which lead to a greater impact and extent of disease (Burreson and Ragone

Calvo 1996; Ford and Smolowitz 2007). This temperature-dependence very likely defines the geographic extent of disease impacts as well. The apparent northward range expansion of *P. marinus* in the early 1990s was associated with above average winter and spring temperatures (Ford 1996; Ford and Chintala 2006), allowing this parasite to establish in areas it had not previously been reported or known to cause mortalities (Ford and Smolowitz 2007).

Infections and the transmission of *P. olsenii* follow a similar annual cycle. Casas (2002) showed that the prevalence and intensity of *P. olsenii* infection in *Ruditapes decussatus* in Galicia, Spain increased in the spring when water temperatures exceeded 15°C, and peaked in the late summer when water temperatures reached annual maxima of 19-21°C. As with infection with *P. marinus* in oysters, remission of *P. olsenii* infections occurred in the population through the winter and spring (Casas 2002; Casas et al. 2002). Although not a bivalve host, higher temperatures also predispose greenlip abalone (*Haliotis laevis*) to *P. olsenii* infection and infection-associated mortalities (Goggin and Lester 1995). Long-term field studies monitoring the prevalence and intensity of infections with *Perkinsus* species other than *P. marinus* and *P. olsenii* have been scarce, but limited sampling has revealed that annual proliferation cycles are not always the case. In British Columbia, Canada, *P. qugwadi* proliferates and remains pathogenic to the cultured Japanese scallop *Patinopecten yessoensis* throughout the winter months, where water temperatures fall to as low as 8°C (Blackbourn et al. 1998). Epizootiological studies suggest that Japanese scallops, introduced into British Columbia in 1983, merely serve as an alternative or even aberrant host for *P. qugwadi*, and this parasite is likely enzootic in an unknown native host and co-adapted to the cool waters of this region

(Bower et al. 1998). It is worth noting that the classification of *P. qugwadi* is uncertain, as this species is the only member of the genus that does not respond to incubation in Ray's fluid thioglycollate media (Dungan and Bushek, this volume).

Like *Perkinsus* spp., microcell haplosporidian parasites such as *Bonamia ostreae*, *B. exitiosa*, *B. roughleyi*, and *Mikrocytos mackini* are presumptively transmitted directly between bivalve hosts (Elston et al. 1986; Grizel et al. 1988; Montes et al. 1994; Culloty et al. 1999). These parasites differ from most haplosporidians by their apparent lack of a spore stage, instead infecting bivalve hemocytes and gill epithelial cells as small (2-3 μm), uninucleate, electron-dense or electron-clear forms, giving the microcell haplosporidians their namesake (Elston et al. 1986; Hine 1991; Carnegie and Cochenne-Laureau 2004; Engelsma et al. 2014). These cell forms reproduce by binary fission, and dense forms appear to be more numerous in heavily infected hosts (Carnegie and Cochenne-Laureau 2004). Dense forms are presumed to be the primary infective stage (Hine 1991) and frequent observations of free dense forms in gill epithelia suggest that parasites are released into the environment through these organs (Montes et al. 1994), but parasites are also likely to be released into the water column in moribund and recently deceased hosts through tissue lysis. Some microcell parasites do express multinucleate plasmodial forms (Hine 1991; Montes et al. 1994; Carnegie and Cochenne-Laureau 2004; Carnegie et al. 2006), but the contribution of these forms to parasite transmission is questionable. Only *B. exitiosa* regularly express multinucleate forms, and *B. ostreae* and *B. roughleyi* plasmodia have very rarely been observed and only in moribund hosts (Hine 1991; Cochenne-Laureau et al. 2003; Carnegie and Cochenne-Laureau 2004).

The transmission and impact of *B. exitiosa* display a strong seasonal association with the gametogenic and reproductive cycles of its host *Ostrea chilensis* in New Zealand (Hine 1991; Hine et al. 2001). Initial infections occur when dense cell forms are ingested during feeding. Within-host parasite proliferation occurs after hosts spawn in December, continuing through the summer and autumn months. Here, dense cell forms proliferate and are shed from hosts, with host mortality and parasite transmission to new hosts following. By winter, many cells appear to grow to larger forms intermediate between dense and plasmodial forms, and plasmodial forms begin to dominate within hosts through the winter and early spring (Hine 1991; Carnegie and Cochenne-Laureau 2004). Plasmodial forms have been observed to develop toward sporogony, but host defenses, and possibly viral infections, are able to overwhelm and eradicate *B. exitiosa* plasmodia before this can occur (Hine 1991). Dense forms present within hosts during this time appear to remain inactive though undetected by host defenses, or at least are able to evade host immune factors (Hine 1991). Overwintering dense forms then incubate within host tissue until hosts spawn the following summer, when the proliferation and transmission cycle repeats (Hine 1991; Hine et al. 2001). The infection cycles of other directly transmitted microcell haplosporidians do not appear to follow host gametogenic or reproductive cycles, but do show some seasonality. Although infections and mortality have been observed to occur year-round, the prevalence and intensity of *B. ostreae* infections in *O. edulis* are generally greatest in the warmer months (Grizel et al. 1988; Montes et al. 1994; Culloty and Mulcahy 1996; Carnegie and Cochenne-Laureau 2004). On the other hand, signs of *B. roughleyi* infection and mortality in *Saccostrea commercialis* in southeastern Australia occur through cooler winter months (Adlard and

Lester 1995). *Mikrocytos mackini* has typically been detected by histology only in the late winter and spring, but more sensitive PCR experiments have shown it to be associated with *Crassostrea gigas* throughout the year (Carnegie et al. 2003).

Whereas most haplosporidians are often only indirectly transmitted among their known hosts, direct transmission of uninucleate stages has been confirmed for *B. ostreae* in *O. edulis* and is well supported for *B. exitiosa*, *B. roughleyi*, and *M. mackini* in their known hosts (Grizel 1988; Hine 1991; Hervio et al. 1995; Culloty et al. 1999; Carnegie and Cochenne-Laureau 2004; Lallias et al. 2008; Engelsma et al. 2014). The microcell parasites are noteworthy for their inconsistent expression of plasmodial forms and apparent lack of a spore stage. Carnegie et al. (2006) did observe sporulation in a novel *Bonamia* sp. infecting *Ostreola equestris* in North Carolina, USA, later characterized as *B. perspora*, but only in a small fraction of confirmed infections and an even smaller fraction of the *O. equestris* examined in the laboratory. The function of plasmodial forms, likely a vestige from an ancestral haplosporidian, seems to have been averted in microcell parasites in their known hosts with the evolution of directly transmitted dense forms (Hine 1991; Carnegie and Cochenne-Laureau 2004; Carnegie et al. 2006). However, the occurrence and utility of plasmodial forms and sporulation should not be overlooked. The longest documented and best-studied relationships between microcell parasites and their hosts are in host species of economic importance, many of which have a long history of being introduced and transported for aquaculture and resource restoration or enhancement. In addition to introducing any associated parasites, the expansion and diversification of susceptible host populations, such as by introducing native or nonnative bivalves for aquaculture, has both modified parasite life cycles and amplified the effects

of parasites on host populations (Burreson 2000; Torchin et al. 20002; Hershberger et al. 2010; Blakeslee et al. 2012). It is possible, likely even, that a more conventional haplosporidian life cycle, including plasmodial forms and sporulation, can be identified if and when microcell parasites are observed in other, presumably natural, hosts.

Haplosporidian parasites introduced into naïve bivalve populations have also appeared to stray from the conventional life cycles characterizing these taxa. *Haplosporidium nelsoni* has caused extensive mortalities in its *Crassostrea virginica* host along the mid-Atlantic coast of the United States (Haskin 1966; Ford and Haskin 1982) since its likely introduction from Asia, where the parasite appears to be enzootic in *C. gigas* (Kern 1976; Burreson 2000; Burreson and Ford 2004; Carnegie and Burreson 2011). This parasite has two life stages within oyster hosts: 1) multinucleate plasmodia, from which the disease caused by infection with *H. nelsoni*, Multinucleate Sphere Unknown (MSX), gets its name, and 2) spores. An infective stage has not been recognized, and efforts to transmit *H. nelsoni* under controlled laboratory conditions have not been successful. The most ambitious effort to date was by Canzonier (1974), implanting infected tissues into uninfected recipients. Canzonier (1968) also reported failure to transmit *H. nelsoni* through cohabitation of infected and uninfected but susceptible oysters. Farley (1967) suggested that the infective stage is a uninucleate sporoplasm escaping from the spore. However, this pathogen is rarely observed to complete sporulation within adult *C. virginica* (Haskin 1966; Andrews 1982), inhibiting experimental efforts to transmit *H. nelsoni*. Limited sporulation has also led some investigators to conclude that *H. nelsoni* must have an intermediate host to complete sporulation and release infective stages into the environment (Farley 1967, Andrews

1982, Ford and Haskin 1982; Sunila et al. 2000). Later efforts have observed sporulation in the digestive tubule epithelium of young oysters less than 30 mm in shell height (Barber et al. 1991; Burreson 1994), typically occurring over a brief two to three week period in late June and early July, which coincides with periods of maximum incidence observed in the field (Andrews 1982; Ford and Haskin 1982). Nevertheless, the complete life cycle of *H. nelsoni* continues to elude investigators and a suspected intermediate host has yet to be identified (Haskin and Andrews 1988).

In contrast to *H. nelsoni*, *H. costale*, which causes Seaside Disease in oysters along the mid-Atlantic and northeastern coasts of the United States, appears to be a well-adapted native species exhibiting a conventional haplosporidian life cycle in its natural host, *C. virginica* (Wood and Andrews 1962; Andrews 1982). Although the timing unquestionably varies year-to-year (Stokes and Burreson 2001), this parasite has a short infection period in the late spring and early summer, followed by an incubation period of up to nine months before clinical signs of diseases can be observed (Andrews 1982). *Haplosporidium costale* sporulates regularly and completely, promptly killing its host following sporulation (Andrews 1982). This leads to the characteristic pattern of acute mortality first observed by Wood and Andrews (1962, pp. 162-163) where “oysters began to die abruptly in mid-May and stopped dying early in July.” Like *H. nelsoni*, efforts to transmit *H. costale* under controlled laboratory conditions have not been successful, leading to speculation that an unknown intermediate host or hosts are required to complete the transmission cycle.

The detection and identification of intermediate hosts is challenging, limited by the tremendous diversity of potential host species to be screened, the broad spatial and

1004 temporal scales required for sufficient screening, and the limitations of tools available for
 1005 detection (Haskin and Andrews 1988; Audemard et al. 2001; Audemard et al. 2002;
 1006 Carrasco et al. 2007; Carrasco et al. 2008). As with the haplosporidian parasites *H.*
 1007 *nelsoni* and *H. costale*, the paramyxean parasites of the genus *Marteilia* infect and
 1008 sporulate in bivalve hosts, but cannot be transmitted directly between hosts, leading
 1009 investigators to speculate that intermediate hosts are required to complete the
 1010 transmission cycle (Grizel 1979; Berthe et al. 1998; Berthe et al. 2004). When working in
 1011 semi-enclosed oyster ponds, locally known as ‘claires’ in Marennes-Oléron Bay, France,
 1012 Berthe et al. (1998) observed new *M. refringens* infections in planted *Ostrea edulis* even
 1013 though no other *O. edulis* were present in the claire. Owing to their limited size and its
 1014 consequences on environmental variables such as acute temperature variability and
 1015 seawater residence time, claire pond systems harbor fewer species than adjacent coastal
 1016 bays and estuaries (Audemard et al. 2001), making them ideal experimental systems for
 1017 detecting potential intermediate hosts. Audemard et al. (2002) systematically screened the
 1018 entire fauna of the pond, finding that the copepod *Paracartia gralli* can serve as a host of
 1019 *M. refringens*. Later surveys have confirmed that *Marteilia* parasites can be associated
 1020 with zooplankton species in larger and more natural bays and estuaries (Carrasco et al.
 1021 2007). Within *O. edulis*, *M. refringens* develops from early stages in the epithelia of the
 1022 upper digestive tract (Berthe et al. 1998; Berthe et al. 2004). Sporulation takes place
 1023 within the epithelium of the digestive gland tubules, releasing sporangia into the lumen of
 1024 the digestive tract (Berthe et al. 1998; Berthe et al. 2004). Sporulation is often associated
 1025 with the destruction of the digestive gland epithelia and shedding of sporangia into the
 1026 water column, becoming available to be ingested by *P. gralli* (Audemard et al. 2002;

Carrasco et al. 2008; Boyer et al. 2013). Within *P. gralli*, *M. refringens* cells migrate to gonadal tissue through adjacent connective tissues, where this parasite then forms pseudoplasmodia containing large numbers of small (2-5 μ m), presumptively infectious cells (Carrasco et al. 2008).

The involvement of *P. grani* in the life cycle of *M. refringens* is consistent with the phenology of this copepod and the epizootiology of disease caused by *M. refringens*, known as Aber disease. Aber disease is seasonal, beginning in late spring, peaking through the summer and early fall, and decreasing in the winter (Grizel 1979). *Paracartia grani* is most commonly observed in coastal waters in the spring and summer, where it can become a dominant member of the zooplankton community (Audemard et al. 2002).

Transmission experiments have demonstrated that *P. gralli* could be infected from infected *O. edulis*, indicating that these two species are contiguous in the life cycle of the parasite (Audemard et al. 2002; Carrasco et al. 2008). It remains open whether and how *M. refringens* can be transmitted from *P. grani* to *O. edulis* though, as attempts at copepod-to-oyster transmissions have failed (Audemard et al. 2002; Carrasco et al. 2008).

These results suggest there may be a second intermediate host, or they may simply reflect a failure for transmission experiments to attain the required inoculum of *M. refringens* in *P. gralli* to lead to successful infection in *O. edulis*. This latter hypothesis is supported by the observations of Boyer et al. (2013), following the dynamics of *M. refringens* in *P. grani* and two bivalve hosts, *Mytilus galloprovincialis* and *Ruditapes decussatus*, in Thau lagoon, southern France. Although *M. galloprovincialis* serve as competent hosts, *M. refringens* was observed only as necrotic cells in the digestive epithelia of *R. decussatus*, suggesting this bivalve is a dead end for *M. refringens*. The prevalence of mature *M.*

refringens sporangia in *M. galloprovincialis* was observed to increase in the spring, declined through the summer, but increased again in the fall (Boyer et al. 2013). The parasite was also detected in eggs and the copepodid stages of *P. grani* throughout the summer and fall, lending support to the hypothesis of the transmission of *M. refringens* from bivalves to copepods and back to bivalves. In addition, it appears this parasite has two cycles per year in Thau lagoon (Boyer et al. 2013).

It is common for parasites with complex life cycles and multiple host species to demonstrate multiple overlapping life cycle phases (Holt et al. 2003; Dobson 2004). Andrews (1982) described irregular patterns of *H. nelsoni* infection in *C. virginica* inhabiting estuaries along the mid-Atlantic coast of the United States. In Virginia, the timing of infections with *H. nelsoni* can be divided into early-summer and late-summer acquisitions that result in quite different mortality patterns (Andrews 1982). The early summer infection period for *H. nelsoni* occurs from mid-May through the early summer, with the earliest infections becoming clinical by the mid-summer and first mortalities occurring by the late summer. Late-summer infections with *H. nelsoni* exhibit patterns similar to those described for *H. costale*, where initial infections occur in the late summer but remain subclinical or localized throughout the winter and early spring. These infections eventually become severe by the following spring and lead to mortality by the early summer (Andrews 1982). In the case of *M. refringens* infection in *M. galloprovincialis*, the increase in prevalence in the early spring observed in Thau lagoon may reflect proliferating overwintered infections that can be shed by bivalve hosts, becoming available to infect intermediate copepod hosts. Parasite development in intermediate hosts lags this event, and infective parasite stages in intermediate hosts then

become available to infect new bivalve hosts by the late summer and early fall. Interestingly, Boyer et al. (2013) also demonstrated high retention efficiency of copepod stages contributing to *M. refringens* transmission in *M. galloprovincialis*, suggesting that exposure to infective parasite stages occurs through direct feeding of copepod stages.

6. Concluding remarks: an ecological perspective

Parasitic diseases cause substantial community- and ecosystem-wide impacts in marine and estuarine bivalves. The long history of diseases impacting bivalve species of ecological and economic importance has produced a rich body of knowledge describing interactions between bivalves and their parasites. Collectively assembled, we can draw a number of conclusions about parasite transmission in bivalve hosts from this diverse body of work.

1. Parasite transmission rarely occurs through direct contact between bivalve hosts, although *Vibrio tapetis* may be transmitted between clams in very close contact. In most cases, the transmission of bivalve parasites occurs by exposure to water-borne parasite stages through suspension feeding processes.
2. Infections with viral parasites are very likely to be common in wild and cultured bivalves, and may be responsible for many of the cryptic mortality events observed in shellfish hatcheries.
3. Many bacterial parasites, including *Nocardia crassostreae* and *Vibrio* spp., and protozoan parasites such as QPX, can be common, though inconspicuous

1096 constituents of ambient marine microbial communities. These facultative parasites
 1097 appear to adapt to parasitic lifestyles when conditions permit.

1098 4. Some stages of protozoan parasites can survive for days, weeks, or even months
 1099 in the water column, and can disperse quite far as passive particles, broadly
 1100 distributing themselves throughout bivalve populations.

1101 5. Many consumed parasites have adapted behaviors to evade direct digestion,
 1102 instead targeting “safer” portals of entry such as pallial organs and pseudo-
 1103 internal extrapallial spaces and peripheral compartments.

1104 6. Parasites often target hemocytes for direct infection or dissemination through host
 1105 tissues.

1106 7. The outcome of interactions between bivalve hosts and their parasites is regulated
 1107 by intrinsic host factors such as innate immunity and physiology, parasite factors
 1108 such as virulence, and the interaction of these two factors with environmental
 1109 variables such as temperature and salinity.

1110 8. Many bivalve parasites, including OsHV-1, *Vibrio tubiashii*, *Perkinsus olseni*,
 1111 *Bonamia ostreae*, *Mikrocytos mackini*, and *Marteilia refringens*, have adapted to
 1112 infect multiple hosts. The susceptibility and tolerance of hosts to parasitic
 1113 infection can vary dramatically by species.

1114

1115 The implications of the final conclusion remain open, and should drive future ecological
 1116 studies of parasites infecting bivalve hosts. A community of bivalves offers parasites
 1117 options for host competence. This point is exemplified by the observations of Boyer et al.
 1118 (2013) in Thau lagoon. There, *Marteilia refringens* infects *Mytilus galloprovincialis*, but

1119 occurs only in digestive epithelia of *Ruditapes decussatus* as necrotic cells. This parasite
 1120 has been observed to infect a diversity of bivalve hosts, including *Ostrea edulis*, *O.*
 1121 *angasi*, *O. puelchana*, *M. galloprovincialis*, and *M. edulis* (Figueras and Montes 1988; Le
 1122 Roux et al. 2001) (Table 1). A diversity of host species, each plausibly offering parasites
 1123 a range of competency, represents the resources supporting a continuum of outcomes for
 1124 parasites, from immediate parasite mortality to successful reproduction and spread
 1125 through host populations. This diversity is not limited to competent hosts. Once released
 1126 into the environment, free-living parasite stages encounter a multitude of factors that
 1127 impact their survival and infection success. While associations with abiotic factors such
 1128 as water temperature and salinity have received much attention here and elsewhere, little
 1129 is known about the role of ambient fauna. This reductionist approach is often intentional,
 1130 as much of what we know about the transmission of bivalve parasites is based on
 1131 experimental systems or models in which hosts and parasites are considered in an
 1132 ecological vacuum. In reality, hosts exist within diverse ecological communities, and
 1133 ecological interactions undoubtedly influence the transmission and impact of parasites
 1134 (Dobson 2004; Hall et al. 2007). Pernet et al. (2014), for example, found the risk of
 1135 OsHV-1 mortality to decrease in *C. gigas* when oysters were held in the vicinity of
 1136 mussel farms. The observation by Pernet et al. (2014) suggests that nonfocal suspension
 1137 feeders can dilute the abundance of suspended particles in the water column, minimizing
 1138 the impact of suspended parasites on their focal hosts. The importance of host diversity
 1139 has become apparent in a number of case studies of bivalve hosts impacted by trematode
 1140 parasites. Studying infections with the larval trematode *Curtuteria australis* in the
 1141 intertidal cockle *Austrovenus stutchburyi*, Mouritsen and Poulin (2003) found that

cockles that provided habitat for epifaunal anemones (*Anthopleura aureoradiata*) were significantly less likely to be infected with the larval trematode *Curtuteria australis*, compared to control groups free of epibionts. In a similar series of experiments, Thieltges et al. (2008) found cockle hosts (*Cerastoderma edule*) held with co-occurring macrofaunal invertebrates acquired a lower load of cercariae of the trematode *Himasthla clongata* compared to cockles held alone. These studies revealed that the effects of parasites were modified when focal hosts were considered within broader ecological communities. In these examples, the effects of parasites were diluted when focal bivalve hosts were considered within a community of abundant alternative hosts with reduced competence. This general phenomenon of a “dilution effect” has received much attention in disease ecology, with relevant examples including Lyme disease (Schmidt and Ostfeld 2001; LoGuidice et al. 2003), West Nile virus (Swaddle and Calos 2008; Allan et al. 2009), and Schistosomiasis (Johnson et al. 2009). In these examples, weakly competent or incompetent hosts play a crucial role in determining the impact of parasites on focal hosts. When these alternative hosts have net negative impacts on focal hosts, via ecological processes such as competition and predation of suspended parasite stages in the water column, the dilution effect is compounded (Schmidt and Ostfeld 2001; Wood and Lafferty 2013).

The relationship between the host diversity and parasite effect is scale-dependent and complex (Randolph and Dobson 2012; Wood and Lafferty 2013). In the case of bivalve hosts, three separate ecological processes can lead to the attenuation of parasite effects in focal hosts: 1) the consumption of free-living parasite stages by predators in the water column, 2) suspension feeding by non-host species (parasite decoys), and 3) the infection

of alternative hosts. Interference competition arising from all three processes, and even between focal hosts, may also result in lower exposure to suspended particles, further lowering the exposure of focal hosts to parasite stages in the water column and diluting the effect of parasites. Connections between the structure of ecological communities and the emergence, spread, and persistence of bivalve diseases are very rarely made, but these connections underscore the importance of understanding the context of and feedbacks to ongoing changes in bivalve populations and their broader ecological communities when considering the impacts of disease.

We encourage future research questions to address these connections directly. How do bivalve diseases respond to changes in host population density? Does the presence of multiple host species influence the impact of bivalve parasites on focal hosts? In turn, how does the abundance and diversity of suspension feeding invertebrates dilute concentrations of parasites in the water column, and what are the effects of parasite dilution on the impact of disease? These questions highlight a critical role for ecological context and host community ecology in the transmission of diseases in populations of bivalve molluscs, which in turn has important consequences for the sustainability of wild and cultured bivalve populations and the function of nearshore ecosystems.

Acknowledgements

The authors would like to thank E. McGurk, R. MacFarlan, B. Allam, D. Proestou, J. Looney, and W. Schroer for constructive discussions on the content and arrangement of this manuscript. We also thank J.E. Ward for clarity on suspension feeding processes in

1188 marine bivalves and we thank one anonymous referee for constructive comments on an
1189 earlier version of this manuscript. TBH was supported by a grant from the National
1190 Science Foundation program in Ecology and Evolution of Infectious Diseases (OCE-
1191 1216220). LH was funded by a National Science Foundation Research Experience for
1192 Undergraduates supplement to OCE-1216220. DAN was funded by MINECON –
1193 NC120086, Chile.

References

- Adlard, R.D., Lester, R.J.G., 1995. Development of a diagnostic test for *Mikrocytos roughleyi*, the aetiological agent of Australian winter mortality of the commercial rock oyster, *Saccostrea commercialis* (Iredale & Roughleyi). J Fish Diseases 18, 609–614.
- Ahmed, H., Schott, E.J., Gauthier, J.D., Vasta, G.R., 2003. Superoxide dismutases from the oyster parasite *Perkinsus marinus*: purification, biochemical characterization, and development of a plate microassay for activity. Anal. Biochem. 318, 132–141.
- Allam, B., Ashton-Alcox, K.A., Ford, S.E., 2001. Haemocyte parameters associated with resistance to brown ring disease in *Ruditapes* spp. clams. Dev. Comp. Immunol. 25, 365–375.
- Allam, B., Carden, W.E., Ward, J.E., Ralph, G., Winnicki, S., Espinosa, E.P., 2013. Early host-pathogen interactions in marine bivalves: Evidence that the alveolate parasite *Perkinsus marinus* infects through the oyster mantle during rejection of pseudofeces. J. Invertebr. Pathol. 113, 26–34.
- Allam, B., Ford, S.E., 2006. Effects of the pathogenic *Vibrio tapetis* on defence factors of susceptible and non-susceptible bivalve species: I. Haemocyte changes following *in vitro* challenge. Fish Shellfish Immun. 20, 374–383.
- Allam, B., Paillard, C., 1998. Defense factors in clam extrapallial fluids. Dis. Aquat. Org. 33, 123–128.
- Allam, B., Paillard, C., Auffret, M., 2000. Alterations in hemolymph and extrapallial fluid parameters in the manila clam, *Ruditapes philippinarum*, challenged with the

- 1217 pathogen *Vibrio tapetis*. J. Invertebr. Pathol. 76, 63–69.
- 1218 Allam, B., Paillard, C., Ford, S.E., 2002. Pathogenicity of *Vibrio tapetis*, the etiological
1219 agent of brown ring disease in clams. Dis. Aquat. Org. 48, 221–231.
- 1220 Allam, B., Paillard, C., Maes, P., 1996. Localization of the pathogen *Vibrio* P1 in clams
1221 affected by Brown Ring Disease. Dis. Aquat. Org. 27, 149–155.
- 1222 Allan, B.F., Langerhans, R.B., Ryberg, W.A., Landesman, W.J., Griffin, N.W., Katz,
1223 R.S., Oberle, B.J., Schutzenhofer, M.R., Smyth, K.N., de St Maurice, A., Clark, L.,
1224 Crooks, K.R., Hernandez, D.E., McLean, R.G., Ostfeld, R.S., Chase, J.M., 2008.
1225 Ecological correlates of risk and incidence of West Nile virus in the United States.
1226 Oecologia 158, 699–708.
- 1227 Amend, A.S., Oliver, T.A., Amaral-Zettler, L.A., Boetius, A., Fuhrman, J.A., Horner-
1228 Devine, M.C., Huse, S.M., Welch, D.B.M., Martiny, A.C., Ramette, A., Zinger, L.,
1229 Sogin, M.L., Martiny, J.B.H., 2012. Macroecological patterns of marine bacteria on a
1230 global scale. J. Biogeogr. 40, 800–811.
- 1231 Anderson, R., May, R., 1981. The population dynamics of microparasites and their
1232 invertebrate hosts. Philos. T. Roy. Soc. B 291, 451–524.
- 1233 Anderson, R.S., 1999. *Perkinsus marinus* secretory products modulate superoxide anion
1234 production by oyster (*Crassostrea virginica*) hemocytes. Fish Shellfish Immun. 9,
1235 51–60.
- 1236 Andrews, J.D., 2015. Epizootiology of late summer and early fall infections of oysters by
1237 *Haplosporidium nelsoni*, and comparison to annual life cycle of *Haplosporidium*
1238 *costalis*, a typical haplosporidian. J. Shellfish Res. 2, 15–23.
- 1239 Arzul, I., Gagnaire, B., Bond, C., Chollet, B., Morga, B., Ferrand, S., Robert, M.,

- Renault, T., 2009. Effects of temperature and salinity on the survival of *Bonamia*
ostreae, a parasite infecting flat oysters *Ostrea edulis*. Dis. Aquat. Org. 85, 67–75.
- Arzul, I., Nicolas, J.-L., Davison, A.J., Renault, T., 2001a. French scallops: a new host
for Ostreid Herpesvirus-1. Virology 290, 342–349.
- Arzul, I., Renault, T., Lipart, C., Davison, A.J., 2001b. Evidence for interspecies
transmission of oyster herpesvirus in marine bivalves. J. Gen. Virol. 82, 865–870.
- Arzul, I., Renault, T.C., Lipart, C., 2001c. Experimental herpes-like viral infections in
marine bivalves: demonstration of interspecies transmission. Dis. Aquat. Org. 46, 1–
6.
- Arzul, I., Renault, T., Thébault, A., Gérard, A., 2002. Detection of oyster herpesvirus
DNA and proteins in asymptomatic *Crassostrea gigas* adults. Virus Res. 84, 151–
160.
- Audemard, C., Barnaud, A., Collins, C.M., Le Roux, F., Sauriau, P.-G., Coustau, C.,
Blachier, P., Berthe, F.C.J., 2001. Claire ponds as an experimental model for
Marteilia refringens life-cycle studies: new perspectives. J. Exp. Mar. Biol. Ecol.
257, 87–108.
- Audemard, C., Kator, H.I., Rhodes, M.W., Gallivan, T., Erskine, A.J., Leggett, A.T.,
Reece, K.S., 2011. High salinity relay as a postharvest processing strategy to reduce
Vibrio vulnificus levels in Chesapeake Bay oysters (*Crassostrea virginica*). J. Food
Prot. 74, 1902–1907.
- Audemard, C., Le Roux, F., Barnaud, A., Collins, C., Sautour, B., Sauriau, P.-G., de
Montadouin, X., Coustau, C., Combes, C., Berthe, F.C.J., 2002. Needle in a
haystack: involvement of the copepod *Paracartia grani* in the life-cycle of the oyster

- 1263 pathogen *Marteilia refringens*. Parasitology 124, 315–323.
- 1264 Audemard, C., Ragone Calvo, L.M., Paynter, K.T., Reece, K.S., Bureson, E.M., 2006.
- 1265 Real-time PCR investigation of parasite ecology: *in situ* determination of oyster
- 1266 parasite *Perkinsus marinus* transmission dynamics in lower Chesapeake Bay.
- 1267 Parasitology 132, 827–842.
- 1268 Auzoux-Bordenave, S., Vigario, A.M., Ruano, F., Domart-Coulon, I., Doumenc, D.,
- 1269 1995. *In vitro* sporulation of the clam pathogen *Perkinsus atlanticus* (Apicomplexa,
- 1270 Perkinsea) under various environmental conditions. J. Shellfish Res. 14, 469–475.
- 1271 Azevedo, C., Conchas, R.F., Montes, J., 2003. Description of *Haplosporidium edule* n.
- 1272 sp. (Phylum Haplosporidia), a parasite of *Cerastoderma edule* (Mollusca, Bivalvia)
- 1273 with complex spore ornamentation. Eur. J. Protistol. 39, 161–167.
- 1274 Azevedo, C., Corral, L., Cachola, R., 1990. Fine structure of zoosporulation in *Perkinsus*
- 1275 *atlanticus* (Apicomplexa: Perkinsea). Parasitology 100, 351–358.
- 1276 Bachali, S., Jager, M., Hassanin, A., Schoentgen, F., Jolles, P., Fiala-Medioni, A.,
- 1277 Deutsch, J.S., 2002. Phylogenetic analysis of invertebrate lysozymes and the
- 1278 evolution of lysozyme function. J. Mol. Evol. 54, 652–664.
- 1279 Baker, K., Morris, J., McCarthy, N., Saldana, L., Lowther, J., Collinson, A., Young, M.,
- 1280 2011. An outbreak of norovirus infection linked to oyster consumption at a UK
- 1281 restaurant, February 2010. J. Public Health 33, 205–211.
- 1282 Balboa, S., Dieguez, A.L., Doce, A., Barja, J.L., Romalde, J.L., 2012. Evaluation of
- 1283 different culture media for the isolation and growth of the fastidious *Vibrio tapetis*,
- 1284 the causative agent of brown ring disease. J. Invertebr. Pathol. 111, 74–81.
- 1285 Baldauf, S.L., 2003. The deep roots of eukaryotes. Science 300, 1703–1706.

- 1286 Barber, B.J., 2004. Neoplastic diseases of commercially important marine bivalves.
1287 Aquat. Living Resour. 17, 449–466.
- 1288 Barber, R.D., Kanaley, S.A., Ford, S.E., 1991. Evidence for regular sporulation by
1289 *Haplosporidium nelsoni* (MSX) (Asctospora; Haplosporidiidae) in spat of the
1290 American oyster, *Crassostrea virginica*. J. Protozool. 34, 305–306.
- 1291 Bayne, C.J., 1990. Phagocytosis and non-self recognition in invertebrates. Bioscience 40,
1292 723–731.
- 1293 Beaz-Hidalgo, R., Balboa, S., Romalde, J.L., Figueras, M.J., 2010. Diversity and
1294 pathogenecity of *Vibrio* species in cultured bivalve molluscs. Environ. Microbiol.
1295 Rep. 2, 34–43.
- 1296 Bellou, M., Kokkinos, P., Vantarakis, A., 2013. Shellfish-borne viral outbreaks: a
1297 systematic review. Food Environ Virol 5, 13–23. Benitez-Nelson, C.R., 2000. The
1298 biogeochemical cycling of phosphorus in marine systems. Earth Sci. Rev. 51, 109–
1299 135.
- 1300 Berthe, F.C.J., Pernas, M., Zerabib, M., Hallner, P., Thébault, A., Figueras, A.J., 1998.
1301 Experimental transmission of *Marteilia refringens* with special consideration of its
1302 life cycle. Dis. Aquat. Org. 34, 135–144.
- 1303 Bertrand, I., Schijven, J.F., Sánchez, G., Wyn-Jones, P., Ottoson, J., Morin, T., Muscillo,
1304 M., Verani, M., Nasser, A., de Roda Husman, A.M., Myrmel, M., Sellwood, J.,
1305 Cook, N., Gantzer, C., 2012. The impact of temperature on the inactivation of enteric
1306 viruses in food and water: a review. J. Appl. Microbiol. 112, 1059–1074.
- 1307 Birkbeck, T.H., McHenry, J., 1982. Degradation of bacteria by *Mytilus edulis*. Mar Biol
1308 72, 7–15.

- 1309 Bitton, G., 1975. Adsorption of viruses on surfaces in soil and water. *Water Res.* 9, 473–
 1310 484.
- 1311 Blackbourn, J., Bower, S.M., Meyer, G.R., 1998. *Perkinsus qugwadi* sp. nov. (incertae
 1312 sedis), a pathogenic protozoan parasite of Japanese scallops, *Patinopecten yessoensis*,
 1313 cultured in British Columbia, Canada. *Can. J. Zool.* 76, 942–953.
- 1314 Blakeslee, A.M.H., Altman, I., Miller, A.W., Byers, J.E., Hamer, C.E., Ruiz, G.M., 2012.
 1315 Parasites and invasions: a biogeographic examination of parasites and hosts in native
 1316 and introduced ranges. *J. Biogeogr.* 39, 609–622.
- 1317 Boettcher, K.J., Barber, B.J., Singer, J.T., 2000. Additional evidence that juvenile oyster
 1318 disease is caused by a member of the *Roseobacter* group and colonization of
 1319 nanaffected animals by *Stappia stellulate*-like strains. *Appl. Environ. Microb.* 66,
 1320 3924–3930.
- 1321 Boettcher, K.J., Geaghan, K., Maloy, A.P., Barber, B.J., 2005. *Roseovarius crassostreae*
 1322 sp. nov., a member of the *Roseobacter* clade and the apparent cause of juvenile oyster
 1323 disease (JOD) in cultured Eastern oysters. *Int. J. Syst. Evol. Micr.* 55, 1531–1537.
- 1324 Borrego, J.J., Castro, D., Luque, A., Paillard, C., Maes, P., Garcia, M.T., Ventosa, A.,
 1325 1996. *Vibrio tapetis* sp. nov., the causative agent of the brown ring disease affecting
 1326 cultured clams. *Int. J. Syst. Bacteriol.* 46, 480–484.
- 1327 Bower, S.M., Blackbourn, J., Meyer, G.R., 1998. Distribution, prevalence, and
 1328 pathogenicity of the protozoan *Perkinsus qugwadi* in Japanese scallops, *Patinopecten*
 1329 *yessoensis*, cultured in British Columbia, Canada. *Can. J. Zool.* 76, 954–959.
- 1330 Bower, S.M., Goh, B., Meyer, G.R., Carnegie, R.B., Gee, A., 2005. Epizootiology and
 1331 detection of nocardiosis in oysters, in: Walker, P., Lester, R., Bondad-Reantaso,

- 1332 M.G. (Eds.), Diseases in Asian Aquaculture V. Diseases in Asian Aquaculture V,
 1333 Fish Health Section, Asian Fisheries Society, Manila, pp. 249–262.
- 1334 Bower, S.M., Hervio, D., Meyer, G.R., 1997. Infectivity of *Mikrocytos mackini*, the
 1335 causative agent of Denman Island disease in Pacific oysters *Crassostrea gigas*, to
 1336 various species of oysters. Dis. Aquat. Org. 29, 111–116.
- 1337 Bower, S.M., McGladdery, S.E., Price, I.M., 1994. Synopsis of infectious diseases and
 1338 parasites of commercially exploited shellfish. Annu. Rev. Fish Dis. 4, 1–199.
- 1339 Boyer, S., Chollet, B., Bonnet, D., Arzul, I., 2013. New evidence for the involvement of
 1340 *Paracartia grani* (Copepoda, Calanoida) in the life cycle of *Marteilia refringens*
 1341 (Paramyxea). Int. J. Parasitol. 43, 1089–1099.
- 1342 Breitbart, M., 2012. Marine viruses: truth or dare. Annu. Rev. Marine. Sci. 4, 425–448.
- 1343 Brilliant, M.G.S., MacDonald, B.A., 2000. Postingestive selection in the sea scallop,
 1344 *Placopecten magellanicus* (Gmelin): the role of particle size and density. J. Exp.
 1345 Mar. Biol. Ecol. 253, 211–227.
- 1346 Brilliant, M.G.S., MacDonald, B.A., 2003. Postingestive sorting of living and heat-killed
 1347 *Chlorella* within the sea scallop, *Placopecten magellanicus* (Gmelin). J. Exp. Mar.
 1348 Biol. Ecol. 290, 81–91.
- 1349 Brown, G.D., Reece, K.S., 2003. Isolation and characterization of serine protease gene(s)
 1350 from *Perkinsus marinus*. Dis. Aquat. Org. 57, 117–126.
- 1351 Brum, J.R., Schenck, R.O., Sullivan, M.B., 2013. Global morphological analysis of
 1352 marine viruses shows minimal regional variation and dominance of non-tailed
 1353 viruses. ISME J. 7, 1738–1751.
- 1354 Buggé, D.M., Allam, B., 2007. Effects of starvation and macroalgae extracts on the

- 1355 survival and growth of quahog parasite unknown (QPX). J. Exp. Mar. Biol. Ecol.
 1356 348, 60–69.
- 1357 Burge, C.A., Griffin, F.J., Friedman, C.S., 2006. Mortality and herpesvirus infections of
 1358 the Pacific oyster *Crassostrea gigas* in Tomales Bay, California, USA. Dis. Aquat.
 1359 Org. 72, 31-43.
- 1360 Burge, C.A. and C.S. Friedman, 2012. Quantifying Ostreid herpesvirus (OsHV-1)
 1361 genome copies and expression during transmission. Microb. Ecol. 63, 596-604.
- 1362 Burge, C.A., Mark Eakin, C., Friedman, C.S., Froelich, B., Hershberger, P.K., Hofmann,
 1363 E.E., Petes, L.E., Prager, K.C., Weil, E., Willis, B.L., Ford, S.E., Harvell, C.D., 2014.
 1364 Climate change influences on marine infectious diseases: implications for
 1365 management and society. Annu. Rev. Marine. Sci. 6, 249–277.
- 1366 Burreson, E.M., Ford, S.E., 2004. A review of recent information on the Haplosporidia,
 1367 with special reference to *Haplosporidium nelsoni* (MSX disease). Aquat. Living
 1368 Resour. 17, 499–517.
- 1369 Burreson, E.M., Ragone Calvo, L.M., 1996. Epizootiology of *Perkinsus marinus* disease
 1370 of oysters in Chesapeake Bay, with emphasis on data since 1985. J. Shellfish Res. 15,
 1371 17–34.
- 1372 Burreson, E.M., Reece, K.S., Dungan, C.F., 2005. Molecular, morphological, and
 1373 experimental evidence support the synonymy of *Perkinsus chesapeaki* and *Perkinsus*
 1374 *andrewsi*. J. Eukaryot. Microbiol. 52, 258–270.
- 1375 Burreson, E.M., Stokes, N.A., Friedman, C.S., 2000. Increased virulence in an introduced
 1376 pathogen: *Haplosporidium nelsoni* (MSX) in the eastern oyster *Crassostrea*
 1377 *virginica*. J. Aquat. Anim. Health 12, 1–8.

- 1378 Bushek, D., Allen, S.K., 2006. Host-parasite interactions among broadly distributed
 1379 populations of the eastern oyster *Crassostrea virginica* and the protozoan *Perkinsus*
 1380 *marinus*. Mar. Ecol. Prog. Ser. 139, 127–141.
- 1381 Bushek, D., Allen, S.K., Ashton-Alcox, K.A., Gustavson, R.G., Ford, S.E., 1997.
 1382 Response of *Crassostrea virginica* to *in vitro* cultured *Perkinsus marinus*:
 1383 preliminary comparison of three inoculation methods. J. Shellfish Res. 16, 479–485.
- 1384 Bushek, D., Ford, S.E., Burt, I., 2012. Long-term patterns of an estuarine pathogen along
 1385 a salinity gradient. J. Mar. Res. 70, 225–251.
- 1386 Bushek, D., Ford, S.E., Chintala, M.M., 2002. Comparison of *in vitro*-cultured and wild-
 1387 type *Perkinsus marinus*. III. Fecal elimination and its role in transmission. Dis.
 1388 Aquat. Org. 51, 217–225.
- 1389 Bushek, D., Landau, B., Scarpa, E., 2008. *Perkinsus chesapeaki* in stout razor clams
 1390 *Tagelus plebeius* from Delaware Bay. Dis. Aquat. Org. 78, 243–247.
- 1391 Canesi, L., Gallo, G., Gavioli, M., Pruzzo, C., 2002. Bacteria-hemocyte interactions and
 1392 phagocytosis in marine bivalves. Microsc. Res. Tech. 57, 469–476.
- 1393 Canesi, L., Pezzati, E., Stauder, M., Grande, C., Bavestrello, M., Papetti, A., Vezzulli, L.,
 1394 Pruzzo, C., 2013. *Vibrio cholerae* interactions with *Mytilus galloprovincialis*
 1395 hemocytes mediated by serum components. Front. Microbiol. 4, 371.
- 1396 Canzonier, W., 1968. Present status of attempts to transmit *Michinia nelsoni* under
 1397 controlled conditions. Proc. Natl. Shellfish Ass. 58, 1.
- 1398 Canzonier, W., 1974. Tissue grafts in the American oyster, *Crassostrea virginica*. Proc.
 1399 Natl. Shellfish Ass. 64, 92–101.
- 1400 Carballal, M., 2003. Virus-like particles associated with large foci of heavy hemocytic

- 1401 infiltration in cockles *Cerastoderma edule* from Galicia (NW Spain). J. Invertebr.
 1402 Pathol. 84, 234–237.
- 1403 Carella, F., Carrasco, N., Andree, K.B., Lacuesta, B., Furones, D., De Vico, G., 2013.
 1404 Nocardiosis in Mediterranean bivalves: First detection of *Nocardia crassostreae* in a
 1405 new host *Mytilus galloprovincialis* and in *Ostrea edulis* from the Gulf of Naples
 1406 (Italy). J. Invertebr. Pathol. 114, 324–328.
- 1407 Carnegie, R.B., Burreson, E.M., 2011. Declining impact of an introduced pathogen:
 1408 *Haplosporidium nelsoni* in the oyster *Crassostrea virginica* in Chesapeake Bay.
 1409 Mar. Ecol. Prog. Ser. 432, 1–15.
- 1410 Carnegie, R.B., Burreson, E.M., Hine, P.M., Stokes, N.A., Audemard, C., Bishop, M.J.,
 1411 Peterson, C.H., 2006. *Bonamia perspora* n. sp. (Haplosporidia), a parasite of the
 1412 oyster *Ostreola equestris*, is the first *Bonamia* species known to produce spores. J
 1413 Eukaryotic Microbiology 53, 232–245.
- 1414 Carnegie, R.B., Cochenne-Laureau, N., 2004. Microcell parasites of oysters: recent
 1415 insights and future trends. Aquat. Living Resour. 17, 519–528.
- 1416 Carnegie, R.B., Meyer, G.R., Blackburn, J., Cochenne-Laureau, N., Berthe, F.C.J.,
 1417 Bower, S.M., 2003. Molecular detection of the oyster parasite *Mikrocytos mackini*,
 1418 and a preliminary phylogenetic analysis. Dis. Aquat. Org. 54, 219–227.
- 1419 Carrasco, N., Arzul, I., Chollet, B., Robert, M., Joly, J.P., Furones, M.D., Berthe, F.C.J.,
 1420 2008. Comparative experimental infection of the copepod *Paracartia grani* with
 1421 *Marteilia refringens* and *Marteilia maurini*. J Fish Diseases 31, 497–504.
- 1422 Carrasco, N., Hine, P.M., Durfort, M., Andree, K.B., Malchus, N., Lacuesta, B.,
 1423 González, J.M., Roque, A., Rodgers, C., Furones, M.D., 2013. *Marteilia cochillia* sp.

- 1424 nov., a new *Marteilia* species affecting the edible cockle *Cerastoderma edule* in
 1425 European waters. *Aquaculture* 412-413, 223–230.
- 1426 Carrasco, N., López-Flores, I., Alcaraz, M., Furones, M.D., Berthe, F.C.J., Arzul, I.,
 1427 2007. First record of a *Marteilia* parasite (Paramyxia) in zooplankton populations
 1428 from a natural estuarine environment. *Aquaculture* 269, 63–70.
- 1429 Carrasco, N., Rojas, M., Aceituno, P., Andree, K.B., Lacuesta, B., Furones, M.D., 2014.
 1430 *Perkinsus chesapeaki* observed in a new host, the European common edible cockle
 1431 *Cerastoderma edule*, in the Spanish Mediterranean coast. *J. Invertebr. Pathol.* 117,
 1432 56–60.
- 1433 Casas, S.M., 2002. Estudio de la perkinsiosis en la almeja fina, *Tapes decussatus*
 1434 (Linnaeus 1758), de Galicia. Ph.D Thesis, University of Santiago de Compostela,
 1435 Spain.
- 1436 Casas, S.M., Grau, A., Reece, K.S., Apakupakul, K., Azevedo, C., Villalba, A., 2004.
 1437 *Perkinsus mediterraneus* n. sp., a protistan parasite of the European flat oyster *Ostrea*
 1438 *edulis* from the Balearic Islands, Mediterranean Sea. *Dis. Aquat. Org.* 58, 231–244.
- 1439 Casas, S.M., Villalba, A., Reece, K.S., 2002. Study of perkinsiosis in the carpet shell clam
 1440 *Tapes decussatus* in Galicia (NW Spain). I. Identification of the aetiological agent
 1441 and *in vitro* modulation of zoosporulation by temperature and salinity. *Dis. Aquat.*
 1442 *Org.* 50, 51–65.
- 1443 Chauvaud, L., Jean, F., Ragueneau, O., Thouzeau, G., 2000. Long-term variation of the
 1444 Bay of Brest ecosystem: benthic-pelagic coupling revisited. *Mar. Ecol. Prog. Ser.*
 1445 200, 35–48.
- 1446 Choi, D., Lee, N., Choi, H., Park, M.A., McGladdery, S.E., Park, M.S., 2004. Viral

- 1447 gametocytic hypertrophy caused by papova-like virus infection in the Pacific oyster
 1448 *Crassostrea gigas* in Korea. Dis. Aquat. Org. 59, 205–209.
- 1449 Choquet, G., Soudant, P., Lambert, C., Nicolas, J.-L., Paillard, C., 2003. Reduction of
 1450 adhesion properties of *Ruditapes philippinarum* hemocytes exposed to *Vibrio tapetis*.
 1451 Dis. Aquat. Org. 57, 109–116.
- 1452 Chu, F.-L.E., 1988. Humoral defense factors in marine bivalves. Am. Fish. Soc. Spec.
 1453 Publ. 18, 178–188.
- 1454 Chu, F.-L.E., 1996. Laboratory investigations of susceptibility, infectivity, and
 1455 transmission of *Perkinsus marinus* in oysters. J. Shellfish Res. 15, 57–66.
- 1456 Chu, F.-L.E., Lund, E., Soudant, P., Harvey, E., 2002. De novo arachidonic acid
 1457 synthesis in *Perkinsus marinus*, a protozoan parasite of the eastern oyster
 1458 *Crassostrea virginica*. Mol. Biochem. Parasit. 119, 179–190.
- 1459 Chu, F.-L.E., Lund, E.D., 2006. Viability, infectivity and fatty acid synthetic activity of
 1460 *Perkinsus marinus* meront cells incubated in estuarine and artificial seawater. Dis.
 1461 Aquat. Org. 71, 131–139.
- 1462 Cochenne, N., 2001. Bonamia ostreae, parasite de l’huître plate, *Ostrea edulis*, sa
 1463 position taxonomique parmi les parasites du groupe “Microcell,” analyses des
 1464 interactions hôte/parasite chez plusieurs populations d’huîtres plates. These de
 1465 Doctorat, Biologie Cellulaire, Universite de la Rochelle, France.
- 1466 Cochenne-Laureau, N., Auffret, M., Renault, T., Langlade, A., 2003. Changes in
 1467 circulating and tissue-infiltrating hemocyte parameters of European flat oysters,
 1468 *Ostrea edulis*, naturally infected with *Bonamia ostreae*. J. Invertebr. Pathol. 83, 23–
 1469 30.

- 1470 Cochlan, W.P., Wikner, J., Steward, G.F., Smith, D.C., Azam, F., 1993. Spatial
 1471 distribution of viruses, bacteria and chlorophyll *a* in neritic, oceanic and estuarine
 1472 environments. *Mar. Ecol. Prog. Ser.* 92, 77–87.
- 1473 Coma, R., Ribes, M., Gili, J.-M., Zabala, M., 2000. Seasonality in coastal benthic
 1474 ecosystems. *Trends Ecol. Evol.* 15, 448–453.
- 1475 Comps, M., Cochenne, N., 1993. A herpes-like virus from the European oyster *Ostrea*
 1476 *edulis*. *J. Invertebr. Pathol.* 62, 201–203.
- 1477 Costerton, J.W., Stewart, P.S., P, G.E., 1999. Bacterial biofilms: a common cause of
 1478 persistent infections. *Science* 284, 1318–1322.
- 1479 Culley, A.I., Lang, A.S., Suttle, C.A., 2003. High diversity of unknown picorna-like
 1480 viruses in the sea. *Nature* 424, 1051–1054.
- 1481 Culloty, S.C., Mulcahy, M.F., 1996. Season-, age-, and sex-related variation in the
 1482 prevalence of bonamiasis in flat oysters (*Ostrea edulis* L.) on the south coast of
 1483 Ireland. *Aquaculture* 144, 53–63.
- 1484 Culloty, S.C., Novoa, B., Pernas, M., Longshaw, M., Mulcahy, M.F., Feist, S.W.,
 1485 Figueras, A., 1999. Susceptibility of a number of bivalve species to the protozoan
 1486 parasite *Bonamia ostreae* and their ability to act as vectors for this parasite. *Dis.*
 1487 *Aquat. Org.* 37, 73–80.
- 1488 Dahl, S.F., Allam, B., 2007. Laboratory transmission studies of QPX disease in the
 1489 northern quahog (=hard clam): development of an infection procedure. *J. Shellfish*
 1490 *Res.* 26, 383–389.
- 1491 Dahl, S.F., Perrigault, M., Allam, B., 2008. Laboratory transmission studies of QPX
 1492 disease in the hard clam: Interactions between different host strains and pathogen

- 1493 isolates. *Aquaculture* 280, 64–70.
- 1494 Dahl, S.F., Thiel, J., Allam, B., 2010. Field performance and QPX disease progress in
 1495 cultured and wild-type strains of *Mercenaria mercenaria* in New York waters. *J.*
 1496 *Shellfish Res.* 29, 83–90.
- 1497 Dame, R.F., 2011. *Ecology of Marine Bivalves: An Ecosystem Approach*. CRC Press,
 1498 Boca Raton, FL.
- 1499 Dame, R.F., Zingmark, R., Haskin, E., 1984. Oyster reefs as processors of estuarine
 1500 materials. *J. Exp. Mar. Biol. Ecol.* 83, 239–247.
- 1501 Daniels, N.A., 2011. *Vibrio vulnificus* oysters: pearls and perils. *Clin. Infect. Dis.* 52,
 1502 788–792.
- 1503 Davis, C.V., Barber, B.J., 1999. Growth and survival of selected lines of eastern oysters,
 1504 *Crassostrea virginica* (Gmelin 1791) affected by juvenile oyster disease. *Aquaculture*
 1505 178, 253–271.
- 1506 Davison, A.J., Trus, B.L., Cheng, N., Steven, A.C., Watson, M.S., Cunningham, C., Le
 1507 Deuff, R.-M., Renault, T., 2005. A novel class of herpesvirus with bivalve hosts. *J.*
 1508 *Gen. Virol.* 86, 41–53.
- 1509 de Freitas Rebelo, M., Figueiredo, E. de S., Mariante, R.M., Nóbrega, A., de Barros,
 1510 C.M., Allodi, S., 2013. New Insights from the oyster *Crassostrea rhizophorae* on
 1511 bivalve circulating hemocytes. *PLoS ONE* 8, e57384.
- 1512 de Simon, M., Tarrago, C., Ferrer, M., 1992. Incidence of *Listeria monocytogenes* in
 1513 fresh foods in Barcelona (Spain). *Int. J. Food Microbiol.* 16, 153–156.
- 1514 Dégrement, L., Guyader, T., Tourbiez, D., Pépin, J.-F., 2013. Is horizontal transmission
 1515 of the Ostreid herpesvirus OsHV-1 in *Crassostrea gigas* affected by unselected or

- 1516 selected survival status in adults to juveniles? Aquaculture 408, 51–57.
- 1517 Di Girolamo, R., Liston, J., Matches, J., 1977. Ionic bonding, the mechanism of viral
1518 uptake by shellfish mucus. Appl. Environ. Microb. 33, 19–25.
- 1519 Dobson, A., 2004. Population dynamics of pathogens with multiple host species. Am Nat
1520 164, S64–S78.
- 1521 Donlan, R.M., Costerton, J.W., 2002. Biofilms: survival mechanisms of clinically
1522 relevant microorganisms. Clin. Microbiol. Rev. 15, 167–193.
- 1523 Dungan, C.F., Bushek, D., in press. Development and applications of Ray's fluid
1524 thioglycollate media for detection and manipulation of *Perkinsus* spp. pathogens of
1525 marine molluscs. J. Invert. Pathol. doi:10.1016/j.jip.2015.05.004
- 1526 Dungan, C.F., Hamilton, R.M., 1995. Use of a tetrazolium-based cell proliferation assay
1527 to measure effects of *in vitro* conditions on *Perkinsus marinus* (Apicomplexa)
1528 proliferation. J. Eukaryot. Microbiol. 42, 379–388.
- 1529 Eisenberg, R., Atanasiu, D., Cairns, T., Gallagher, J.R., Krummenacher, C., Cohen, G.H.,
1530 2012. Herpes virus fusion and entry: a story with many characters. Viruses 4, 800–
1531 832.
- 1532 Elston, R.A., 1997. Special topic review: bivalve mollusc viruses. World J. Microb. Biot.
1533 13, 393–403.
- 1534 Elston, R.A., Farley, C.A., Kent, M.L., 1986. Occurrence and significance of bonamiasis
1535 in European flat oysters *Ostrea edulis* in North America. Dis. Aquat. Org. 2, 49–54.
- 1536 Elston, R.A., Hasegawa, H., Humphrey, K.L., Polyak, I.K., Häse, C.C., 2008. Re-
1537 emergence of *Vibrio tubiashii* in bivalve shellfish aquaculture: severity,
1538 environmental drivers, geographic extent and management. Dis. Aquat. Org. 82,

- 1539 119–134.
- 1540 Elston, R.A., Moore, J.D., Brooks, K., 1992. Disseminated neoplasia in bivalve molluscs.
- 1541 Rev. Aquat. Sci. 6, 405–466.
- 1542 Elston, R.A., Wilkinson, M., 1985. Pathology, management and diagnosis of oyster velar
- 1543 virus disease (OVVD). Aquaculture 48, 189–210.
- 1544 Engelsma, M.Y., Culloty, S.C., Lynch, S.A., Arzul, I., Carnegie, R.B., 2014. *Bonamia*
- 1545 parasites: a rapidly changing perspective on a genus of important mollusc pathogens.
- 1546 Dis. Aquat. Org. 110, 5–23.
- 1547 Engelsma, M.Y., Roozenburg, I., Joly, J.P., 2008. First isolation of *Nocardia*
- 1548 *crassostreae* from Pacific oyster *Crassostrea gigas* in Europe. Dis. Aquat. Org. 80,
- 1549 229–234.
- 1550 Farley, C.A., 1967. A proposed life cycle of *Minchinia nelsoni* (Haplosporidia,
- 1551 Haplosporidiidae) in the American oyster *Crassostrea virginica*. J. Protozool. 14,
- 1552 616–625.
- 1553 Farley, C.A., 1972. Oyster herpes-type virus. Science 178, 759–760.
- 1554 Fayer, R., Dubey, J.P., Lindsay, D.S., 2004. Zoonotic protozoa: from land to sea. Trends
- 1555 Parasitol. 20, 531–536.
- 1556 Fernández Robledo, J.A., Vasta, G.R., Record, N.R., 2014. Protozoan parasites of bivalve
- 1557 molluscs: literature follows culture. PLoS ONE 9, e100872.
- 1558 Figueras, A., Montes, J., 1988. Aber disease of edible oysters caused by *Marteilia*
- 1559 *refringens*. Am. Fish. Soc. Spec. Publ. 18, 38–46.
- 1560 Fischer, A.M., Ryan, J.P., Levesque, C., Welschmeyer, N., 2014. Characterizing
- 1561 estuarine plume discharge into the coastal ocean using fatty acid biomarkers and

- 1562 pigment analysis. Mar. Environ. Res. 99, 106–116.
- 1563 Flye-Sainte-Marie, J., Soudant, P., Lambert, C., Le Goïc, N., Goncalvez, M., Travers,
1564 M.-A., Paillard, C., Jean, F., 2009. Variability of the hemocyte parameters of
1565 *Ruditapes philippinarum* in the field during an annual cycle. J. Exp. Mar. Biol. Ecol.
1566 377, 1–11.
- 1567 Ford, S.E., 1996. Range extension by the oyster parasite *Perkinsus marinus* into
1568 northeastern US: response to climate change? J. Shellfish Res. 15, 45–56.
- 1569 Ford, S.E., Borrero, F.J., 2001. Epizootiology and pathology of juvenile oyster disease in
1570 the eastern oyster, *Crassostrea virginica*. J. Invertebr. Pathol. 78, 141–154.
- 1571 Ford, S.E., Chintala, M.M., 2006. Northward expansion of a marine parasite: Testing the
1572 role of temperature adaptation. J. Exp. Mar. Biol. Ecol. 339, 226–235.
- 1573 Ford, S.E., Haskin, H., 1982. History and epizootiology of *Haplosporidium nelsoni*
1574 (MSX), an oyster pathogen in Delaware Bay, 1957-1980. J. Invertebr. Pathol. 40,
1575 118–141.
- 1576 Ford, S.E., Kraeuter, J.N., Barber, R.D., Mathis, G., 2002. Aquaculture-associated factors
1577 in QPX disease of hard clams: density and seed source. Aquaculture 208, 23–38.
- 1578 Ford, S.E., Schotthoefer, A., Spruck, C., 1999. *In vivo* dynamics of the microparasite
1579 *Perkinsus marinus* and regression of infections in eastern oysters. J. Parasitol. 85,
1580 273–282.
- 1581 Ford, S.E., Smolowitz, R., 2007. Infection dynamics of an oyster parasite in its newly
1582 expanded range. Mar Biol 151, 119–133.
- 1583 Forns, X., Thimme, R., Govindarajan, S., Emerson, S.U., Purcell, R.H., Chisari, F.V.,
1584 Bukh, J., 2000. Hepatitis C virus lacking the hypervariable region 1 of the second

- 1585 envelope protein is infectious and causes acute resolving or persistent infection in
- 1586 chimpanzees. P Natl. Acad. Sci. USA 97, 13318–13323.
- 1587 Friedman, C.S., Beaman, B.L., Chun, J., Goodfellow, M., Gee, A., Hedrick, R.P., 1998.
- 1588 *Nocardia crassostreae* sp. nov., the causal agent of nocardiosis in Pacific oysters. Int.
- 1589 J. Syst. Bacteriol. 48, 237–246.
- 1590 Friedman, C.S., Estes, R.M., Stokes, N.A., Burge, C.A., Hargrove, J.S., Barber, B.J.,
- 1591 Elston, R.A., Burrenson, E.M., Reece, K.S., 2005. Herpes virus in juvenile Pacific
- 1592 oysters *Crassostrea gigas* from Tomales Bay, California, coincides with summer
- 1593 mortality episodes. Dis. Aquat. Org. 63, 33–41.
- 1594 Friedman, C.S., Hedrick, R.P., 1991. Pacific oyster nocardiosis: isolation of the bacterium
- 1595 and induction of laboratory infections. J. Invertebr. Pathol. 57, 109–120.
- 1596 Garreis, K.A., La Peyre, J.F., Faisal, M., 1996. The effects of *Perkinsus marinus*
- 1597 extracellular products and purified proteases on oyster defense parameters *in vitro*.
- 1598 Fish Shellfish Immun. 6, 581–597.
- 1599 Garza, D.R., Suttle, C.A., 1998. The effect of cyanophages on the mortality of
- 1600 *Synechococcus* spp. and selection for UV resistant viral communities. Microb Ecol
- 1601 36, 281–292.
- 1602 Gast, R.J., Moran, D.M., Audemard, C., Lyons, M.M., DeFavari, J., Reese, K.S., Leavitt,
- 1603 D., Smolowitz, R., 2008. Environmental distribution and persistence of Quahog
- 1604 Parasite Unknown (QPX). Dis. Aquat. Org. 81, 219–229.
- 1605 Gauthier, J.D., Vasta, G.R., 1995. *In vitro* culture of the eastern oyster parasite *Perkinsus*
- 1606 *marinus*: optimization of the methodology. J. Invertebr. Pathol. 66, 156–168.
- 1607 Gentry, J., Vinje, J., Guadagnoli, D., Lipp, E.K., 2009. Norovirus distribution within an

- 1608 estuarine environment. Appl. Environ. Microb. 75, 5474–5480.
- 1609 Geyer, W.R., Signell, R.P., 1992. A reassessment of the role of tidal dispersion in
1610 estuaries and bays. Estuaries. 15, 97-108.
- 1611 Gili, J.-M., Coma, R., 1998. Benthic suspension feeders: their paramount role in littoral
1612 marine food webs. Trends Ecol. Evol. 13, 316–321.
- 1613 Goggin, C.L., Lester, R.J.G., 1995. *Perkinsus*, a protistan parasite of abalone in Australia:
1614 a review. Mar. Freshwater Res. 46, 639–646.
- 1615 Goggin, C.L., Sewell, K.B., Lester, R.J.G., 1989. Cross-infection experiments with
1616 Australian *Perkinsus* species. Dis. Aquat. Org. 7, 53–59.
- 1617 González, J.M., Moran, M.A., 1997. Numerical dominance of a group of marine bacteria
1618 in the α -subclass of the class Proteobacteria in coastal seawater. Appl. Environ.
1619 Microb. 63, 4237–4242.
- 1620 Greenwood, M., Winnard, G., Bagot, B., 1998. An outbreak of *Salmonella* enteritidis
1621 phage type 19 infection associated with cockles. Comm. Dis. Publ. Health 1, 35–37.
- 1622 Grizel, H., 1979. *Marteilia refringens* and oyster disease: recent observations. Mar. Fish
1623 Rev. 41, 38–39.
- 1624 Grizel, H., Mialhe, E., Chagot, D., Boulo, V., Bachere, E., 1988. Bonamiasis : a model
1625 study of diseases in marine molluscs. Am. Fish. Soc. Spec. Publ. 18, 1–4.
- 1626 Guidi-Rontani, C., 2002. The alveolar macrophage: the Trojan horse of *Bacillus*
1627 *anthracis*. Trends Microbiol. 10, 405–409.
- 1628 Hada, H.S., West, P.A., Lee, J.V., Stemmler, J., Colwell, R.R., 1984. *Vibrio tubiashii* sp.
1629 nov., a pathogen of bivalve mollusks. Int. J. Syst. Bacteriol. 34, 1–4.
- 1630 Hall, S.R., Sivars-Becker, L., Becker, C., Duffy, M.A., Tessier, A.J., Caceres, C.E., 2007.

- 1631 Eating yourself sick: transmission of disease as a function of foraging ecology. Ecol.
 1632 Letters 10, 207–218.
- 1633 Harvell, C.D., Kim, K., Burkholder, J.M., Colwell, R.R., Epstein, P.R., Grimes, D.J.,
 1634 Hofmann, E.E., Lipp, E.K., Osterhaus, A.D.M.E., Overstreet, R.M., Porter, J.W.,
 1635 Smith, G.W., Vasta, G.R., 1999. Emerging marine diseases: climate links and
 1636 anthropogenic factors. Science 285, 1505–1510.
- 1637 Haskin, H., 1966. *Minchinia nelsoni* n. sp. (Haplosporida, Haplosporidiidae): causitive
 1638 agent of the Delaware Bay oyster epizootic. Science 153, 1414–1416.
- 1639 Haskin, H., Andrews, J.D., 1988. Uncertainties and speculations about the life cycle of
 1640 the eastern oyster pathogen *Haplosporidium nelsoni* (MSX). Am. Fish. Soc. Spec.
 1641 Publ. 18, 5–22.
- 1642 Heldal, M., Bratbak, G., 1991. Production and decay of viruses in aquatic environments.
 1643 Mar. Ecol. Prog. Ser. 72, 205–212.
- 1644 Hershberger, P.K., van der Leeuw, B.K., Gregg, J.L., Grady, C.A., Lujan, K.M.,
 1645 Gutenberger, S.K., Purcell, M.K., Woodson, J.C., Winton, J.R., Parsley, M.J., 2010.
 1646 Amplification and transport of an endemic fish disease by an introduced species. Biol
 1647 Invasions 12, 3665–3675.
- 1648 Hervio, D., Bachere, E., Boulo, V., Cochenne, N., Vuillemin, V., Le Coguic, Y.,
 1649 Cailletaux, G., Mazurie, J., Mialhe, E., 1995. Establishment of an experimental
 1650 infection protocol for the flat oyster, *Ostrea edulis*, with the intrahaemocytic
 1651 protozoan parasite, *Bonamia ostreae*: application in the selection of parasite-resistant
 1652 oysters. Aquaculture 132, 183–194.
- 1653 Hervio, D., Chagot, D., Godin, P., Grizel, H., Mialhe, E., 1991. Localization and

- 1654 characterization of acid phosphatase activity in *Bonamia ostreae* (Ascetospora), an
 1655 intrahemocytic protozoan parasite of the flat oyster *Ostrea edulis* (Bivalvia). Dis.
 1656 Aquat. Org. 12, 67–70.
- 1657 Hine, P.M., 1991. Ultrastructural observations on the annual infection pattern of *Bonamia*
 1658 sp. in flat oysters *Tiostrea chilensis*. Dis. Aquat. Org. 11, 163–171.
- 1659 Hine, P.M., 1999. The inter-relationships of bivalve haemocytes. Fish Shellfish Immun.
 1660 9, 367–385.
- 1661 Hine, P.M., Cochenne-Laureau, N., Berthe, F.C.J., 2001. *Bonamia exitiosa* n. sp.
 1662 (Haplosporidia) infecting flat oysters, *Ostrea chilensis*, in New Zealand. Dis. Aquat.
 1663 Org. 47, 63–72.
- 1664 Hine, P.M., Wesney, B., Besant, P., 1998. Replication of a herpes-like virus in larvae of
 1665 the flat oyster *Tiostrea chilensis* at ambient temperatures. Dis. Aquat. Org. 32, 181–
 1666 171.
- 1667 Holt, R.D., Dobson, A.P., Begon, M., Bowers, R.G., Schaubert, E.M., 2003. Parasite
 1668 establishment in host communities. Ecol. Letters 6, 837–842.
- 1669 Honess, R.W., Roizman, B., 1974. Regulation of herpesvirus macromolecular synthesis:
 1670 I. cascade regulation of the synthesis of three groups of viral proteins. J. Virol. 14, 8–
 1671 19.
- 1672 Hughes, F.M., Foster, B., Grewal, S., Sokolova, I.M., 2010. Apoptosis as a host defense
 1673 mechanism in *Crassostrea virginica* and its modulation by *Perkinsus marinus*. Fish
 1674 Shellfish Immun. 29, 247–257.
- 1675 Iwamoto, M., Ayers, T., Mahon, B.E., Swerdlow, D.L., 2010. Epidemiology of seafood-
 1676 associated infections in the United States. Clin. Microbiol. Rev. 23, 399–411.

- 1677 Jensen, S., Samulsen, O.B., Andersen, K., Torkildsen, L., Lambert, C., Choquet, G.,
 1678 Paillard, C., Bergh, O., 2003. Characterization of strains of *Vibrio splendidus* and *V.*
 1679 *tapetis* isolated from corkwing wrasse *Symphodus melops* suffering vibriosis. Dis.
 1680 Aquat. Org. 2003, 25–31.
- 1681 Johnson, P.T.J., Dobson, A., Lafferty, K.D., Marcogliese, D.J., Memmott, J., Orlofske,
 1682 S.A., Poulin, R., Thieltges, D.W., 2010. When parasites become prey: ecological and
 1683 epidemiological significance of eating parasites. Trends Ecol. Evol. 25, 362–371.
- 1684 Johnson, P.T.J., Lund, P.J., Hartson, R.B., Yoshino, T.P., 2009. Community diversity
 1685 reduces *Schistosoma mansoni* transmission, host pathology and human infection risk.
 1686 P. Roy. Soc. Lond. B Bio. 276, 1657–1663.
- 1687 Johnson, P.T.J., Thieltges, D.W., 2010. Diversity, decoys and the dilution effect: how
 1688 ecological communities affect disease risk. J. Exp. Biol. 213, 961–970.
- 1689 Jones, J., Scotti, P., Dearing, S., Wesney, B., 1996. Virus-like particles associated with
 1690 marine mussel mortalities in New Zealand. Dis. Aquat. Org. 25, 143–149.
- 1691 Jorgensen, C.B., 1996. Bivalve filter feeding revisited. Mar. Ecol. Prog. Ser. 142, 287–
 1692 302.
- 1693 Jouaux, A., Lafont, M., Blin, J.-L., Houssin, M., Mathieu, M., Lelong, C., 2013.
 1694 Physiological change under OsHV-1 contamination in Pacific oyster *Crassostrea*
 1695 *gigas* through massive mortality events on fields. BMC Genomics 14, 590.
- 1696 Kang, Y.-S., Kim, Y.-M., Park, K.-I., Kim Cho, S., Choi, K.S., Cho, M., 2006. Analysis
 1697 of EST and lectin expressions in hemocytes of Manila clams (*Ruditapes*
 1698 *philippinarum*) (Bivalvia: Mollusca) infected with *Perkinsus olseni*. Dev. Comp.
 1699 Immunol. 30, 1119–1131.

- 1700 Keesing, F., Belden, L.K., Daszak, P., Dobson, A., Harvell, C.D., Holt, R.D., Hudson, P.,
 1701 Jolles, A., Jones, K.E., Mitchell, C.E., Myers, S.S., Bogich, T., Ostfeld, R.S., 2010.
 1702 Impacts of biodiversity on the emergence and transmission of infectious diseases.
 1703 Nature 468, 647–652.
- 1704 Kellogg, M.L., Cornwell, J.C., Owens, M.S., Paynter, K.T., 2013. Denitrification and
 1705 nutrient assimilation on a restored oyster reef. Mar. Ecol. Prog. Ser. 480, 1–19.
- 1706 Kern, F., 1976. Sporulation of *Minchinia* sp. (Haplosporida, Haplosporidiidae) in the
 1707 Pacific oyster *Crassostrea gigas* (Thunberg) from the Republic of Korea. J.
 1708 Protozool. 23, 498–500.
- 1709 Kim, J.Y., Adhya, M., Cho, S.K., Choi, K.S., Cho, M., 2008. Characterization, tissue
 1710 expression, and immunohistochemical localization of MCL3, a C-type lectin
 1711 produced by *Perkinsus olseni*-infected Manila clams (*Ruditapes philippinarum*). Fish
 1712 Shellfish Immun. 25, 598–603.
- 1713 Kiss, T., 2010. Apoptosis and its functional significance in molluscs. Apoptosis 15, 313–
 1714 321.
- 1715 Kleeman, S.N., Adlard, R.D., Lester, R.J.G., 2002. Detection of the initial infective
 1716 stages of the protozoan parasite *Marteilia sydneyi* in *Saccostrea glomerata* and their
 1717 development through to sporogenesis. Int. J. Parasitol. 32, 767–784.
- 1718 Kleinschuster, S.J., Smolowitz, R., Parent, J., 1998. *In vitro* life cycle and propagation of
 1719 Quahog Parasite Unknown. J. Shellfish Res. 17, 75–78.
- 1720 Kach, D.J., Ward, J.E., 2008. The role of marine aggregates in the ingestion of
 1721 picoplankton-size particles by suspension-feeding molluscs. Mar. Biol. 153, 797–805.
- 1722 Kramer, A.M., Lyons, M.M., Dobbs, F.C., Drake, J.M., 2013. Bacterial colonization and

- 1723 extinction on marine aggregates: stochastic model of species presence and
 1724 abundance. *Ecol Evol* 3, 4300–4309.
- 1725 La Peyre, J.F., Chu, F.-L.E., Meyers, J.M., 1995. Haemocytic and humoral activities of
 1726 eastern and Pacific oysters following challenge by the protozoan *Perkinsus marinus*.
 1727 *Fish Shellfish Immun.* 5, 179–190.
- 1728 La Peyre, J.F., Xue, Q.-G., Itoh, N., Li, Y., Cooper, R.K., 2010. Serine protease inhibitor
 1729 cvSI-1 potential role in the eastern oyster host defense against the protozoan parasite
 1730 *Perkinsus marinus*. *Dev. Comp. Immunol.* 34, 84–92.
- 1731 La Peyre, M.K., Casas, S.M., Gayle, W., La Peyre, J.F., 2010. The combined influence of
 1732 sub-optimal temperature and salinity on the *in vitro* viability of *Perkinsus marinus*, a
 1733 protistan parasite of the eastern oyster *Crassostrea virginica*. *J. Invertebr. Pathol.*
 1734 105, 176–181.
- 1735 La Peyre, M.K., Casas, S.M., La Peyre, J.F., 2006. Salinity effects on viability, metabolic
 1736 activity and proliferation of three *Perkinsus* species. *Dis. Aquat. Org.* 71, 59–74.
- 1737 La Peyre, M.K., Casas, S.M., Villalba, A., La Peyre, J.F., 2008. Determination of the
 1738 effects of temperature on viability, metabolic activity and proliferation of two
 1739 *Perkinsus* species, and its significance to understanding seasonal cycles of
 1740 perkinsosis. *Parasitology* 135, 505–519.
- 1741 Labreuche, Y., Soudant, P., Gonclaves, M., Lambert, C., Nicolas, J.-L., 2006. Effects of
 1742 extracellular products from the pathogenic *Vibrio aestuarianus* strain 01/32 on
 1743 lethality and cellular immune responses of the oyster *Crassostrea gigas*. *Dev. Comp.*
 1744 *Immunol.* 30, 367–379.
- 1745 Lacoste, A., Jalabert, F., Cueff, A., Gelebart, F., Cordevant, C., Lange, M., Poulet, S.A.,

- 1746 2001. A *Vibrio splendidus* strain is associated with summer mortality of juvenile
 1747 oysters *Crassostrea gigas* in the Bay of Morlaix (North Brittany, France). Dis. Aquat.
 1748 Org. 46, 139–145.
- 1749 Lacoste, A., Malham, S.K., Gelebart, F., Cueff, A., Poulet, S.A., 2002. Stress-induced
 1750 immune changes in the oyster *Crassostrea gigas*. Dev. Comp. Immunol. 26, 181–188.
- 1751 Lafferty, K.D., Dobson, A.P., Kuris, A.M., 2006. Parasites dominate food web links. P.
 1752 Natl. Acad. Sci. USA 103, 11211–11216.
- 1753 Lallias, D., Arzul, I., Heurtebise, S., Ferrand, S., Chollet, B., Robert, M., Beaumont,
 1754 A.R., Boudry, P., Morga, B., Lapègue, S., 2008. *Bonamia ostreae*-induced
 1755 mortalities in one-year old European flat oysters *Ostrea edulis*: experimental
 1756 infection by cohabitation challenge. Aquat. Living Resour. 21, 423–439.
- 1757 Lambert, C., Nicolas, J.-L., Bultel, V., 2001. Toxicity to bivalve hemocytes of pathogenic
 1758 *Vibrio* cytoplasmic extract. J. Invertebr. Pathol. 77, 165–172.
- 1759 Lambert, H., Barragan, A., 2010. Modelling parasite dissemination: host cell subversion
 1760 and immune evasion by *Toxoplasma gondii*. Cell. Microbiol. 12, 292–300.
- 1761 Lane, E., Birkbeck, T.H., 2000. Species specificity of some bacterial pathogens of
 1762 bivalve molluscs is correlated with their interaction with bivalve haemocytes. J Fish
 1763 Diseases 23, 275–279.
- 1764 Langdon, C.J., Newell, R.I.E., 1990. Utilization of detritus and bacteria as food sources
 1765 by two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel
 1766 *Geukensia demissa*. Mar. Ecol. Prog. Ser. 58, 299–310.
- 1767 Le Guyader, F.S., Loisy, F., Atmar, R.L., Hutson, A.M., Estes, M.K., Ruvoen-Clouet, N.,
 1768 Pommeypuy, M., Le Pendu, J., 2006. Norwalk virus-specific binding to oyster

- 1769 digestive tissues. *Emerg. Infect. Diseases* 12, 931–936.
- 1770 Le Roux, F., Lorenzo, G., Peyret, P., Audemard, C., Figueras, A., Vivares, C., Gouy, M.,
 1771 Berthe, F.C.J., 2001. Molecular evidence for the existence of two species of
 1772 *Marteilia* in Europe. *J. Eukaryot. Microbiol.* 48, 449–454.
- 1773 Lee, H.S., Sobsey, M.D., 2011. Survival of prototype strains of somatic coliphage
 1774 families in environmental waters and when exposed to UV low-pressure
 1775 monochromatic radiation or heat. *Water Res.* 45, 3723–3734.
- 1776 Lees, D., 2000. Viruses and bivalve shellfish. *Int. J. Food Microbiol.* 59, 81–116.
- 1777 Lewis, E.J., Farley, C.A., Small, E.B., Baya, A.M., 1996. A synopsis of juvenile oyster
 1778 disease (JOD) experimental studies in *Crassostrea virginica*. *Aquat. Living Resour.*
 1779 9, 169–178.
- 1780 LoGiudice, K., Ostfeld, R.S., Schmidt, K.A., Keesing, F., 2003. The ecology of
 1781 infectious disease: effects of host diversity and community composition on Lyme
 1782 disease risk. *P. Natl. Acad. Sci. USA* 100, 567–571.
- 1783 Lopez, G.R., Cheng, I.-J., 1983. Synoptic measurements of ingestion rate, ingestion
 1784 selectivity, and absorption efficiency of natural foods in the deposit-feeding molluscs
 1785 *Nucula annulata* (Bivalvia) and *Hydrobia totteni* (Gastropoda). *Mar. Ecol. Prog. Ser.*
 1786 11, 55–62.
- 1787 Lopez-Cortes, L., Castro, D., Navas, J.I., Borrego, J.J., 1999a. Phagocytic and
 1788 chemotactic responses of manila and carpet shell clam haemocytes against *Vibrio*
 1789 *tapetis*, the causative agent of brown ring disease. *Fish Shellfish Immun.* 1999, 543–
 1790 555.
- 1791 Lopez-Cortes, L., Luque, A., Martinez-Manzanares, E., Castro, D., Borrego, J.J., 1999b.

- 1792 Adhesion of *Vibrio tapetis* to clam shells. J. Shellfish Res. 18, 91–97.
- 1793 Lund, E.D., Chu, F.-L.E., Harvey, E., 2004. *In vitro* effects of temperature and salinity on
- 1794 fatty acid synthesis in the oyster protozoan parasite *Perkinsus marinus*. J. Exp. Mar.
- 1795 Biol. Ecol. 307, 111–126.
- 1796 Lynch, S.A., Carlsson, J., Reilly, A.O., Cotter, E., Culloty, S.C., 2012. A previously
- 1797 undescribed ostreid herpes virus 1 (OsHV-1) genotype detected in the pacific oyster,
- 1798 *Crassostrea gigas*, in Ireland. Parasitology 139, 1526–1532.
- 1799 Lyons, M.M., Lau, Y.-T., Carden, W.E., Ward, J.E., Roberts, S.B., Smolowitz, R.,
- 1800 Vallino, J., Allam, B., 2007a. Characteristics of marine aggregates in shallow-water
- 1801 ecosystems: implications for disease ecology. EcoHealth 4, 406–420.
- 1802 Lyons, M.M., Smolowitz, R., Gomez-Chiarri, M., Ward, J.E., 2007b. Epizootiology of
- 1803 Quahog Parasite Unknown (QPX) disease in northern quahogs (=hard clams)
- 1804 *Mercenaria mercenaria*. J. Shellfish Res. 26, 371–381.
- 1805 Lyons, M.M., Ward, J.E., Gaff, H., Hicks, R.E., Drake, J.M., Dobbs, F.C., 2010. Theory
- 1806 of island biogeography on a microscopic scale: organic aggregates as islands for
- 1807 aquatic pathogens. Aquat. Microb. Ecol. 60, 1–13.
- 1808 Lyons, M.M., Ward, J.E., Smolowitz, R., Uhlinger, K.R., Gast, R.J., 2005. Lethal marine
- 1809 snow: pathogen of bivalve mollusc concealed in marine aggregates. Limnol.
- 1810 Oceanogr. 50, 1983–1988.
- 1811 Lucas, M.I., Newell, R.C., Shumway, S.E., Seiderer, L.J., Bally, R., 1987. Particle
- 1812 clearance and yield in relation to bacterioplankton and suspended particulate
- 1813 availability in estuarine and open coast population of the mussel *Mytilus edulis*. Mar.
- 1814 Ecol. Prog. Ser. 36, 215–224.

- 1815 MacCready, P., Geyer, W.R., 2010. Advances in estuarine physics. *Ann. Rev. Mar. Sci.*
 1816 2, 35-58.
- 1817 Maes, P., n.d. Pathologie bactérienne chez deux invertébrés marins d'intérêt commercial,
 1818 *Ruditapes philippinarum* et *Paracentrotus lividus*. These de Doctorat, Université de
 1819 Brest.
- 1820 Maloy, A.P., Barber, B.J., Boettcher, K.J., 2007a. Use of the 16S-23S rDNA internal
 1821 transcribed spacer of *Roseovarius crassostreae* for epizootiological studies of
 1822 juvenile oyster disease (JOD). *Dis. Aquat. Org.* 76, 151–161.
- 1823 Maloy, A.P., Ford, S.E., Karney, R.C., Boettcher, K.J., 2007b. *Roseovarius crassostreae*,
 1824 the etiological agent of Juvenile Oyster Disease (now to be known as *Roseovarius*
 1825 Oyster Disease) in *Crassostrea virginica*. *Aquaculture* 269, 71–83.
- 1826 Mann, R., Harding, J.M., Southworth, M.J., 2009. Reconstructing pre-colonial oyster
 1827 demographics in the Chesapeake Bay, USA. *Estuar. Coast. Shelf S.* 85, 217–222.
- 1828 Mann, R., Powell, E.N., 2007. Why oyster restoration goals in the Chesapeake Bay are
 1829 not and probably cannot be achieved. *J. Shellfish Res.* 26, 905–917.
- 1830 Marranger, R., Bird, D.F., 1995. Viral abundance in aquatic systems: a comparison
 1831 between marine and fresh waters. *Mar. Ecol. Prog. Ser.* 121, 217–226.
- 1832 Martenot, C., Fourour, S., Oden, E., Jouaux, A., Travaillé, E., Malas, J.P., Houssin, M.,
 1833 2012. Detection of the OsHV-1 μ Var in the Pacific oyster *Crassostrea gigas* before
 1834 2008 in France and description of two new microvariants of the Ostreid Herpesvirus
 1835 1 (OsHV-1). *Aquaculture* 338-341, 293–296.
- 1836 Martenot, C., Oden, E., Travaillé, E., Malas, J.-P., Houssin, M., 2011. Detection of
 1837 different variants of Ostreid Herpesvirus 1 in the Pacific oyster, *Crassostrea gigas*

- 1838 between 2008 and 2010. *Virus Res.* 160, 25–31.
- 1839 Martin, L.B., Weil, Z.M., Nelson, R.J., 2006. Refining approaches and diversifying
1840 directions in ecoimmunology. *Integr. Comp. Biol.* 46, 1030–1039.
- 1841 Martinez-Manzanares, E., Castro, D., Navas, J.I., Lopez-Cortes, L., Borrego, J.J., 1998.
1842 Transmission routes and treatment of brown ring disease affecting Manila clams
1843 (*Tapes philippinarum*). *J. Shellfish Res.* 17, 1051–1056.
- 1844 Mathias, C.B., Kirschner, A.K.T., Velimirov, B., 1995. Seasonal variations of virus
1845 abundance and viral control of the bacterial production in a backwater system of the
1846 Danube River. *Appl. Environ. Microb.* 61, 3734–3740.
- 1847 McCullough, C.B., Albright, B.W., Abbe, G.R., Barker, L.S., Dungan, C.F., 2007.
1848 Acquisition and progression of *Perkinsus marinus* infections by specific-pathogen-
1849 free juvenile oysters (*Crassostrea virginica* Gmelin) in a mesohaline Chesapeake
1850 Bay tributary. *J. Shellfish Res.* 26, 465–477.
- 1851 McHenry, J., Allen, J., Birkbeck, T.H., 1986. Distribution of lysozyme-like activity in 30
1852 bivalve species. *Comp. Biochem. Phys. B* 85, 581–584.
- 1853 Mcleod, C., Hay, B., Grant, C., Greening, G., Day, D., 2009. Localization of norovirus
1854 and poliovirus in Pacific oysters. *J. Appl. Microbiol.* 106, 1220–1230.
- 1855 Mehle, N., Ravnkar, M., 2012. Plant viruses in aqueous environment - survival, water
1856 mediated transmission and detection. *Water Res.* 46, 4902–4917.
- 1857 Mele, A., Rastelli, M., Gill, O., Di Biscelglie, D., Rosmini, F., Pardelli, G., Valtriani, C.,
1858 Patriarchi, P., 1989. Recurrent epidemic hepatitis A associated with consumption of
1859 raw shellfish, probably controlled through public health measures. *Am. J. Epidemiol.*
1860 130, 540–546.

- 1861 Meyers, T.R., 1979. A reo-like virus isolated from juvenile American oysters
 1862 (*Crassostrea virginica*). J. Gen. Virol. 43, 203–212.
- 1863 Meyers, T.R., 1984. Marine bivalve mollusks as reservoirs of viral finfish pathogens:
 1864 significance to marine and anadromous finfish aquaculture. Mar. Fish Rev. 46, 14–
 1865 17.
- 1866 Montes, J., Anadon, R., Azevedo, C., 1994. A possible life cycle for *Bonamia ostreae* on
 1867 the basis of electron microscopy studies. J. Invertebr. Pathol. 63, 1–6.
- 1868 Morse, D.L., Guzewich, J.J., Hanrahan, J.P., Stricof, R., Shayegani, M., Deibel, R.,
 1869 Grabau, J.C., Nowak, N.A., Hermann, J.E., Cukor, G., Blacklow, N.R., 1986.
 1870 Widespread outbreaks of clam- and oyster-associated gastroenteritis: role of Norwalk
 1871 virus. New Engl. J. Med. 314, 678–681.
- 1872 Mouritsen, K.N., Poulin, R., 2002. Parasitism, community structure and biodiversity in
 1873 intertidal ecosystems. Parasitology 124, S101–S117.
- 1874 Mouritsen, K.N., Poulin, R., 2003. The mud flat anemone-cockle association: mutualism
 1875 in the intertidal zone? Oecologia 135, 131–137.
- 1876 Muehlstein, L.K., Porter, D., T, S.F., 1988. *Labyrinthula* sp., a marine slime mold
 1877 producing the symptoms of wasting diseases in eelgrass, *Zostera marina*. Mar Biol
 1878 99, 465–472.
- 1879 Munoz, P., Vance, K., Gomez-Chiarri, M., 2003. Protease activity in the plasma of
 1880 American oysters, *Crassostrea virginica*, experimentally infected with the protozoan
 1881 parasite *Perkinsus marinus*. J. Parasitol. 89, 941–951.
- 1882 Narváez, D.A., Klinck, J.M., Powell, E.N., Hofmann, E.E., Wilkin, J., Haidvogel, D.B.,
 1883 2012. Circulation and behavior controls on dispersal of eastern oyster *Crassostrea*

- 1884 virginica larvae in Delaware Bay. J. Mar. Res. 70, 411-440.
- 1885 Newell, R.I.E., 2004. Ecosystem influences of natural and cultivated populations of
- 1886 suspension-feeding bivalve molluscs: a review. J. Shellfish Res. 23, 51–61.
- 1887 Nguyen, L., Pieters, J., 2005. The Trojan horse: survival tactics of pathogenic
- 1888 mycobacteria in macrophages. Trends Cell Biol. 15, 269–276.
- 1889 Novoa, B., Figueras, A., 2000. Virus-like particles associated with mortalities of the
- 1890 carpet-shell clam *Ruditapes decussatus*. Dis. Aquat. Org. 39, 147–149.
- 1891 Nyholm, S.V., Stabb, E.V., Ruby, E.G., McFall-Ngai, M.J., 2000. Establishment of an
- 1892 animal–bacterial association: recruiting symbiotic vibrios from the environment. P
- 1893 Natl. Acad. Sci. USA 97, 10231–10235.
- 1894 Oprandy, J.J., Chang, P.W., Pronovost, A.D., Cooper, K.R., Brown, R.S., Yates, V.J.,
- 1895 1981. Isolation of a viral agent causing hematopoietic neoplasia in the soft shell clam
- 1896 *Mya arenaria*. J. Invertebr. Pathol. 38, 45–51.
- 1897 Paillard, C., 2004. A short-review of brown ring disease, a vibriosis affecting clams,
- 1898 *Ruditapes philippinarum* and *Ruditapes decussatus*. Aquat. Living Resour. 17, 467–
- 1899 475.
- 1900 Paillard, C., Jean, F., Ford, S.E., Powell, E.N., Klinck, J.M., Hofmann, E.E., Flye-Sainte-
- 1901 Marie, J., 2014. A theoretical individual-based model of Brown Ring Disease in
- 1902 Manila clams, *Venerupis philippinarum*. J. Sea Res. 91, 15–34.
- 1903 Paillard, C., Le Roux, F., Borrego, J.J., 2004. Bacterial disease in marine bivalves, a
- 1904 review of recent studies: trends and evolution. Aquat. Living Resour. 17, 477–498.
- 1905 Paillard, C., Maes, P., 1994. Brown ring disease in the Manila clam *Ruditapes*
- 1906 *philippinarum*: establishment of a classification system. Dis. Aquat. Org. 19, 137–

- 1907 146.
- 1908 Paillard, C., Maes, P., Oubella, R., 1994. Brown ring disease in clams. *Annu. Rev. Fish*
- 1909 *Dis.* 4, 219–240.
- 1910 Pales Espinosa, E., Winnicki, S., Allam, B., 2013. Early host–pathogen interactions in a
- 1911 marine bivalve: *Crassostrea virginica* pallial mucus modulates *Perkinsus marinus*
- 1912 growth and virulence. *Dis. Aquat. Org.* 104, 237–247.
- 1913 Pedrós-Alió, C., 2006. Marine microbial diversity: can it be determined? *Trends*
- 1914 *Microbiol.* 14, 257–263.
- 1915 Percher, W., Alavi, M.R., Schott, E.J., Fernández Robledo, J.A., Roth, L., Berg, S.T.,
- 1916 Vasta, G.R., 2008. Assessment of the northern distribution range of selected
- 1917 *Perkinsus* species in eastern oysters (*Crassostrea virginica*) and hard clams
- 1918 (*Mercenaria mercenaria*). *J. Parasitol.* 94, 410–422.
- 1919 Perkins, F., 1988. Structure of protistan parasites found in bivalve molluscs. *Am. Fish.*
- 1920 *Soc. Spec. Publ.* 18, 93–111.
- 1921 Perkins, F., 1996. The structure of *Perkinsus marinus* (Mackin, Owen and Collier, 1950)
- 1922 Levinem, 1978 with comments on taxonomy and phylogeny of *Perkinsus* spp. *J.*
- 1923 *Shellfish Res.* 15, 67–87.
- 1924 Pernet, F., Barret, J., Le Gall, P., Corporeau, C., Dégremont, L., Lagarde, F., Pepin, J.F.,
- 1925 Keck, N., 2012. Mass mortalities of Pacific oysters *Crassostrea gigas* reflect
- 1926 infectious diseases and vary with farming practices in the Mediterranean Thau
- 1927 lagoon, France. *Aquacult. Environ. Interact.* 2, 215–237.
- 1928 Pernet, F., Lagarde, F., Le Gall, P., Roque D’Orbcastel, E., 2014. Associations between
- 1929 farming practices and disease mortality of Pacific oyster *Crassostrea gigas* in a

- 1930 Mediterranean lagoon. *Aquacult. Environ. Interact.* 5, 99-106.
- 1931 Pernet, F., Tamayo, D., Petton, B., 2015. Influence of low temperature on the survival of
 1932 the Pacific oyster (*Crassostrea gigas*) infected with Ostreid herpes virus type 1.
 1933 *Aquaculture* 445, 57-62.
- 1934 Perrigault, M., Buggé, D.M., Allam, B., 2010. Effect of environmental factors on survival
 1935 and growth of quahog parasite unknown (QPX) *in vitro*. *J. Invertebr. Pathol.* 104, 83–
 1936 89.
- 1937 Perrigault, M., Dahl, S.F., Espinosa, E.P., Gambino, L., Allam, B., 2011. Effects of
 1938 temperature on hard clam (*Mercenaria mercenaria*) immunity and QPX (Quahog
 1939 Parasite Unknown) disease development: II. Defense parameters. *J. Invertebr. Pathol.*
 1940 106, 322–332.
- 1941 Petton, B., Pernet, F., Robert, R., Boudry, P., 2013. Temperature influence on pathogen
 1942 transmission and subsequent mortalities in juvenile Pacific oysters *Crassostrea gigas*.
 1943 *Aquac. Environ. Interact.* 3, 257-273.
- 1944 Petton, B., Boudry, P., Alunno-Bruscia, M., Pernet, F., 2015. Factors influencing disease-
 1945 induced mortality of Pacific oysters *Crassostrea gigas*. *Aquac. Environ. Interact.* 6,
 1946 205-222.
- 1947 Poranen, M.M., Daugelavičius, R., Bamford, D.H., 2002. Common principles in viral
 1948 entry. *Annu. Rev. Microbiol.* 56, 521–538.
- 1949 Potasman, I., Paz, A., Odeh, M., 2002. Infectious outbreaks associated with bivalve
 1950 shellfish consumption: a worldwide perspective. *Clin. Infect. Dis.* 35, 921–928.
- 1951 Poulin, R., Morand, S., 2000. The diversity of parasites. *Q. Rev. Biol.* 75, 277–293.
- 1952 Powell, A., Baker-Austin, C., Wagley, S., Bayley, A., Hartnell, R., 2013. Isolation of

- 1953 pandemic *Vibrio parahaemolyticus* from UK Water and shellfish produce. Microb
- 1954 Ecol 65, 924–927.
- 1955 Power, U.F., Collins, J.K., 1989. Differential depuration of poliovirus, *Escherichia coli*,
- 1956 and a coliphage by the common mussel, *Mytilus edulis*. Appl. Environ. Microb. 55,
- 1957 1386–1390.
- 1958 Power, U.F., Collins, J.K., 1990. Tissue distribution of a coliphage and *Escherichia coli*
- 1959 in mussels after contamination and depuration. Appl. Environ. Microb. 56, 803–807.
- 1960 Prins, T.C., Smaal, A.C., Dame, R.F., 1998. A review of the feedbacks between bivalve
- 1961 grazing and ecosystem processes. Aquat. Ecol. 31, 349–359.
- 1962 Provost, K., Dancho, B.A., Ozbay, G., Anderson, R.S., Richards, G.P., Kingsley, D.H.,
- 1963 2011. Hemocytes are sites of enteric virus persistence within oysters. Appl. Environ.
- 1964 Microb. 77, 8360–8369.
- 1965 Pruzzo, C., Gallo, G., Canesi, L., 2005. Persistence of vibrios in marine bivalves: the role
- 1966 of interactions with haemolymph components. Environ. Microbiol. 7, 761–772.
- 1967 Pujalte, M.J., Ortigosa, M., Macian, M.C., Garay, E., 1999. Aerobic and facultative
- 1968 anaerobic heterotrophic bacteria associated to Mediterranean oysters and seawater.
- 1969 Internatl. Microbiol. 2, 259–266.
- 1970 Ragan, M.A., MacCallum, G.S., Murphy, C.A., Cannone, J.J., Gutell, R.R., McGladdery,
- 1971 S.E., 2000. Protistan parasite QPX of hard-shell clam *Mercenaria mercenaria* is a
- 1972 member of Labyrinthulomycota. Dis. Aquat. Org. 42, 185–190.
- 1973 Raghukumar, S., Damare, V.S., 2011. Increasing evidence for the important role of
- 1974 Labyrinthulomycetes in marine ecosystems. Bot. Mar. 54, 3–11.
- 1975 Ragone Calvo, L.M., Dungan, C.F., Robertson, B.S., Burreson, E.M., 2003. Systematic

- 1976 evaluation of factors controlling *Perkinsus marinus* transmission dynamics in lower
- 1977 Chesapeake Bay. Dis. Aquat. Org. 56, 75–86.
- 1978 Ragone Calvo, L.M., Walker, J.G., Burreson, E.M., 1998. Prevalence and distribution of
- 1979 QPX, Quahog Parasite Unknown, in hard clams *Mercenaria mercenaria* in Virginia,
- 1980 USA. Dis. Aquat. Org. 33, 209–219.
- 1981 Randolph, S.E., Dobson, A., 2012. Pangloss revisited: a critique of the dilution effect and
- 1982 the biodiversity-buffers-disease paradigm. Parasitology 139, 847–863.
- 1983 Reece, K.S., Dungan, C.F., Burreson, E.M., 2008. Molecular epizootiology of *Perkinsus*
- 1984 *marinus* and *P. chesapeaki* infections among wild oysters and clams in Chesapeake
- 1985 Bay, USA. Dis. Aquat. Org. 82, 237–248.
- 1986 Reid, H.I., Duncan, H.L., Laidler, L.A., Hunter, D., Birkbeck, T.H., 2003. Isolation of
- 1987 *Vibrio tapetis* from cultivated Atlantic halibut (*Hippoglossus hippoglossus* L.).
- 1988 Aquaculture 221, 65–74.
- 1989 Renault, T., Bouquet, A.L., Maurice, J.T., Lupo, C., Blachier, P., 2014. Ostreid
- 1990 herpesvirus 1 Infection among Pacific oyster (*Crassostrea gigas*) spat: relevance of
- 1991 water temperature to virus replication and circulation prior to the onset of mortality.
- 1992 Appl. Environ. Microb. 80, 5419–5426.
- 1993 Renault, T., Lipart, C., Arzul, I., 2001. A herpes-like virus infects a non-ostreid bivalve
- 1994 species: virus replication in *Ruditapes philippinarum* larvae. Dis. Aquat. Org. 45, 1–
- 1995 7.
- 1996 Renault, T., Novoa, B., 2004. Viruses infecting bivalve molluscs. Aquat. Living Resour.
- 1997 17, 397–409.
- 1998 Renault, T.C., 2008. Genomics and mollusc pathogens: trends and perspective. J. Vet.

- 1999 Clin. Sci. 1, 4–14.
- 2000 Renault, T.C., Arzul, I., 2001. Herpes-like virus infections in hatchery-reared bivalve
- 2001 larvae in Europe: specific viral detection by PCR. J Fish Diseases 24, 161–167.
- 2002 Richards, G.P., McLeod, C., Le Guyader, F.S., 2010. Processing strategies to inactivate
- 2003 enteric viruses in shellfish. Food Environ Virol 2, 183–193.
- 2004 Riisgard, H.U., Larsen, P.S., 1995. Filter-feeding in marine macro-invertebrates: pump
- 2005 characteristics, modeling and energy cost. Biol. Rev. 70, 67–106.
- 2006 Rippey, S.R., 1994. Infectious diseases associated with molluscan shellfish consumption.
- 2007 Clin. Microbiol. Rev. 7, 419–425.
- 2008 Robertson, L.J., 2007. The potential for marine bivalve shellfish to act as transmission
- 2009 vehicles for outbreaks of protozoan infections in humans: a review. Int. J. Food
- 2010 Microbiol. 120, 201–216.
- 2011 Rodrigues, S., Paillard, C., Dufour, A., Bazire, A., 2014. Antibiofilm activity of the
- 2012 marine bacterium *Pseudoalteromonas* sp. 3J6 against *Vibrio tapetis*, the causative
- 2013 Agent of Brown Ring Disease. Probiotics & Antimicro. Prot. 7, 45–51.
- 2014 Rodriguez, F., Navas, J.I., 1995. A comparison of gill and hemolymph assays for the
- 2015 thioglycollate diagnosis of *Perkinsis atlanticus* (Apicomplexa, Perkinsea) in clams,
- 2016 *Ruditapes decussatus* (L.) and *Ruditapes philippinarum* (Adams et Reeve).
- 2017 Aquaculture 132, 145–152.
- 2018 Romalde, J.L., Luz Vilariño, M., Beaz, R., Rodríguez, J.M., Díaz, S., Villalba, A.,
- 2019 Carballal, M.J., 2007. Evidence of retroviral etiology for disseminated neoplasia in
- 2020 cockles (*Cerastoderma edule*). J. Invertebr. Pathol. 94, 95–101.
- 2021 Romero, J., Garcia-Varela, M., Laclette, J.P., Espejo, R.T., 2002. Bacterial 16S rRNA

- 2022 gene analysis revealed that bacteria related to *Arcobacter* spp. constitute an Abundant
 2023 and common component of the oyster microbiota (*Tiostrea chilensis*). Microb Ecol
 2024 44, 365–371.
- 2025 Roubal, F.R., Masel, J., Lester, R.J.G., 1989. Studies on *Marteilia sydneyi*, agent of QX
 2026 disease in the Sydney rock oyster, *Saccostrea commercialis*, with implications for its
 2027 life cycle. Aus. J. Mar. Fresh Res. 40, 155–167.
- 2028 Roy, S., Wainberg, M.A., 1988. Role of the mononuclear phagocyte system in the
 2029 development of acquired immunodeficiency syndrome (AIC). J. Leukocyte Biol. 43,
 2030 91–97.
- 2031 Rubenstein, D.I., Koehl, M.A.R., 1977. Mechanisms of filter-feeding: some theoretical
 2032 considerations. Am Nat 111, 981–994.
- 2033 Sainz-Hernandez, J.C., Maeda-Martinez, A.N., 2005. Sources of *Vibrio* bacteria in
 2034 mollusc hatcheries and control methods: a case study. Aquac Research 36, 1611–
 2035 1618.
- 2036 Sandifer, P.A., Sutton-Grier, A.E., 2014. Connecting stressors, ocean ecosystem services,
 2037 and human health. Nat Resour Forum 38, 157–167.
- 2038 Sauvage, C., Pépin, J.-F., Lapègue, S., Boudry, P., Renault, T., 2009. Ostreid herpes virus
 2039 1 infection in families of the Pacific oyster, *Crassostrea gigas*, during a summer
 2040 mortality outbreak: Differences in viral DNA detection and quantification using real-
 2041 time PCR. Virus Res. 142, 181–187.
- 2042 Savin, K.W., Cocks, B.G., Wong, F., Sawbridge, T., Cogan, N., Savage, D., Warner, S.,
 2043 2010. A neurotropic herpesvirus infecting the gastropod, abalone, shares ancestry
 2044 with oyster herpesvirus and a herpesvirus associated with the amphioxus genome.

- 2045 Virology 7, 308.
- 2046 Scanlon, C., Ragone Calvo, L.M., Burrenson, E.M., 1997. The potential for transmission
2047 of *Perkinsus marinus* by faecal matter from the eastern oyster *Crassostrea virginica*.
2048 J. Shellfish Res. 16, 332.
- 2049 Schikorski, D., Faury, N., Pepin, J.F., Saulnier, D., Tourbiez, D., Renault, T., 2011.
2050 Experimental ostreid herpesvirus 1 infection of the Pacific oyster *Crassostrea gigas*:
2051 kinetics of virus DNA detection by q-PCR in seawater and in oyster samples. Virus
2052 Res. 155, 28–34.
- 2053 Schmader, K., 2001. Herpes zoster in older adults. Clin. Infect. Dis. 32, 1481–1486.
- 2054 Schmidt, K.A., Ostfeld, R.S., 2001. Biodiversity and the dilution effect in disease
2055 ecology. Ecology 82, 609–619.
- 2056 Schott, E.J., Vasta, G.R., 2003. The PmSOD1 gene of the protistan parasite *Perkinsus*
2057 *marinus* complements the *sod2Δ* mutant of *Saccharomyces cerevisiae*, and directs an
2058 iron superoxide dimutase to mitochondria. Mol. Biochem. Parasit. 126, 81–92.
- 2059 Segarra, A., Pépin, J.F., Arzul, I., Morga, B., Faury, N., Renault, T., 2010. Detection and
2060 description of a particular *Ostreid herpesvirus 1* genotype associated with massive
2061 mortality outbreaks of Pacific oysters, *Crassostrea gigas*, in France in 2008. Virus
2062 Res. 153, 92–99.
- 2063 Segarra, A., Mauduit, F., Faury, N., Trancart, S., Dégremont, L., Tourbiez, D., Haffner,
2064 P., Barbosa-Solomieu, V., Pépin, J.F., Travers, M.-A., Renault, T., 2014a. Dual
2065 transcriptomics of virus-host interactions: comparing two Pacific oyster families
2066 presenting contrasted susceptibility to Ostreid herpesvirus 1. BMC Genomics 15,
2067 580.

- 2068 Segarra, A., Faury, N., Pépin, J.F., Renault, T., 2014b. Transcriptomic study of 39
 2069 Ostreid herpesvirus 1 genes during an experimental infection. J Invertebr. Pathol.
 2070 119, 5-11.
- 2071 Segarra, A., Mauduit, F., Faury, N., Trancart, S., Dégremont, L., Toubiez, D., Haffner, P.,
 2072 Barbosa-Solomieu, V., Pépin, J.F., Travers, M.A., Renault, T., 2014c. Dual
 2073 transcriptomics of virus-host interactions: comparing two Pacific oyster families
 2074 presenting contrasting susceptibility to Ostreid herpesvirus 1. BMC Genomics 15,
 2075 580.
- 2076 Shimenta, J., Jumars, P.A., 1991. Physical mechanisms and rates of particle capture by
 2077 suspension-feeders. Oceanogr. Mar. Biol. Annu. Rev. 29, 191–257.
- 2078 Shumway, S.E., 1985. Particle selection, ingestion, and adsorption in filter-feeding
 2079 bivalves. J. Exp. Mar. Biol. Ecol. 91, 77–92.
- 2080 Silverman, H., Achberger, E.C., Lynn, J.W., Dietz, T.H., 1995. Filtration and utilization
 2081 of laboratory-cultured bacteria by *Dreissena polymorpha*, *Corbicula fluminea*, and
 2082 *Carunculina texasensis*. Biol. Bull. 189, 308-319.
- 2083 Simon, M., Grossart, H.-P., Schweitzer, B., Ploug, H., 2002. Microbial ecology of
 2084 organic aggregates in aquatic ecosystems. Aquat. Microb. Ecol. 28, 175–211.
- 2085 Sindermann, C.J., Rosenfield, A., 1967. Principal diseases of commercially important
 2086 marine bivalve mollusca and crustacea. Fish B-NOAA 66, 335–385.
- 2087 Smolowitz, R., Leavitt, D., Perkins, F., 1998. Observations of a protistan disease similar
 2088 to QPX in *Mercenaria mercenaria* (hard clams) from the coast of Massachusetts. J.
 2089 Invertebr. Pathol. 71, 9–25.
- 2090 Smyth, A.R., Thompson, S.P., Siporin, K.N., Gardner, W.S., McCarthy, M.J., Piehler,

- 2091 M.F., 2012. Assessing nitrogen dynamics throughout the estuarine landscape. *Estuar.*
 2092 *Coast.* 36, 44–55.
- 2093 Soniat, T., 1996. Epizootiology of *Perkinsus marinus* disease of eastern oysters in the
 2094 Gulf of Mexico. *J. Shellfish Res.* 15, 35–43.
- 2095 Soudant, P., Chu, F.-L.E., 2001. Lipid class and fatty acid composition of the protozoan
 2096 parasite of oysters, *Perkinsus marinus* cultivated in two different media. *J. Eukaryot.*
 2097 *Microbiol.* 48, 309–319.
- 2098 Soudant, P., Chu, F.-L.E., Volety, A.K., 2013. Host-parasite interactions: marine bivalve
 2099 molluscs and protozoan parasites, *Perkinsus* species. *J. Invertebr. Pathol.* 114, 196–
 2100 216.
- 2101 Stokes, N.A., Burreson, E.M., 2001. Differential diagnosis of mixed *Haplosporidium*
 2102 *nelsoni* infections in the western oyster, *Crassostrea virginica*, using DNA probes. *J.*
 2103 *Shellfish Res.* 20, 207–213.
- 2104 Stokes, N.A., Ragone Calvo, L.M., Reece, K.S., Burreson, E.M., 2002. Molecular
 2105 diagnostics, field validation, and phylogenetic analysis of Quahog Parasite Unknown
 2106 (QPX), a pathogen of the hard clam *Mercenaria mercenaria*. *Dis. Aquat. Org.* 52,
 2107 233–247.
- 2108 Sunila, I., Hamilton, R.M., Dungan, C.F., 2001. Ultrastructural characteristics of the *in*
 2109 *vitro* cell cycle of the protozoan pathogen of oysters *Perkinsus marinus*. *J. Eukaryot.*
 2110 *Microbiol.* 48, 348–361.
- 2111 Sunila, I., Karolus, J., Lang, E.P., Mroczka, M.E., Volk, J., 2000. Transmission of the
 2112 haplosporidian parasite MSX *Haplosporidium nelsoni* to the eastern oyster
 2113 *Crassostrea virginica* in an upweller system. *Dis. Aquat. Org.* 42, 152–155.

- 2114 Sunila, I., LaBanca, J., 2003. Apoptosis in the pathogenesis of infectious diseases of the
 2115 eastern oyster *Crassostrea virginica*. Dis. Aquat. Org. 56, 163–170.
- 2116 Suttle, C.A., 2005. Viruses in the sea. Nature 437, 356–361.
- 2117 Suttle, C.A., 2007. Marine viruses — major players in the global ecosystem. Nat. Rev.
 2118 Micro. 5, 801–812.
- 2119 Suttle, C.A., Chen, F., 1992. Mechanisms and rates of decay of marine viruses in
 2120 seawater. Appl. Environ. Microb. 58, 3721–3729.
- 2121 Swaddle, J.P., Calos, S.E., 2008. Increased avian diversity is associated with lower
 2122 incidence of human West Nile infection: observation of the dilution effect. PLoS
 2123 ONE 3, e2488.
- 2124 Taghon, G.L., Self, R.F., Jumars, P.A., 1978. Predicting particle selection by deposit
 2125 feeders: a model and its implications. Limnol. Oceanogr. 23, 752–759.
- 2126 Tasumi, S., Vasta, G.R., 2007. A galectin of unique domain organization from hemocytes
 2127 of the eastern oyster (*Crassostrea virginica*) Is a receptor for the protistan parasite
 2128 *Perkinsus marinus*. J. Immunol. 179, 3086–3098.
- 2129 Thieltges, D.W., Amundsen, P.-A., Hechinger, R.F., Johnson, P.T.J., Lafferty, K.D.,
 2130 Mouritsen, K.N., Preston, D.L., Reise, K., Zander, C.D., Poulin, R., 2013. Parasites
 2131 as prey in aquatic food webs: implications for predator infection and parasite
 2132 transmission. Oikos 122, 1473–1482.
- 2133 Thieltges, D.W., Bordalo, M.D., Caballero Hernandez, A., Prinz, K., Jensen, K.T., 2008.
 2134 Ambient fauna impairs parasite transmission in a marine parasite-host system.
 2135 Parasitology 135, 1111–1116.
- 2136 Thompson, R.M., Mouritsen, K.N., Poulin, R., 2005. Importance of parasites and their

- 2137 life cycle characteristics in determining the structure of a large marine food web. J.
 2138 Anim. Ecol. 74, 77–85.
- 2139 Thongchan, J., Bhoopong, P., Yingkajorn, M., Nishibuchi, M., Vuddhakul, V., 2013.
 2140 Total number, virulence genes, and heterogeneity of *Vibrio parahaemolyticus* in a
 2141 single shellfish. ScienceAsia 39, 230–235.
- 2142 Tsui, C.K.M., Marshall, W., Yokoyama, R., Honda, D., Lippmeier, J.C., Craven, K.D.,
 2143 Peterson, P.D., Berbee, M.L., 2009. Labyrinthulomycetes phylogeny and its
 2144 implications for the evolutionary loss of chloroplasts and gain of ectoplasmic gliding.
 2145 Mol. Phylogenet. Evol. 50, 129–140.
- 2146 Ueki, Y., Shoji, M., Suto, A., Tanabe, T., Okimura, Y., Kikuchi, Y., Saito, N., Sano, D.,
 2147 Omura, T., 2007. Persistence of caliciviruses in artificially contaminated oysters
 2148 during depuration. Appl. Environ. Microb. 73, 5698–5701.
- 2149 Valle Levinson, A., Wong, K., 2000. Fortnightly variability in the transverse dynamics of
 2150 a coastal plain estuary. J. Geophys. Res. 3413–3423.
- 2151 Vigneron, V., Sollicec, G., Montanie, H., Renault, T., 2004. Detection of Ostreid
 2152 Herpesvirus 1 (OsHV-1) DNA in seawater by PCR: influence of water parameters in
 2153 bioassays. Dis. Aquat. Org. 62, 35–44.
- 2154 Villalba, A., Casas, S.M., Lopez, C., Carballal, M.J., 2005. Study of perkinsosis in the
 2155 carpet shell clam *Tapes decussatus* in Galicia (NW Spain). II. Temporal pattern of
 2156 disease dynamics and association with clam mortality. Dis. Aquat. Org. 65, 257–267.
- 2157 Villalba, A., Reece, K.S., Camino Ordás, M., Casas, S.M., Figueras, A., 2004.
 2158 Perkinsosis in molluscs: a review. Aquat. Living Resour. 17, 411–432.
- 2159 Volety, A.K., Chu, F.-L.E., 1995. Suppression of chemiluminescence of eastern oyster

- 2160 (*Crassostrea virginica*) hemocytes by the protozoan parasite *Perkinsus marinus*.
 2161 Dev. Comp. Immunol. 19, 135–142.
- 2162 Wagner-Dobler, I., Biebl, H., 2006. Environmental biology of the marine Reseobacter
 2163 lineage. Annu. Rev. Microbiol. 60, 255–280.
- 2164 Ward, J.E., 1996. Biodynamics of suspension-feeding in adult bivalve molluscs: particle
 2165 capture, processing, and fate. Invertebr. Biol. 115, 218–231.
- 2166 Ward, J.E., Levinton, J.S., Shumway, S.E., Cucci, T., 1998. Particle sorting in bivalves:
 2167 *in vivo* determination of the pallial organs of selection. Mar Biol 131, 283–292.
- 2168 Ward, J.E., Shumway, S.E., 2004. Separating the grain from the chaff: particle selection
 2169 in suspension- and deposit-feeding bivalves. J. Exp. Mar. Biol. Ecol. 300, 83–130.
- 2170 Weinbauer, M.G., Bettarel, Y., Cattaneo, R., Luef, B., Maier, C., Motegi, C., Peduzzi, P.,
 2171 Mari, X., 2009. Viral ecology of organic and inorganic particles in aquatic systems:
 2172 avenues for further research. Aquat. Microb. Ecol. 57, 321–341.
- 2173 Wesche, S.J., Adlard, R.D., Lester, R.J.G., 1999. Survival of spores of the oyster
 2174 pathogen *Marteilia sydneyi* (Protozoa, Paramyxia) as assessed using fluorogenic
 2175 dyes. Dis. Aquat. Org. 36, 221–226.
- 2176 White, M.E., Powell, E.N., Ray, S.M., Wilson, E.A., 1987. Host-to-host transmission of
 2177 *Perkinsus marinus* in oyster *Crassostrea virginica* populations by the ectoparasitic
 2178 snail *Boonea impressa* (Pyramidellidae). J. Shellfish Res. 6, 1–5.
- 2179 Whittington, R.J., Hick, P.M., Evans, O., Rubio, A., Alford, B., Dhand, N., Paul-Pont, I.,
 2180 2015a. Protection of Pacific oyster (*Crassostrea gigas*) spat from mortality due to
 2181 Ostreid herpesvirus 1 (OsHV-1 μ var) using simple treatments of incoming seawater
 2182 in land-based upwellers. Aquaculture 437, 10–20.

- 2183 Whittington, R.J., Dhand, N.K., Evans, O., Paul-Pont, I., 2015b. Further observations on
 2184 the influence of husbandry practices on OsHV-1 μ Var mortality in Pacific oysters
 2185 *Crassostrea gigas*: age, cultivation structures and growing height. Aquaculture 438,
 2186 82–97.
- 2187 Wilcox, R.M., Fuhrman, J.A., 1994. Bacterial viruses in coastal seawater: lytic rather
 2188 than lysogenic production. Mar. Ecol. Prog. Ser. 114, 35–45.
- 2189 Willhelm, S.W., Weinbauer, M.G., Suttle, C.A., Pledger, R.J., Mitchell, D.L., 1998.
 2190 Measurements of DNA damage and photoreactivation imply that most viruses in
 2191 marine surface waters are infective. Aquat. Microb. Ecol. 14, 215–222.
- 2192 Wolf, K., Darlington, R.W., 1971. Channel catfish virus: a new herpesvirus of ictaurid
 2193 fish. J. Virol. 8, 525–533.
- 2194 Wommack, K.E., Colwell, R.R., 2000. Virioplankton: viruses in aquatic ecosystems.
 2195 Microbiol. Mol. Biol. R. 46, 69–114.
- 2196 Wommack, K.E., Hill, R.T., Muller, T., Colwell, R.R., 1996. Effects of sunlight on
 2197 bacteriophage viability and structure. Appl. Environ. Microb. 62, 1336–1341.
- 2198 Wong, K.-C., Gavine, R.W., 1984. Observations of wind-induced subtidal variability in
 2199 the Delaware estuary. J. Geophys. Res. 89, 10589–10597.
- 2200 Wong, K.C., 1994. On the nature of transverse variability in a coastal plain estuary. J.
 2201 Geophys. Res. 99, 14209–14222.
- 2202 Wood, C.L., Lafferty, K.D., 2013. Biodiversity and disease: a synthesis of ecological
 2203 perspectives on Lyme disease transmission. Trends Ecol. Evol. 28, 239–247.
- 2204 Wood, J.L., Andrews, J.D., 1962. *Haplosporidium costale* (Sporozoa) asociated with a
 2205 disease of Virginia oysters. Science 136, 710–711.

- 2206 Wootton, E.C., Dyrinda, E.A., Ratcliffe, N.A., 2003. Bivalve immunity: comparisons
 2207 between the marine mussel (*Mytilus edulis*), the edible cockle (*Cerastoderma edule*)
 2208 and the razor-shell (*Ensis siliqua*). Fish Shellfish Immun. 15, 195–210.
- 2209 Wright, A.C., Ahmed, H., Gauthier, J.D., Silva, A.M., Vasta, G.R., 2002. cDNA cloning
 2210 and characterization of two iron superoxide dismutases from the oyster parasite
 2211 *Perkinsus marinus*. Mol. Biochem. Parasit. 123, 73–77.
- 2212 Wyatt, R., Sodroski, J., 1998. The HIV-1 envelope glycoproteins: fusogens, antigens, and
 2213 immunogens. Science 280, 1884–1888.
- 2214 Yildiz, F.H., Visick, K.L., 2009. *Vibrio* biofilms: so much the same yet so different.
 2215 Trends Microbiol. 17, 109–118.
- 2216 Zhang, L., Li, L., Zhu, Y., Zhang, G., Guo, X., 2014. Transcriptome analysis reveals a
 2217 rich gene set related to innate immunity in the eastern oyster (*Crassostrea virginica*).
 2218 Mar Biotechnol. 16, 17–33.
- 2219 Zhang, L., Li, L., Guo, X., Litman, G.W., Dishaw, L.J., Zhang, G., 2015. Massive
 2220 expansion and functional divergence of innate immune genes in a protostome. Sci.
 2221 Rep. 5, 8693.
- 2222 Zlotkin, A., Chilmonczyk, S., Eyngor, M., Hurvitz, A., Ghittino, C., Eldar, A., 2003.
 2223 Trojan Horse Effect: phagocyte-mediated *Streptococcus iniae* Infection of fish. Infect.
 2224 Immun. 71, 2318–2325.

Table Caption

Table 1: Parasites that have impacted bivalve molluscs, by host species, mode of transmission, and key references selected by the author.

Figure Captions

Figure 1: A) Relative abundance of bacteria, protozoans, and viruses in coastal oceans (data from Cochlan et al. 1993, Maranger et al. 1995, and Suttle 2007). B) Relative reporting of bacterial, protozoan, and viral parasites of bivalve molluscs from a search of the SCOPUS database (<http://www.info.sciverse.com/scopus/>) of peer-reviewed articles published between 1950 until 2014 with titles or abstracts containing parasite and host string names presented in Table S1 (16,846 records). C) Relative reporting of bacterial, protozoan, and viral parasites of bivalve molluscs (same as above) but removing parasites infectious to human hosts from the search results (11,229 records).

Figure 2: Life cycle of Ostreid herpesvirus 1 (OsHV-1) in *Crassostrea gigas*.

Figure 3: Life cycle of *Vibrio tapetis* in *Ruditapes philippinarum*.

Figure 4: Life cycle of *Perkinsus marinus* in *Crassostrea virginica*.

Parasite	Host species	Mode of transmission	Reproduction outside hosts?	Key references
DNA Viruses				
Herpes-like viruses	<i>Crassostrea virginica</i> <i>Ostrea angasi</i> <i>Tiostrea chilensis</i>	Direct	No	Farley et al. (1979) Hine and Thorne (1997) Hine et al. (1998)
Ostreid herpesvirus 1 (OsHV-1)	<i>Crassostrea gigas</i> <i>Crassostrea angulata</i> <i>Crassostrea ariakensis</i> <i>Ostrea edulis</i> <i>Ruditapes philippinarum</i> <i>Pecten maximus</i>	Direct	No	Renault et al. (2000) Arzul et al. (2001a) Arzul et al. (2001b)
Papova-like viruses	<i>Crassostrea gigas</i>	Direct	No	Choi et al. (2004)
RNA Viruses				
Reo-like viruses	<i>Crassostrea virginica</i>	Direct	No	Meyers (1979)
Irido-like viruses	<i>Crassostrea gigas</i>	Direct	No	Elston and Wilkinson (1985)
Picorna-like viruses	<i>Mytilus edulis</i> <i>Perna canaliculis</i> <i>Ruditapes decussatus</i>	Direct	No	Rasmussen (1986) Jones et al. (1996) Novoa and Figueras (2000)
Retroviruses				
Retro-like viruses	<i>Mya arenaria</i>	Direct	No	Oprandy et al. (1981)
Bacteria				
<i>Vibrio tapetis</i>	<i>Ruditapes philippinarum</i>	Direct	Yes	Paillard et al. (1994) Borrego et al. (1996) Allam et al. (2000)
<i>Vibrio pectenicida</i>	<i>Pecten maximum</i>	Direct	Yes	Lambert et al. (1998) Lambert et al. (2001)
<i>Vibrio tubiashii</i>	<i>Crassostrea gigas</i> <i>Ostrea edulis</i> <i>Crassostrea sikamea</i> <i>Panope abrupta</i>	Direct	Yes	Hada et al. (1984) Elston et al. (2008)
<i>Vibrio aestuarianus</i> <i>Vibrio splendidus</i> <i>Vibrio harveyi</i>	<i>Crassostrea gigas</i>	Direct	Yes	Lacoste et al. (2001) Labreuche et al. (2006)
<i>Roseovarius crassostreae</i>	<i>Crassostrea virginica</i>	Direct	Unknown but likely	Ford and Borrero (2001) Maloy et al. (2007)
<i>Nocardia crassostreae</i>	<i>Crassostrea gigas</i> <i>Ostrea edulis</i> <i>Mytilus galloprovincialis</i>	Direct	Unknown but likely	Friedman et al. (1998) Eglesma et al. (2008) Carella et al. (2013)
Protozoans				
<i>Perkinsus chesapeaki</i>	<i>Cerastoderma edule</i> <i>Tagelus plebeius</i> <i>Mya arenaria</i> <i>Macoma baltica</i>	Direct	No	Burreson et al. (2005) Bushek et al. (2008)

Parasite	Host species	Mode of transmission	Reproduction outside hosts?	Key references
<i>Perkinsus marinus</i>	<i>Crassostrea virginica</i>	Direct	No	Perkins (1988) Perkins (1996)
<i>Perkinsus qugwadi</i>	<i>Patinopecten yessoensis</i>	Direct	Unknown but likely	Blackbourn et al. (1998)
<i>Perkinsus olseni</i>	<i>Austrovenus stutchburyi</i> <i>Venerupis pullastra</i> <i>Venerupis aurea</i> <i>Ruditapes decussatus</i> <i>Ruditapes philippinarum</i> <i>Paphies australis</i> <i>Crassostrea gigas</i> <i>Crassostrea ariakensis</i>	Direct	No	Goggin et al. (1989) Auzoux-Bordenave et al. (1995) Casas et al. (2002)
<i>Perkinsus mediterraneus</i>	<i>Ostrea edulis</i>	Direct	Unknown	Casas et al. (2004)
<i>Bonamia exitiosa</i>	<i>Ostrea angasi</i> <i>Ostrea chilensis</i>	Direct	Unknown	Berthe and Hine (2003)
<i>Bonamia ostreae</i>	<i>Ostrea edulis</i> <i>Ostrea angasi</i> <i>Ostrea puelchana</i> <i>Ostrea chilensis</i> <i>Ostrea denselammellosa</i> <i>Crassostrea ariakensis</i>	Direct	Unknown	Montes et al. (1994)
<i>Bonamia roughleyi</i>	<i>Saccostrea commercialis</i>	Direct	Unknown	Farley et al. (1988)
<i>Bonamia perspora</i>	<i>Ostreola equestris</i>	Unknown	Unknown	Carnegie et al. (2006)
<i>Mikrocytos mackini</i>	<i>Crassostrea gigas</i> <i>Crassostrea virginica</i> <i>Ostrea edulis</i> <i>Ostreola conchaphila</i>	Direct	Unknown	Bower et al. (1997)
<i>Marteilia sydneyi</i>	<i>Saccostrea commercialis</i>	Intermediate host likely	Unknown but unlikely	Roubal et al. (1989)
<i>Marteilia refringens</i>	<i>Ostrea edulis</i> <i>Ostrea angasi</i> <i>Ostrea puelchana</i> <i>Ostrea chilensis</i> <i>Mytilus edulis</i> <i>Mytilus galloprovincialis</i>	Intermediate host	Unknown but unlikely	Figueras and Montes (1988)
<i>Marteilia maurini</i>	<i>Mytilus galloprovincialis</i> <i>Mytilus edulis</i>	Intermediate host likely	Unknown but unlikely	Comps et al. (1982) Le Roux et al. (2001)
<i>Marteilia conchillia</i>	<i>Cerastoderma edule</i>	Intermediate host likely	Unknown but unlikely	Carrasco et al. (2013)
<i>Haplosporidium nelsoni</i>	<i>Crassostrea virginica</i> <i>Crassostrea gigas</i>	Intermediate host likely	Unknown but unlikely	Haskin et al. (1966)
<i>Haplosporidium costale</i>	<i>Crassostrea virginica</i>	Intermediate host likely	Unknown but likely	Wood and Andrews (1962)
QPX	<i>Mercenaria mercenaria</i>	Direct	Yes	Maas et al.(1999) Stokes et al. (2002)

Figure1

Figure2

Figure3

Figure4

Infective meronts and zoospores can survive in seawater for weeks or more

Parasite infects through the mantle as rejected pseudofeces

Vegetative multiplication within host

Infective meronts are continuously shed from live hosts and both meronts and hypnospores are released from decaying moribund hosts

Parasite transmission in bivalve hosts