

Parasite transmission through suspension feeding

Tal Ben-Horin ^{a,†}, Gorka Bidegain ^b, Lauren Huey ^a, Diego A. Narvaez ^c, David Bushek ^a

^a Haskin Shellfish Research Laboratory, Department of Marine and Coastal Sciences, Rutgers University, 6959 Miller Ave, Port Norris, NJ 08349, United States

^b Gulf Coast Research Laboratory, University of Southern Mississippi, 703 East Beach Dr, Ocean Springs, MS 39564, United States ^c Department of Aquatic Systems, Faculty of Environmental Sciences, Center EULA-Chile, Universidad de Concepción, Concepción, Chile

Abstract

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

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 (Pedrós-Alió, 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 and Larsen, 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; Chauvaud 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, 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., 1992), *Salmonella* (Greenwood et al., 1998), *Shigella* (Iwamoto et al., 2010) 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; 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 (Baldauf, 2003; Tsui et al., 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* (Grizel et al., 1988; Ragone Calvo et al., 2003;

Carnegie and Cochenec-Laureau, 2004; Villalba et al., 2004; Fernández Robledo et al., 2014; Soudant et al., 2013). 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; Fernández 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, 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, 1972; Comps and Cochenec, 1993; Hine et al., 1998; Arzul et al., 2001a,b,c; Renault et al., 2001; Renault and Arzul, 2001; Friedman et al., 2005; Burge et al., 2006) and Papovaviridae (Choi et al., 2004), RNA viruses such as Reoviridae (Meyers, 1979), Iridoviridae (Elston and Wilkinson, 1985), and Picornaviridae (Jones et al., 1996; Novoa and Figueras, 2000; Carballal, 2003), and retroviruses (Oprandy et al., 1981). As with many viruses infecting vertebrate hosts, most bivalve viruses are host-specific (Davison et al., 2005; Suttle, 2007), although this is not always the case (Arzul et al., 2001b,c). Herpes-like viruses infecting oysters have been classified as a member of the Herpesviridae family under the name Ostreid herpesvirus-1 (OsHV-1; Davison et al., 2005; Savin et al., 2010; Jouaux et al., 2013). The apparent lack of host specificity of OsHV-1 is unique among the Herpesviridae, and this herpesvirus has been found to infect

at least seven bivalve species across four genera (Arzul et al., 2001b,c) where putative hosts include *Crassostrea gigas*, *Crassostrea angulata*, *Crassostrea ariakensis*, *Ostrea edulis*, *Ruditapes philippinarum*, *Ruditapes decussatus*, and *Pecten maximus* (Renault et al., 2001; Arzul et al., 2001a,b; Lynch et al., 2012). In the case of OsHV-1, it is suspected that the virus uses common, conserved systems among bivalves to enter and establish within this diverse host community, having emerged as a consequence of selection arising from intensive aquaculture of multiple bivalve species (Arzul et al., 2001b,c). Further studies seem to indicate that OsHV-1 has the capacity to evolve rapidly, and an increase in mortalities of *C. gigas* linked with OsHV-1 infection along French coasts since 2008 has been associated with the detection of a virulent OsHV-1 variant, designated OsHV-1 Ivar (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, 2012).

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

Parasite	Host species	Mode of transmission	Reproduction outside hosts?	Key references
<i>DNA viruses</i>				
Herpes-like viruses	<i>Crassostrea virginica</i> <i>Ostrea angasi</i> <i>Tiostrea chilensis</i>	Direct	No	Farley (1972) 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. (2001) Arzul et al. (2001a) Arzul et al. (2001b)
Papova-like viruses	<i>Crassostrea gigas</i>	Direct	No	Choi et al. (2004)
<i>RNA viruses</i>				
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	Jones et al. (1996) Novoa and Figueras (2000)
<i>Retroviruses</i>				
Retro-like viruses	<i>Mya arenaria</i>	Direct	No	Oprandy et al. (1981)
<i>Bacteria</i>				
<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 maximus</i>	Direct	Yes	Lambert et al. (2001)
<i>Vibrio tubiashii</i>	<i>Crassostrea gigas</i> <i>Ostrea edulis</i> <i>Crassostrea sikamea</i> <i>Panope abrupta</i> <i>Crassostrea gigas</i>	Direct Direct	Yes 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. (2007a,b)
<i>Nocardia crassostreae</i>	<i>Crassostrea gigas</i> <i>Ostrea edulis</i> <i>Mytilus galloprovincialis</i>	Direct	Unknown but likely	Friedman et al. (1998) Carella et al. (2013)
<i>Protozoans</i>				
<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)
<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)

Table 1 (continued)

Parasite	Host species	Mode of transmission	Reproduction outside hosts?	Key references
<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	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 (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	Stokes et al. (2002)

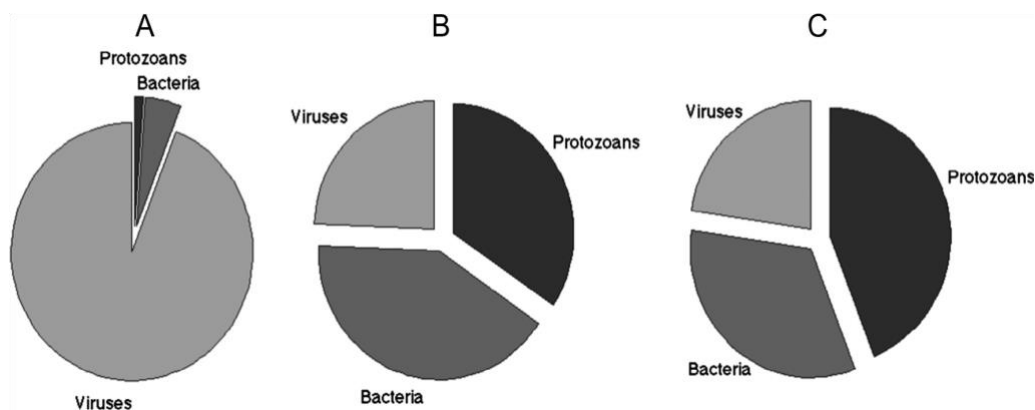


Fig. 1. (A) Relative abundance of bacteria, protozoans, and viruses in coastal oceans (data from Cochlan et al. (1993), Maranger and Bird, 1995 and Suttle (2007)). (B) Relative reporting of bacterial, protozoan, and viral parasites of bivalve molluscs from a search of the SCOPUS database (<http://www.info.sciverse.com/scopus/>) 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).

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 (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., 2007a,b), *Nocardia crassostreae*, the causal agent of Pacific Oyster Nocardiosis (Friedman and Hedrick, 1991; Friedman et al., 1998; Bower et al., 2005), and *Vibrio pectenicida* and *Vibrio tubiashii*, significant parasites of *P. maximus* and *C. gigas* larvae in hatcheries (Hada et al., 1984; 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 *R. philippinarum* in France, England, Ireland, Spain and Italy (Allam et al., 2000; Paillard, 2004). Signs of the characteristic brown deposit on the inner surface of the valve caused by colonization of *V. tapetis* have also been detected in a number of bivalves, including *R. decussatus*, *Venerupis aurea*, *Dosinia exoleta* and *P. 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, *V. tapetis* acts initially as an external microparasite and does not directly enter host tissues (Allam et al., 2001, 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, 2007a,b). Whittington et al. (2015a) observed that mortality of *C. 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 μ m. 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., *N. crassostreae*, and *R. crassostreae*, are common, though inconspicuous, members of coastal marine ecosystems (González 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 (González 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, 2007a,b), 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 and Stewart, 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, 2014; Elston et al., 2008; Beaz-Hidalgo et al., 2010), 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 *P. marinus*, *P. olseni*, *Marteilia sydneyi*, and *B. 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 and Porter, 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; Buggé 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 (Buggé 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; Buggé 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, 2008, 2010a,b; Arzul et al., 2009; 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 to 32 C and optimal salinity ranges from 24 to 36 ppt (Dungan and Hamilton, 1995; Gauthier and Vasta, 1995; La Peyre et al., 2010a,b). 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 *M. 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 been demonstrated to occur though, often in association with high river runoff (Narváez et al., 2012). Below average flows, on the other hand, allow particles to move further into upstream regions (Narváez et al., 2012). Short term changes in river flows can therefore be crucial for the transmission of parasites that remain infective in the water column often for weeks or more.

Other physical mechanisms, such as tides and winds, are equally important. Dispersion by tides is dependent on the size of the estuary relative to the tidal excursion length. In small bays and estuaries, or areas with abrupt changes in topography, the consequences of tidal dispersion can be significant (Geyer and Signell, 1992). In mid-latitude regions, ebb and flood tide velocities can range between 0.5 and 1 m s⁻¹ potentially transporting particles up to 4 km per hour (Valle Levinson and Wong, 2000). This estimate is supported somewhat by observations of new infections with *P. marinus* in disease-free oyster cohorts as early as ten days after placement in disease-enzootic waters of Chesapeake Bay. These new *P. marinus* infections occurred despite approximately 5 km of separation from naturally infected oyster reefs (McCollough et al., 2007). Wind patterns can also exert significant controls on estuary circulation, having both local and remote effects (Wong and Garvine, 1984). Local winds have an immediate effect on local circulation, but remote winds can also result in an elevation or depression of regional sea level, with cascading consequences on local circulation in estuaries (Wong, 1994). Furthermore, vertical mixing by winds can be important in the redistribution of particles in the water column, exposing displaced particles to different and often opposing 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 (Shimeta and Jumars, 1991; Chauvaud et al., 2000; Ward and Shumway, 2004). Combined with studies of nutrient assimilation and biodeposition (Newell, 2004; Kellogg et al., 2013; Smyth et al., 2012), 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 (Shimeta 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, 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; Wootton 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; Canesi et al., 2013; 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; Zhang et al., 2014, 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 500–600 nm apart. It has been suggested that viral particles can both become electrostatically bound to sulfates on the mucopolysaccharides of shellfish mucus (Di Girolamo 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, 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 *C. 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. More recent evidence from the dynamics of OsHV-1 infections within *C. 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., 2014a). 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., 2014a,b). 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., 1995) and with respect to the relative abundance of bacteria in the water column. Nevertheless, bivalves have demonstrated the ability to efficiently clear natural bacterioplankton assemblages in experimental systems (Lucas et al., 1987). *Mytilus edulis*, for example, have demonstrated the capacity to degrade bacteria at rates up to 10^9 cells h^{-1} (Birkbeck and McHenry, 1982). Lysozymes, in particular, play a central role in the digestion and defense against bacterial parasites due to the ability of these humoral defense factors to break down bacterial cell walls (McHenry et al., 1986; Bachali et al., 2002). The susceptibility of bacterial parasites to elimination is a product of the sensitivity of bacteria to humoral defense factors and the capability of hemocytes to bind to and encapsulate different bacteria, reflecting both the ability for bacteria to evade hemocytes as well as the sensitivity of bacteria to intracellular elimination (Chu, 1988; Allam et al., 2002; Bachali et al., 2002; Canesi et al., 2002). As a consequence, the pathogenicity of bacterial parasites is often directly associated with the capacity for these parasites to evade and avoid host defense factors (Allam et al., 2002; Canesi et al., 2002; Pruzzo et al., 2005). For example, the persistence of pathogenic vibrios in hemolymph depends largely on their sensitivity to the bactericidal activity of circulating hemocytes and soluble humoral factors

(reviewed by Pruzzo et al. (2005)). The pathogenic effects of *Vibrio splendidus*, the likely cause of summer mortalities of juvenile *C. gigas* in Brittany, has shown to result from the capacity for some strains to interfere with the signaling pathways involved in hemocyte function (Lacoste et al., 2001; Labreuche et al., 2006). *V. tapetis* avoids phagocytic encapsulation by impairing the adhesion properties of hemocytes (Choquet et al., 2003). The physiological state and immunity of hosts is also an important factor determining the capacity for parasites to evade defense factors and establish within hosts (Lacoste et al., 2002). *N. crassostreae* appears to enter oyster 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 *R. 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 et al., 1996; Paillard et al., 2004; Boettcher et al., 2000; Boardman et al., 2008). Roseovariouss *crassostreae*, for example, colonizes the inner shell surfaces of *C. 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 et al., 1996, 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 and Stewart, 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 and Stewart, 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, 2002; Paillard, 2004).

Some protozoan parasites, such as *B. 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 *C. virginica* and *B. ostreae* in *O. edulis*, as these parasites initially infect the gill epithelium before invading nearby connective tissues (Andrews, 1982; Montes et al., 1994; Sunila et al., 2000; Burreson 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 *M. 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., 1990; 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, 2010a,b; Volety and Chu, 1995; Kang et al., 2006; Tasumi and Vasta, 2007) 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). *P. marinus* can inhibit phagocytic processes (Anderson, 1999; Schott and Vasta, 2003), allowing this parasite to hijack circulating hemocytes for dissemination throughout host tissues by

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

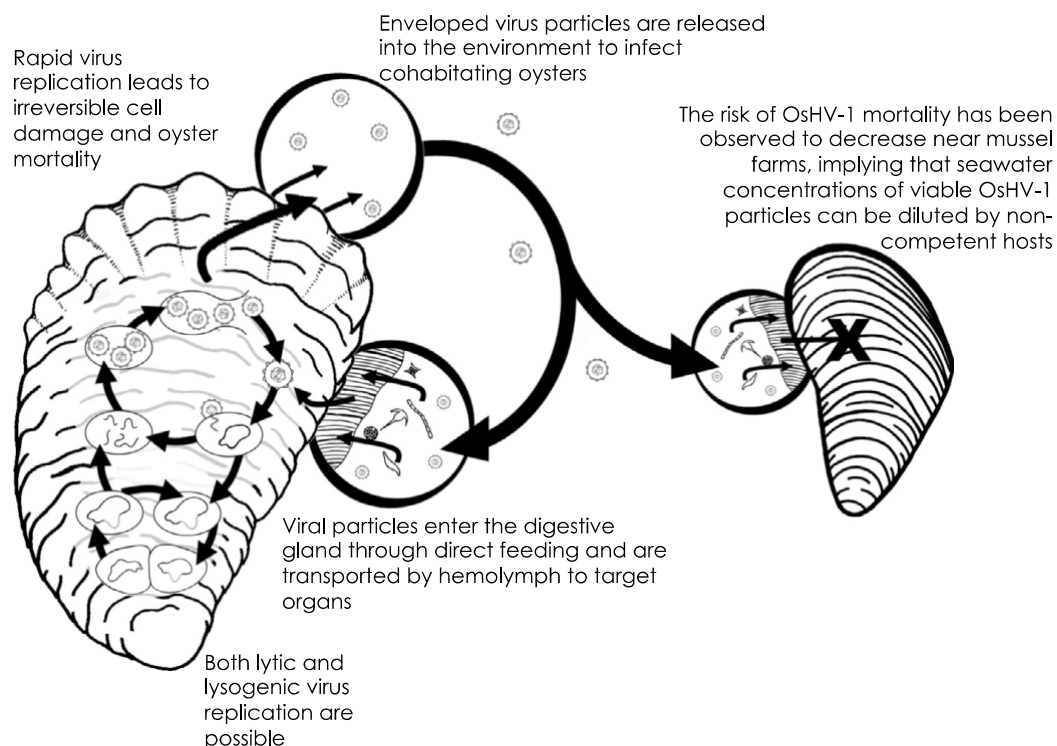


Fig. 2. Life cycle of Ostreid herpesvirus 1 (OsHV-1) in *Crassostrea gigas*

The concentration of OsHV-1 particles in the environment can be substantial during herpesvirus epizootics, reaching $6\text{--}7 \times 10^9$ 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 *N. crassostreae* have been associated with summer mortalities in wild populations of *C. gigas*, *O. edulis*, and *M. 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 and Hedrick, 1991). Since most terrestrial *Nocardia* are soil saprophytes, it is suggested that *N. 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 and Hedrick, 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, *N.*

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. *R. 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 *R. crassostreae* is known to be pathogenic (Boardman et al., 2008). After colonizing the inner shell surface of *C. 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).

R. 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 *R. crassostreae* (Maloy et al., 2007a,b). This result, combined with repeated summer epizootics, suggests that *R. 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 *V. 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 et al., 1996; Lopez-Cortes et al., 1999a; Paillard, 2004). Parasit 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, 2002; Lopez-Cortes et al., 1999a; 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). *V. 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).

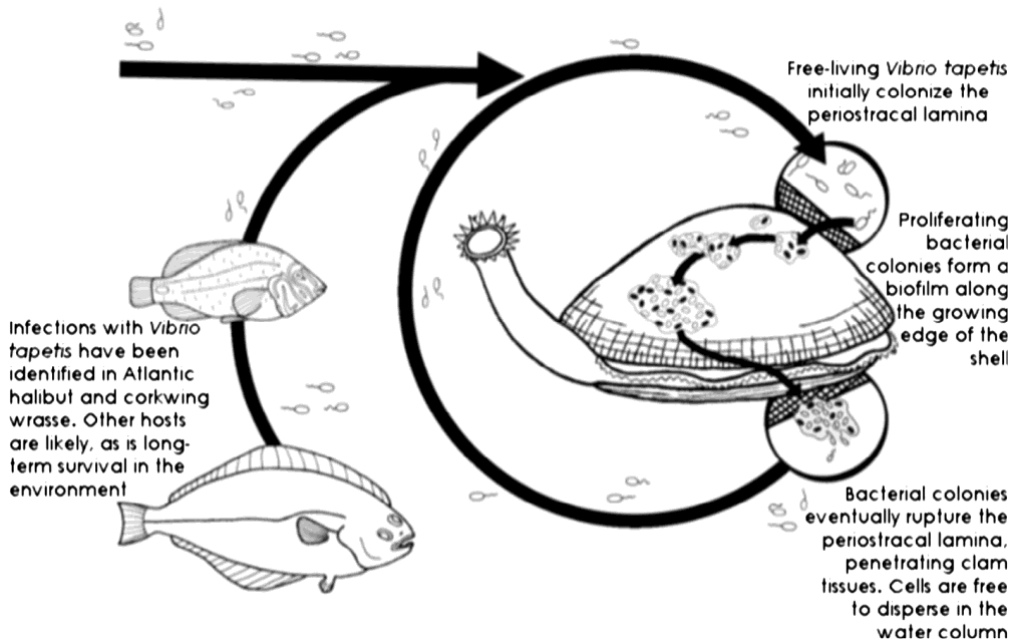


Fig. 3. Life cycle of *Vibrio tapetis* in *Ruditapes philippinarum*.

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 and Maes, 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 (Buggé 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 et al., 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., 1998) 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., 1998), which is unusual for labyrinthulomycetes (Kleinschuster et al., 1998). QPX thalli, endospores, and zoospores have demonstrated the ability to produce the ectoplasmic net however (Kleinschuster et al., 1998). This occurs when QPX cells are suspended in seawater and adhere to substrate, such as culture flasks (Kleinschuster et al., 1998). Vegetative reproduction occurs within hosts and in culture media, where growth and maturation of thalli results in the formation of sporangia containing many vegetative endospores. Endospores are released when sporangia are ruptured, and the endospores form into thalli, repeating the life cycle (Kleinschuster et al., 1998). In seawater, motile zoospores can also form from endospores and thalli; however, zoospores have not been identified in infected clam tissue (Smolowitz et al., 1998). Vegetative reproduction can commence when zoospores are placed in culture media though (Kleinschuster et al., 1998). These observations suggest that zoospores occur in seawater and can infect hosts but do not commonly occur in the internal host environment. These observations of the QPX life cycle in infected hosts and culture media suggest that QPX can alternate between vegetative reproduction within clam hosts and survival and growth on substrates outside clam hosts, which is supported by the detection of QPX in association with a wide range of environmental samples, including seawater, marine aggregates (Lyons et al., 2005), marsh grasses, macroalgae, gastropods, ascidians, amphibians, barnacles, polychaetes, annelids, and bivalves other than quahogs (Gast et al., 2008). Detection of QPX in association with environmental samples increases in regions where QPX disease in quahogs is prevalent, suggesting that alternating between saprotrophy and parasitism may increase the distribution and spread of this labyrinthulomycete. In the absence of quahog hosts, or when environmental conditions do not favor infections within these hosts, QPX is likely to remain a common 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, 2006; Goggin et al., 1989; Casas et al., 2002; Ragone Calvo et al., 2003; Villalba et al., 2004, 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, 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), where numerous zoospores form by multiple fission within the prezoosporangia cell wall (Sunila et al., 2001) and emerge through a single discharge tube (Azevedo et al., 1990; Casas et al., 2002). Prezoosporangia have also been observed in decaying tissues of moribund hosts, and begin zoosporulation when placed in seawater (Perkins, 1996; Casas et al., 2002). Zoosporulation following transfer to seawater or culture media has been observed in all *Perkinsus* spp. except for *P. qugwadi*. In the case of *P. qugwadi*, the development of *P. qugwadi* zoospores has been observed to occur only within the interstitial spaces of scallops infected by this parasite (Blackbourn et al., 1998; Bower et al., 1998). All life stages of *Perkinsus* spp. have been shown experimentally to cause infection in bivalves, and all species can be transmitted directly, without intermediate or secondary hosts. Viable *Perkinsus* spp. trophozoites are released into the water column from live infected bivalves through diapedesis and in feces (Scanlon et al., 1997; Bushek et al., 2002; Casas et al., 2002). Infected hosts appear to provide a continuous source of infectious stages to new hosts, which are exposed to these parasites through feeding processes (Fig. 4). Maximum rates of transmission of *P. marinus* in oysters appear to occur during periods of maximum mortality caused by this parasite, most often in the late summer and early autumn when infection intensities peak (Ragone Calvo et al., 2003). This observation suggests that prezoosporangia, forming in moribund hosts and releasing numerous zoospores into the water column, contribute substantially to *P. marinus* transmission. White et al. (1987) also demonstrated that *P. marinus* could be transmitted through hemolymph meals taken by the ectoparasitic 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 et al., 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 et al., 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, 2012).

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

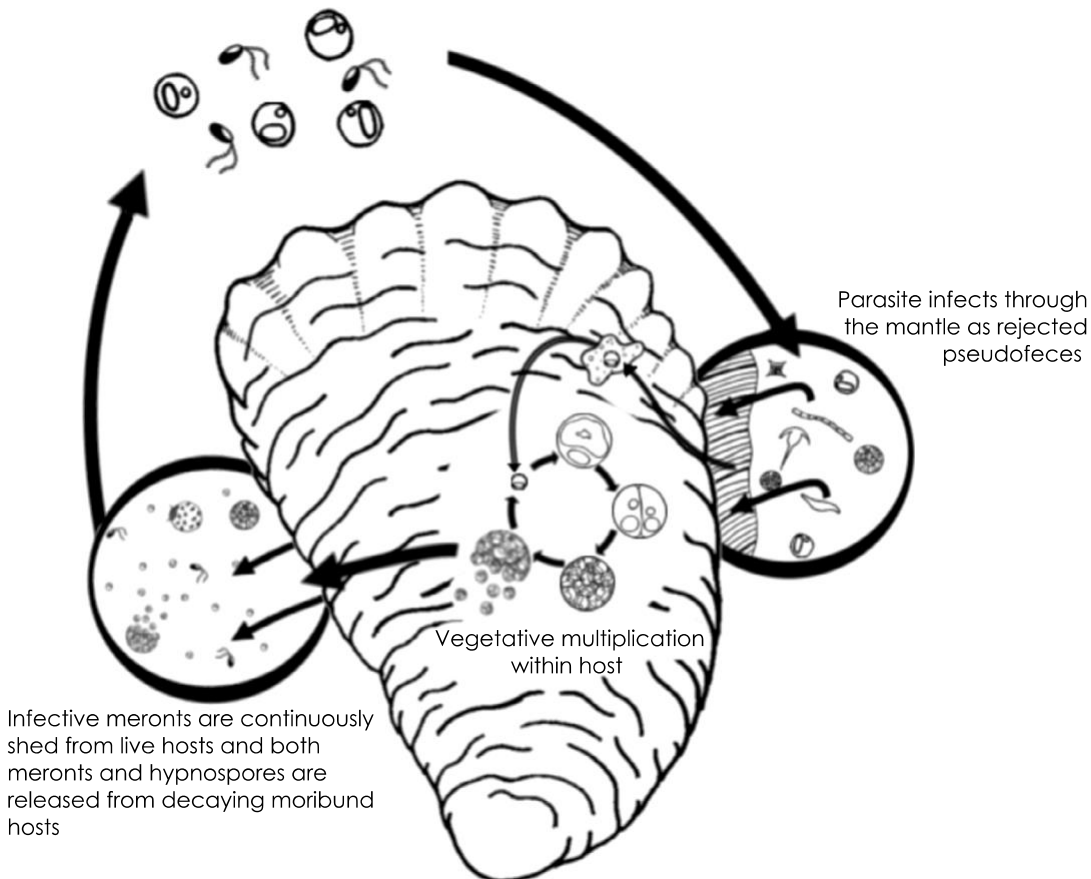


Fig. 4. Life cycle of *Perkinsus marinus* in *Crassostrea virginica*.

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 et al., 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. olseni* follow a similar annual cycle. Casas (2002) showed that the prevalence and intensity of *P. olseni* infection in *R. 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. olseni* 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 laevigata*) to *P. olseni* 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. olseni* 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 *B. ostreae*, *B. exitiosa*, *B. roughleyi*, and *M. 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 Cochenec-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 Cochenec-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 Cochenec-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; Cochenec-Laureau et al., 2003; Carnegie and Cochenec-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 Cochenec-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

Cochennec-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). *M. 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 *C. 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 et al., 1988; Hine, 1991; Hervio et al., 1995; Culloty et al., 1999; Carnegie and Cochennec-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 Cochennec-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 (Burrenson et al., 2000; 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. *H. nelsoni* has caused extensive mortalities in its *C. 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; Burrenson et al., 2000; Burrenson and Ford, 2004; Carnegie and Burrenson, 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), 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 temporal scales required for sufficient screening, and the limitations of tools available for detection (Haskin and Andrews, 1988; Audemard et al., 2001, 2002; Carrasco et al., 2007, 2008). As with the haplosporidian parasites *H. nelsoni* and *H. costale*, the paramyxean parasites of the genus *Marteilia* infect and sporulate in bivalve hosts, but cannot be transmitted directly between hosts, leading investigators to speculate that intermediate hosts are required to complete the transmission cycle (Grizel, 1979; Berthe et al., 1998). When working in semi-enclosed oyster ponds, locally known as ‘claires’ in Marennes-Oléron Bay, France, Berthe et al. (1998) observed new *M. refringens* infections in planted *O. edulis* even though no other *O. edulis* were present in the claire. Owing to their limited size and its consequences on environmental variables such as acute temperature variability

and seawater residence time, clear pond systems harbor fewer species than adjacent coastal bays and estuaries (Audemard et al., 2001), making them ideal experimental systems for detecting potential intermediate hosts. Audemard et al. (2002) systematically screened the entire fauna of the pond, finding that the copepod *Paracartia gralli* can serve as a host of *M. refringens*. Later surveys have confirmed that *Marteilia* parasites can be associated with zooplankton species in larger and more natural bays and estuaries (Carrasco et al., 2007). Within *O. edulis*, *M. refringens* develops from early stages in the epithelia of the upper digestive tract (Berthe et al., 1998). Sporulation takes place within the epithelium of the digestive gland tubules, releasing sporangia into the lumen of the digestive tract (Berthe et al., 1998). Sporulation is often associated with the destruction of the digestive gland epithelia and shedding of sporangia into the 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 μm), 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, *M. galloprovincialis* and *R. 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 *V. 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 *N. crassostreae* and *Vibrio* spp., and protozoan parasites such as QPX, can be common, though inconspicuous constituents of ambient marine microbial communities. These facultative parasites appear to adapt to parasitic lifestyles when conditions permit.
4. Some stages of protozoan parasites can survive for days, weeks, or even months in the water column, and can disperse quite far as passive particles, broadly distributing themselves throughout bivalve populations.
5. Many consumed parasites have adapted behaviors to evade direct digestion, instead targeting “safer” portals of entry such as pallial organs and pseudo-internal extrapallial spaces and peripheral compartments.
6. Parasites often target hemocytes for direct infection or dissemination through host tissues.
7. The outcome of interactions between bivalve hosts and their parasites is regulated by intrinsic host factors such as innate immunity and physiology, parasite factors such as virulence, and the interaction of these two factors with environmental variables such as temperature and salinity.
8. Many bivalve parasites, including OsHV-1, *V. tubiashii*, *P. olsenii*, *B. ostreae*, *M. mackini*, and *M. refringens*, have adapted to infect multiple hosts. The susceptibility and tolerance of hosts to parasitic infection can vary dramatically by species.

The implications of the final conclusion remain open, and should drive future ecological studies of parasites infecting bivalve hosts. A community of bivalves offers parasites options for host competence. This point is exemplified by the observations of Boyer et al. (2013) in Thau lagoon. There, *M. refringens* infects *M. galloprovincialis*, but occurs only in digestive epithelia of *R. decussatus* as necrotic cells. This parasite has been observed to infect a diversity of bivalve hosts, including *O. edulis*, *O. angasi*, *O. puelchana*, *M. galloprovincialis*, and *M. edulis* (Figueras and Montes, 1988; Le Roux et al., 2001) (Table 1). A diversity of host species, each plausibly offering parasites a range of competency, represents the resources supporting a continuum of outcomes for parasites, from immediate parasite mortality to successful reproduction and spread through host populations. This diversity is not limited to competent hosts. Once released into the environment, free-living parasite stages encounter a multitude of factors that impact their survival and infection success. While associations with abiotic factors such as water temperature and salinity have received much attention here and elsewhere, little is known about the role of ambient fauna. This reductionist approach is often intentional, as much of what we know about the transmission of bivalve parasites is based on experimental systems or models in which hosts and parasites are considered in an ecological vacuum. In reality, hosts exist within diverse ecological communities, and ecological interactions undoubtedly influence the transmission and impact of parasites (Dobson, 2004; Hall

et al., 2007). Pernet et al. (2014), for example, found the risk of OsHV-1 mortality to decrease in *C. gigas* when oysters were held in the vicinity of mussel farms. The observation by Pernet et al. (2014) suggests that nonfocal suspension feeders can dilute the abundance of suspended particles in the water column, minimizing the impact of suspended parasites on their focal hosts. The importance of host diversity has become apparent in a number of case studies of bivalve hosts impacted by trematode parasites. Studying infections with the larval trematode *Curtuteria australis* in the 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 *C. 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 elongata* 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; LoGiudice et al., 2003), West Nile virus (Swaddle and Calos, 2008; Allan et al., 2008), 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.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jip.2015.07.006>.

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 Dis.* 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 pathogen *Vibrio tapetis*. *J. Invertebr. Pathol.* 76, 63–69.
- Allam, B., Paillard, C., Ford, S.E., 2002. Pathogenicity of *Vibrio tapetis*, the etiological agent of brown ring disease in clams. *Dis. Aquat. Org.* 48, 221–231.
- Allam, B., Paillard, C., Maes, P., 1996. Localization of the pathogen *Vibrio* P1 in clams affected by Brown Ring Disease. *Dis. Aquat. Org.* 27, 149–155.
- Allan, B.F., Langerhans, R.B., Ryberg, W.A., Landesman, W.J., Griffin, N.W., Katz, R.S., Oberle, B.J., Schutzenhofer, M.R., Smyth, K.N., de St Maurice, A., Clark, L., Crooks, K.R., Hernandez, D.E., McLean, R.G., Ostfeld, R.S., Chase, J.M., 2008. Ecological correlates of risk and incidence of West Nile virus in the United States. *Oecologia* 158, 699–708.
- Amend, A.S., Oliver, T.A., Amaral-Zettler, L.A., Boetius, A., Fuhrman, J.A., HornerDevine, M.C., Huse, S.M., Welch, D.B.M., Martiny, A.C., Ramette, A., Zinger, L., Sogin, M.L., Martiny, J.B.H., 2012. Macroecological patterns of marine bacteria on a global scale. *J. Biogeogr.* 40, 800–811.
- Anderson, R.S., 1999. *Perkinsus marinus* secretory products modulate superoxide anion production by oyster (*Crassostrea virginica*) hemocytes. *Fish Shellfish Immun.* 9, 51–60.
- Andrews, J.D., 1982. Epizootiology of late summer and early fall infections of oysters by *Haplosporidium nelsoni*, and comparison to annual life cycle of *Haplosporidium costalis*, a typical haplosporidian. *J. Shellfish Res.* 2, 15–23.
- 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 pathogen *Marteilia refringens*. *Parasitology* 124, 315–323.
- Audemard, C., Ragone Calvo, L.M., Paynter, K.T., Reece, K.S., Bureson, E.M., 2006. Real-time PCR investigation of parasite ecology: in situ determination of oyster parasite *Perkinsus marinus* transmission dynamics in lower Chesapeake Bay. *Parasitology* 132, 827–842.
- Auzoux-Bordenave, S., Vigario, A.M., Ruano, F., Domart-Coulon, I., Doumenc, D., 1995. In vitro sporulation of the clam pathogen *Perkinsus atlanticus* (Apicomplexa, Perkinsea) under various environmental conditions. *J. Shellfish Res.* 14, 469–475.
- Azevedo, C., Corral, L., Cachola, R., 1990. Fine structure of zoosporulation in *Perkinsus atlanticus* (Apicomplexa: Perkinsea). *Parasitology* 100, 351–358.
- Bachali, S., Jager, M., Hassanin, A., Schoentgen, F., Jolles, P., Fiala-Medioni, A., Deutsch, J.S., 2002. Phylogenetic analysis of invertebrate lysozymes and the evolution of lysozyme function. *J. Mol. Evol.* 54, 652–664.
- Baker, K., Morris, J., McCarthy, N., Saldana, L., Lowther, J., Collinson, A., Young, M., 2011. An outbreak of norovirus infection linked to oyster consumption at a UK restaurant, February 2010. *J. Public Health* 33, 205–211.
- Balboa, S., Dieguez, A.L., Doce, A., Barja, J.L., Romalde, J.L., 2012. Evaluation of different culture media for the isolation and growth of the fastidious *Vibrio tapetis*, the causative agent of brown ring disease. *J. Invertebr. Pathol.* 111, 74–81.
- Baldauf, S.L., 2003. The deep roots of eukaryotes. *Science* 300, 1703–1706.

- Barber, B.J., 2004. Neoplastic diseases of commercially important marine bivalves. *Aquat. Living Resour.* 17, 449–466.
- Barber, R.D., Kanaley, S.A., Ford, S.E., 1991. Evidence for regular sporulation by *Haplosporidium nelsoni* (MSX) (Asctospora; Haplosporidiidae) in spat of the American oyster, *Crassostrea virginica*. *J. Protozool.* 34, 305–306.
- Bayne, C.J., 1990. Phagocytosis and non-self recognition in invertebrates. *Bioscience* 40, 723–731.
- Beaz-Hidalgo, R., Balboa, S., Romalde, J.L., Figueras, M.J., 2010. Diversity and pathogenicity of *Vibrio* species in cultured bivalve molluscs. *Environ. Microbiol. Rep.* 2, 34–43.
- Bellou, M., Kokkinos, P., Vantarakis, A., 2013. Shellfish-borne viral outbreaks: a systematic review. *Food Environ. Virol.* 5, 13–23.
- Benitez-Nelson, C.R., 2000. The biogeochemical cycling of phosphorus in marine systems. *Earth Sci. Rev.* 51, 109–135.
- Berthe, F.C.J., Hine, P.M., 2003. *Bonamia exitiosa* Hine et al., 2001 is proposed instead of *B. exitiosus* as the valid name of *Bonamia* sp. infecting flat oysters *Ostrea chilensis* in New Zealand. *Dis. Aquat. Org.* 57, 181.
- Berthe, F.C.J., Pernas, M., Zerabib, M., Hallner, P., Thébault, A., Figueras, A.J., 1998. Experimental transmission of *Marteilia refringens* with special consideration of its life cycle. *Dis. Aquat. Org.* 34, 135–144.
- Birkbeck, T.H., McHenry, J., 1982. Degradation of bacteria by *Mytilus edulis*. *Mar. Biol.* 72, 7–15.
- Bitton, G., 1975. Adsorption of viruses on surfaces in soil and water. *Water Res.* 9, 473–484.
- Blackbourn, J., Bower, S.M., Meyer, G.R., 1998. *Perkinsus qugwadi* sp. nov. (incertae sedis), a pathogenic protozoan parasite of Japanese scallops, *Patinopecten yessoensis*, cultured in British Columbia, Canada. *Can. J. Zool.* 76, 942–953.
- Blakeslee, A.M.H., Altman, I., Miller, A.W., Byers, J.E., Hamer, C.E., Ruiz, G.M., 2012. Parasites and invasions: a biogeographic examination of parasites and hosts in native and introduced ranges. *J. Biogeogr.* 39, 609–622.
- Boardman, C.L., Maloy, A.P., Boettcher, K.J., 2008. Localization of the bacterial agent of juvenile oyster disease (*Roseavarios crassostreae*) within affected eastern oysters (*Crassostrea virginica*). *J. Invert. Pathol.* 97, 150–158.
- Boettcher, K.J., Barber, B.J., Singer, J.T., 2000. Additional evidence that juvenile oyster disease is caused by a member of the *Roseobacter* group and colonization of unaffected animals by *Stappia stellulate*-like strains. *Appl. Environ. Microbiol.* 66, 3924–3930.

- Boettcher, K.J., Geaghan, K., Maloy, A.P., Barber, B.J., 2005. *Roseovarius crassostreae* sp. nov., a member of the Roseobacter clade and the apparent cause of juvenile oyster disease (JOD) in cultured Eastern oysters. *Int. J. Syst. Evol. Microbiol.* 55, 1531–1537.
- Borrego, J.J., Castro, D., Luque, A., Paillard, C., Maes, P., Garcia, M.T., Ventosa, A., 1996. *Vibrio tapetis* sp. nov., the causative agent of the brown ring disease affecting cultured clams. *Int. J. Syst. Bacteriol.* 46, 480–484.
- Bougrier, S., Geairon, P., Deslous-Paoli, J.M., Bacher, C., Jonquières, G., 1995. Allometric relationships and effects of temperature on clearance and oxygen consumption rates of *Crassostrea gigas* (Thunberg). *Aquaculture* 134, 143–154.
- Bower, S.M., Blackbourn, J., Meyer, G.R., 1998. Distribution, prevalence, and pathogenicity of the protozoan *Perkinsus qugwadi* in Japanese scallops, *Patinopecten yessoensis*, cultured in British Columbia, Canada. *Can. J. Zool.* 76, 954–959.
- Bower, S.M., Goh, B., Meyer, G.R., Carnegie, R.B., Gee, A., 2005. Epizootiology and detection of nocardiosis in oysters. In: Walker, P., Lester, R., Bondad-Reantaso, M.G. (Eds.), *Diseases in Asian Aquaculture V. Diseases in Asian Aquaculture V*, Fish Health Section. Asian Fisheries Society, Manila, pp. 249–262.
- Bower, S.M., Hervio, D., Meyer, G.R., 1997. Infectivity of *Mikrocytos mackini*, the causative agent of Denman Island disease in Pacific oysters *Crassostrea gigas*, to various species of oysters. *Dis. Aquat. Org.* 29, 111–116.
- Bower, S.M., McGladdery, S.E., Price, I.M., 1994. Synopsis of infectious diseases and parasites of commercially exploited shellfish. *Annu. Rev. Fish Dis.* 4, 1–199.
- Boyer, S., Chollet, B., Bonnet, D., Arzul, I., 2013. New evidence for the involvement of *Paracartia grani* (Copepoda, Calanoida) in the life cycle of *Marteilia refringens* (Paramyxia). *Int. J. Parasitol.* 43, 1089–1099.
- Breitbart, M., 2012. Marine viruses: truth or dare. *Annu. Rev. Mar. Sci.* 4, 425–448.
- Brillant, M.G.S., MacDonald, B.A., 2000. Postingestive selection in the sea scallop, *Placopecten magellanicus* (Gmelin): the role of particle size and density. *J. Exp. Mar. Biol. Ecol.* 253, 211–227.
- Brillant, M.G.S., MacDonald, B.A., 2003. Postingestive sorting of living and heat-killed *Chlorella* within the sea scallop, *Placopecten magellanicus* (Gmelin). *J. Exp. Mar. Biol. Ecol.* 290, 81–91.
- Brown, G.D., Reece, K.S., 2003. Isolation and characterization of serine protease gene(s) from *Perkinsus marinus*. *Dis. Aquat. Org.* 57, 117–126.
- Brum, J.R., Schenck, R.O., Sullivan, M.B., 2013. Global morphological analysis of marine viruses shows minimal regional variation and dominance of non-tailed viruses. *ISME J.* 7, 1738–1751.

- Buggé, D.M., Allam, B., 2007. Effects of starvation and macroalgae extracts on the survival and growth of quahog parasite unknown (QPX). *J. Exp. Mar. Biol. Ecol.* 348, 60–69.
- Burge, C.A., Griffin, F.J., Friedman, C.S., 2006. Mortality and herpesvirus infections of the Pacific oyster *Crassostrea gigas* in Tomales Bay, California, USA. *Dis. Aquat. Org.* 72, 31–43.
- Burge, C.A., Friedman, C.S., 2012. Quantifying Ostreid herpesvirus (OsHV-1) genome copies and expression during transmission. *Microb. Ecol.* 63, 596–604.
- Burge, C.A., Mark Eakin, C., Friedman, C.S., Froelich, B., Hershberger, P.K., Hofmann, E.E., Petes, L.E., Prager, K.C., Weil, E., Willis, B.L., Ford, S.E., Harvell, C.D., 2014. Climate change influences on marine infectious diseases: implications for management and society. *Annu. Rev. Mar. Sci.* 6, 249–277.
- Burreson, E.M., Ford, S.E., 2004. A review of recent information on the Haplosporidia, with special reference to *Haplosporidium nelsoni* (MSX disease). *Aquat. Living Resour.* 17, 499–517.
- Burreson, E.M., Ragone Calvo, L.M., 1996. Epizootiology of *Perkinsus marinus* disease of oysters in Chesapeake Bay, with emphasis on data since 1985. *J. Shellfish Res.* 15, 17–34.
- Burreson, E.M., Reece, K.S., Dungan, C.F., 2005. Molecular, morphological, and experimental evidence support the synonymy of *Perkinsus chesapeaki* and *Perkinsus andrewsi*. *J. Eukaryot. Microbiol.* 52, 258–270.
- Burreson, E.M., Stokes, N.A., Friedman, C.S., 2000. Increased virulence in an introduced pathogen: *Haplosporidium nelsoni* (MSX) in the eastern oyster *Crassostrea virginica*. *J. Aquat. Anim. Health* 12, 1–8.
- Bushek, D., Allen, S.K., 2006. Host-parasite interactions among broadly distributed populations of the eastern oyster *Crassostrea virginica* and the protozoan *Perkinsus marinus*. *Mar. Ecol. Prog. Ser.* 139, 127–141.
- Bushek, D., Ford, S.E., Burt, I., 2012. Long-term patterns of an estuarine pathogen along a salinity gradient. *J. Mar. Res.* 70, 225–251.
- Bushek, D., Ford, S.E., Chintala, M.M., 2002. Comparison of in vitro-cultured and wild-type *Perkinsus marinus*. III. Fecal elimination and its role in transmission. *Dis. Aquat. Org.* 51, 217–225.
- Bushek, D., Landau, B., Scarpa, E., 2008. *Perkinsus chesapeaki* in stout razor clams *Tagelus plebeius* from Delaware Bay. *Dis. Aquat. Org.* 78, 243–247.
- Canesi, L., Gallo, G., Gavioli, M., Pruzzo, C., 2002. Bacteria–hemocyte interactions and phagocytosis in marine bivalves. *Microsc. Res. Tech.* 57, 469–476.
- Canesi, L., Pezzati, E., Stauder, M., Grande, C., Bavestrello, M., Papetti, A., Vezzulli, L., Pruzzo, C., 2013. *Vibrio cholerae* interactions with *Mytilus galloprovincialis* hemocytes mediated by serum components. *Front. Microbiol.* 4, 371.

- Canzonier, W., 1968. Present status of attempts to transmit *Michinia nelsoni* under controlled conditions. Proc. Natl. Shellfish Ass. 58, 1.
- Canzonier, W., 1974. Tissue grafts in the American oyster, *Crassostrea virginica*. Proc. Natl. Shellfish Ass. 64, 92–101.
- Carballal, M., 2003. Virus-like particles associated with large foci of heavy hemocytic infiltration in cockles *Cerastoderma edule* from Galicia (NW Spain). J. Invertebr. Pathol. 84, 234–237.
- Carella, F., Carrasco, N., Andree, K.B., Lacuesta, B., Furones, D., De Vico, G., 2013. Nocardiosis in Mediterranean bivalves: first detection of *Nocardia crassostreae* in a new host *Mytilus galloprovincialis* and in *Ostrea edulis* from the Gulf of Naples (Italy). J. Invertebr. Pathol. 114, 324–328.
- Carnegie, R.B., Burreson, E.M., 2011. Declining impact of an introduced pathogen: *Haplosporidium nelsoni* in the oyster *Crassostrea virginica* in Chesapeake Bay. Mar. Ecol. Prog. Ser. 432, 1–15.
- Carnegie, R.B., Burreson, E.M., Hine, P.M., Stokes, N.A., Audemard, C., Bishop, M.J., Peterson, C.H., 2006. *Bonamia perspora* n. sp. (Haplosporidia), a parasite of the oyster *Ostreola equestris*, is the first *Bonamia* species known to produce spores. J. Eukaryotic Microbiol. 53, 232–245.
- Carnegie, R.B., Cochenec-Laureau, N., 2004. Microcell parasites of oysters: recent insights and future trends. Aquat. Living Resour. 17, 519–528.
- Carnegie, R.B., Meyer, G.R., Blackburn, J., Cochenec-Laureau, N., Berthe, F.C.J., Bower, S.M., 2003. Molecular detection of the oyster parasite *Mikrocytos mackini*, and a preliminary phylogenetic analysis. Dis. Aquat. Org. 54, 219–227.
- Carrasco, N., Arzul, I., Chollet, B., Robert, M., Joly, J.P., Furones, M.D., Berthe, F.C.J., 2008. Comparative experimental infection of the copepod *Paracartia grani* with *Marteilia refringens* and *Marteilia maurini*. J. Fish Dis. 31, 497–504.
- Carrasco, N., Hine, P.M., Durfort, M., Andree, K.B., Malchus, N., Lacuesta, B., González, J.M., Roque, A., Rodgers, C., Furones, M.D., 2013. *Marteilia cochillia* sp. nov., a new *Marteilia* species affecting the edible cockle *Cerastoderma edule* in European waters. Aquaculture 412–413, 223–230.
- Carrasco, N., López-Flores, I., Alcaraz, M., Furones, M.D., Berthe, F.C.J., Arzul, I., 2007. First record of a *Marteilia* parasite (Paramyxea) in zooplankton populations from a natural estuarine environment. Aquaculture 269, 63–70.
- Casas, S.M., 2002. Estudio de la perkinsiosis en la almeja fina, *Tapes decussatus* (Linnaeus 1758), de Galicia. Ph.D Thesis, University of Santiago de Compostela, Spain.

- Casas, S.M., Grau, A., Reece, K.S., Apakupakul, K., Azevedo, C., Villalba, A., 2004. *Perkinsus mediterraneus* n. sp., a protistan parasite of the European flat oyster *Ostrea edulis* from the Balearic Islands, Mediterranean Sea. *Dis. Aquat. Org.* 58, 231–244.
- Casas, S.M., Villalba, A., Reece, K.S., 2002. Study of perkinsosis in the carpet shell clam *Tapes decussatus* in Galicia (NW Spain). I. Identification of the aetiological agent and in vitro modulation of zoosporulation by temperature and salinity. *Dis. Aquat. Org.* 50, 51–65.
- Chauvaud, L., Jean, F., Ragueneau, O., Thouzeau, G., 2000. Long-term variation of the Bay of Brest ecosystem: benthic-pelagic coupling revisited. *Mar. Ecol. Prog. Ser.* 200, 35–48.
- Choi, D., Lee, N., Choi, H., Park, M.A., McGladdery, S.E., Park, M.S., 2004. Viral gametocytic hypertrophy caused by papova-like virus infection in the Pacific oyster *Crassostrea gigas* in Korea. *Dis. Aquat. Org.* 59, 205–209.
- Choquet, G., Soudant, P., Lambert, C., Nicolas, J.-L., Paillard, C., 2003. Reduction of adhesion properties of *Ruditapes philippinarum* hemocytes exposed to *Vibrio tapetis*. *Dis. Aquat. Org.* 57, 109–116.
- Chu, F.-L.E., 1988. Humoral defense factors in marine bivalves. *Am. Fish. Soc. Spec. Publ.* 18, 178–188.
- Chu, F.-L.E., 1996. Laboratory investigations of susceptibility, infectivity, and transmission of *Perkinsus marinus* in oysters. *J. Shellfish Res.* 15, 57–66.
- Chu, F.-L.E., Lund, E., Soudant, P., Harvey, E., 2002. De novo arachidonic acid synthesis in *Perkinsus marinus*, a protozoan parasite of the eastern oyster *Crassostrea virginica*. *Mol. Biochem. Parasitol.* 119, 179–190.
- Chu, F.-L.E., Lund, E.D., 2006. Viability, infectivity and fatty acid synthetic activity of *Perkinsus marinus* meront cells incubated in estuarine and artificial seawater. *Dis. Aquat. Org.* 71, 131–139.
- Cochennec, N., 2001. *Bonamia ostreae*, parasite de l’huître plate, *Ostrea edulis*, sa position taxonomique parmi les parasites du groupe “Microcell,” analyses des interactions hôte/parasite chez plusieurs populations d’huîtres plates. These de Doctorat, Biologie Cellulaire, Universite de la Rochelle, France.
- Cochennec-Laureau, N., Auffret, M., Renault, T., Langlade, A., 2003. Changes in circulating and tissue-infiltrating hemocyte parameters of European flat oysters, *Ostrea edulis*, naturally infected with *Bonamia ostreae*. *J. Invertebr. Pathol.* 83, 23–30.
- Cochlan, W.P., Wikner, J., Steward, G.F., Smith, D.C., Azam, F., 1993. Spatial distribution of viruses, bacteria and chlorophyll a in neritic, oceanic and estuarine environments. *Mar. Ecol. Prog. Ser.* 92, 77–87.
- Comps, M., Cochennec, N., 1993. A herpes-like virus from the European oyster *Ostrea edulis*. *J. Invertebr. Pathol.* 62, 201–203.
- Costerton, J.W., Stewart, P.S.P.G.E., 1999. Bacterial biofilms: a common cause of persistent infections. *Science* 284, 1318–1322.

- Culloty, S.C., Mulcahy, M.F., 1996. Season-, age-, and sex-related variation in the prevalence of bonamiasis in flat oysters (*Ostrea edulis* L.) on the south coast of Ireland. *Aquaculture* 144, 53–63.
- Culloty, S.C., Novoa, B., Pernas, M., Longshaw, M., Mulcahy, M.F., Feist, S.W., Figueras, A., 1999. Susceptibility of a number of bivalve species to the protozoan parasite *Bonamia ostreae* and their ability to act as vectors for this parasite. *Dis. Aquat. Org.* 37, 73–80.
- Dahl, S.F., Allam, B., 2007. Laboratory transmission studies of QPX disease in the northern quahog (=hard clam): development of an infection procedure. *J. Shellfish Res.* 26, 383–389.
- Dahl, S.F., Perrigault, M., Allam, B., 2008. Laboratory transmission studies of QPX disease in the hard clam: interactions between different host strains and pathogen isolates. *Aquaculture* 280, 64–70.
- Dahl, S.F., Thiel, J., Allam, B., 2010. Field performance and QPX disease progress in cultured and wild-type strains of *Mercenaria mercenaria* in New York waters. *J. Shellfish Res.* 29, 83–90.
- Dame, R.F., 2011. *Ecology of Marine Bivalves: An Ecosystem Approach*. CRC Press, Boca Raton, FL.
- Dame, R.F., Zingmark, R., Haskin, E., 1984. Oyster reefs as processors of estuarine materials. *J. Exp. Mar. Biol. Ecol.* 83, 239–247.
- Daniels, N.A., 2011. *Vibrio vulnificus* oysters: pearls and perils. *Clin. Infect. Dis.* 52, 788–792.
- Davis, C.V., Barber, B.J., 1999. Growth and survival of selected lines of eastern oysters, *Crassostrea virginica* (Gmelin 1791) affected by juvenile oyster disease. *Aquaculture* 178, 253–271.
- Davison, A.J., Trus, B.L., Cheng, N., Steven, A.C., Watson, M.S., Cunningham, C., Le Deuff, R.-M., Renault, T., 2005. A novel class of herpesvirus with bivalve hosts. *J. Gen. Virol.* 86, 41–53.
- de Freitas Rebelo, M., Figueiredo, E.deS., Mariante, R.M., Nóbrega, A., de Barros, C.M., Allodi, S., 2013. New Insights from the oyster *Crassostrea rhizophorae* on bivalve circulating hemocytes. *PLoS One* 8, e57384.
- de Simon, M., Tarrago, C., Ferrer, M., 1992. Incidence of *Listeria monocytogenes* in fresh foods in Barcelona (Spain). *Int. J. Food Microbiol.* 16, 153–156.
- Dégremont, L., Guyader, T., Tourbiez, D., Pépin, J.-F., 2013. Is horizontal transmission of the Ostreid herpesvirus OsHV-1 in *Crassostrea gigas* affected by unselected or selected survival status in adults to juveniles? *Aquaculture* 408, 51–57.
- Di Girolamo, R., Liston, J., Matches, J., 1977. Ionic bonding, the mechanism of viral uptake by shellfish mucus. *Appl. Environ. Microbiol.* 33, 19–25.
- Dobson, A., 2004. Population dynamics of pathogens with multiple host species. *Am. Nat.* 164, S64–S78.
- Donlan, R.M., Costerton, J.W., 2002. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin. Microbiol. Rev.* 15, 167–193.

- Dungan, C.F., Bushek, D., this volume. Development and applications of Ray's fluid thioglycollate media for detection and manipulation of *Perkinsus* spp. pathogens of marine molluscs. *J. Invert. Pathol.* <http://dx.doi.org/10.1016/j.jip.2015.05.004>.
- Dungan, C.F., Hamilton, R.M., 1995. Use of a tetrazolium-based cell proliferation assay to measure effects of in vitro conditions on *Perkinsus marinus* (Apicomplexa) proliferation. *J. Eukaryot. Microbiol.* 42, 379–388.
- Eisenberg, R., Atanasiu, D., Cairns, T., Gallagher, J.R., Krummenacher, C., Cohen, G.H., 2012. Herpes virus fusion and entry: a story with many characters. *Viruses* 4, 800–832.
- Elston, R.A., 1997. Special topic review: bivalve mollusc viruses. *World J. Microbiol. Biotechnol.* 13, 393–403.
- Elston, R.A., Farley, C.A., Kent, M.L., 1986. Occurrence and significance of bonamiasis in European flat oysters *Ostrea edulis* in North America. *Dis. Aquat. Org.* 2, 49–54.
- Elston, R.A., Hasegawa, H., Humphrey, K.L., Polyak, I.K., Häse, C.C., 2008. Reemergence of *Vibrio tubiashii* in bivalve shellfish aquaculture: severity, environmental drivers, geographic extent and management. *Dis. Aquat. Org.* 82, 119–134.
- Elston, R.A., Moore, J.D., Brooks, K., 1992. Disseminated neoplasia in bivalve molluscs. *Rev. Aquat. Sci.* 6, 405–466.
- Elston, R.A., Wilkinson, M., 1985. Pathology, management and diagnosis of oyster velar virus disease (OVVD). *Aquaculture* 48, 189–210.
- Engelsma, M.Y., Culloty, S.C., Lynch, S.A., Arzul, I., Carnegie, R.B., 2014. *Bonamia* parasites: a rapidly changing perspective on a genus of important mollusc pathogens. *Dis. Aquat. Org.* 110, 5–23.
- Engelsma, M.Y., Roozenburg, I., Joly, J.P., 2008. First isolation of *Nocardia crassostreae* from Pacific oyster *Crassostrea gigas* in Europe. *Dis. Aquat. Org.* 80, 229–234.
- Farley, C.A., 1967. A proposed life cycle of *Minchinia nelsoni* (Haplosporidia, Haplosporidiidae) in the American oyster *Crassostrea virginica*. *J. Protozool.* 14, 616–625.
- Farley, C.A., 1972. Oyster herpes-type virus. *Science* 178, 759–760.
- Farley, C.A., Wolf, P.H., Elston, R.A., 1988. A long-term study of "microcell" disease in oysters with a description of a new genus, *Mikrocytos* (g.n.), and two new species, *Mikrocytos mackini* and *Mikrocytos roughleyi* (sp. n.). *Fish Bull.* 86, 581–593.
- Fayer, R., Dubey, J.P., Lindsay, D.S., 2004. Zoonotic protozoa: from land to sea. *Trends Parasitol.* 20, 531–536.
- Fernández Robledo, J.A., Vasta, G.R., Record, N.R., 2014. Protozoan parasites of bivalve molluscs: literature follows culture. *PLoS One* 9, e100872.

- Figueras, A., Montes, J., 1988. Aber disease of edible oysters caused by *Marteilia refringens*. *Am. Fish. Soc. Spec. Publ.* 18, 38–46.
- Fischer, A.M., Ryan, J.P., Levesque, C., Welschmeyer, N., 2014. Characterizing estuarine plume discharge into the coastal ocean using fatty acid biomarkers and pigment analysis. *Mar. Environ. Res.* 99, 106–116.
- Flye-Sainte-Marie, J., Soudant, P., Lambert, C., Le Goïc, N., Goncalvez, M., Travers, M.A., Paillard, C., Jean, F., 2009. Variability of the hemocyte parameters of *Ruditapes philippinarum* in the field during an annual cycle. *J. Exp. Mar. Biol. Ecol.* 377, 1–11.
- Ford, S.E., 1996. Range extension by the oyster parasite *Perkinsus marinus* into northeastern US: response to climate change? *J. Shellfish Res.* 15, 45–56.
- Ford, S.E., Borrero, F.J., 2001. Epizootiology and pathology of juvenile oyster disease in the eastern oyster, *Crassostrea virginica*. *J. Invertebr. Pathol.* 78, 141–154.
- Ford, S.E., Chintala, M.M., 2006. Northward expansion of a marine parasite: testing the role of temperature adaptation. *J. Exp. Mar. Biol. Ecol.* 339, 226–235.
- Ford, S.E., Haskin, H., 1982. History and epizootiology of *Haplosporidium nelsoni* (MSX), an oyster pathogen in Delaware Bay, 1957–1980. *J. Invertebr. Pathol.* 40, 118–141.
- Ford, S.E., Kraeuter, J.N., Barber, R.D., Mathis, G., 2002. Aquaculture-associated factors in QPX disease of hard clams: density and seed source. *Aquaculture* 208, 23–38.
- Ford, S.E., Schotthoefer, A., Spruck, C., 1999. In vivo dynamics of the microparasite *Perkinsus marinus* and regression of infections in eastern oysters. *J. Parasitol.* 85, 273–282.
- Ford, S.E., Smolowitz, R., 2007. Infection dynamics of an oyster parasite in its newly expanded range. *Mar. Biol.* 151, 119–133.
- Forns, X., Thimme, R., Govindarajan, S., Emerson, S.U., Purcell, R.H., Chisari, F.V., Bukh, J., 2000. Hepatitis C virus lacking the hypervariable region 1 of the second envelope protein is infectious and causes acute resolving or persistent infection in chimpanzees. *Proc. Natl. Acad. Sci. USA* 97, 13318–13323.
- Friedman, C.S., Beaman, B.L., Chun, J., Goodfellow, M., Gee, A., Hedrick, R.P., 1998. *Nocardia crassostreae* sp. nov., the causal agent of nocardiosis in Pacific oysters. *Int. J. Syst. Bacteriol.* 48, 237–246.
- Friedman, C.S., Estes, R.M., Stokes, N.A., Burge, C.A., Hargrove, J.S., Barber, B.J., Elston, R.A., Burrenson, E.M., Reece, K.S., 2005. Herpes virus in juvenile Pacific oysters *Crassostrea gigas* from Tomales Bay, California, coincides with summer mortality episodes. *Dis. Aquat. Org.* 63, 33–41.
- Friedman, C.S., Hedrick, R.P., 1991. Pacific oyster nocardiosis: isolation of the bacterium and induction of laboratory infections. *J. Invertebr. Pathol.* 57, 109–120.
- Garreis, K.A., La Peyre, J.F., Faisal, M., 1996. The effects of *Perkinsus marinus* extracellular products and purified proteases on oyster defense parameters in vitro. *Fish Shellfish Immun.* 6, 581–597.

- Garza, D.R., Suttle, C.A., 1998. The effect of cyanophages on the mortality of *Synechococcus* spp. and selection for UV resistant viral communities. *Microb. Ecol.* 36, 281–292.
- Gast, R.J., Moran, D.M., Audemard, C., Lyons, M.M., DeFavari, J., Reese, K.S., Leavitt, D., Smolowitz, R., 2008. Environmental distribution and persistence of Quahog Parasite Unknown (QPX). *Dis. Aquat. Org.* 81, 219–229.
- Gauthier, J.D., Vasta, G.R., 1995. In vitro culture of the eastern oyster parasite *Perkinsus marinus*: optimization of the methodology. *J. Invertebr. Pathol.* 66, 156–168.
- Gentry, J., Vinje, J., Guadagnoli, D., Lipp, E.K., 2009. Norovirus distribution within an estuarine environment. *Appl. Environ. Microbiol.* 75, 5474–5480.
- Geyer, W.R., Signell, R.P., 1992. A reassessment of the role of tidal dispersion in estuaries and bays. *Estuaries* 15, 97–108.
- Goggin, C.L., Lester, R.J.G., 1995. *Perkinsus*, a protistan parasite of abalone in Australia: a review. *Mar. Freshwater Res.* 46, 639–646.
- Goggin, C.L., Sewell, K.B., Lester, R.J.G., 1989. Cross-infection experiments with Australian *Perkinsus* species. *Dis. Aquat. Org.* 7, 53–59.
- González, J.M., Moran, M.A., 1997. Numerical dominance of a group of marine bacteria in the a-subclass of the class Proteobacteria in coastal seawater. *Appl. Environ. Microbiol.* 63, 4237–4242.
- Greenwood, M., Winnard, G., Bagot, B., 1998. An outbreak of *Salmonella enteritidis* phage type 19 infection associated with cockles. *Comm. Dis. Publ. Health* 1, 35–37.
- Grizel, H., 1979. *Marteilia refringens* and oyster disease: recent observations. *Mar. Fish Rev.* 41, 38–39.
- Grizel, H., Mialhe, E., Chagot, D., Boulo, V., Bachere, E., 1988. Bonamiasis: a model study of diseases in marine molluscs. *Am. Fish. Soc. Spec. Publ.* 18, 1–4.
- Guidi-Rontani, C., 2002. The alveolar macrophage: the Trojan horse of *Bacillus anthracis*. *Trends Microbiol.* 10, 405–409.
- Hada, H.S., West, P.A., Lee, J.V., Stemmler, J., Colwell, R.R., 1984. *Vibrio tubiashii* sp. nov., a pathogen of bivalve mollusks. *Int. J. Syst. Bacteriol.* 34, 1–4.
- Hall, S.R., Sivars-Becker, L., Becker, C., Duffy, M.A., Tessier, A.J., Caceres, C.E., 2007. Eating yourself sick: transmission of disease as a function of foraging ecology. *Ecol. Lett.* 10, 207–218.
- Harvell, C.D., Kim, K., Burkholder, J.M., Colwell, R.R., Epstein, P.R., Grimes, D.J., Hofmann, E.E., Lipp, E.K., Osterhaus, A.D.M.E., Overstreet, R.M., Porter, J.W., Smith, G.W., Vasta, G.R., 1999. Emerging marine diseases: climate links and anthropogenic factors. *Science* 285, 1505–1510.
- Haskin, H., 1966. *Minchinia nelsoni* n. sp. (Haplosporida, Haplosporidiidae): causative agent of the Delaware Bay oyster epizootic. *Science* 153, 1414–1416.

- Haskin, H., Andrews, J.D., 1988. Uncertainties and speculations about the life cycle of the eastern oyster pathogen *Haplosporidium nelsoni* (MSX). *Am. Fish. Soc. Spec. Publ.* 18, 5–22.
- Heldal, M., Bratbak, G., 1991. Production and decay of viruses in aquatic environments. *Mar. Ecol. Prog. Ser.* 72, 205–212.
- Hershberger, P.K., van der Leeuw, B.K., Gregg, J.L., Grady, C.A., Lujan, K.M., Gutenberger, S.K., Purcell, M.K., Woodson, J.C., Winton, J.R., Parsley, M.J., 2010. Amplification and transport of an endemic fish disease by an introduced species. *Biol. Invasions* 12, 3665–3675.
- Hervio, D., Bachere, E., Boulo, V., Cochenec, N., Vuillemin, V., Le Coguic, Y., Cailletaux, G., Mazurie, J., Mialhe, E., 1995. Establishment of an experimental infection protocol for the flat oyster, *Ostrea edulis*, with the intrahaemocytic protozoan parasite, *Bonamia ostreae*: application in the selection of parasiteresistant oysters. *Aquaculture* 132, 183–194.
- Hervio, D., Chagot, D., Godin, P., Grizel, H., Mialhe, E., 1991. Localization and characterization of acid phosphatase activity in *Bonamia ostreae* (Asctospora), an intrahemocytic protozoan parasite of the flat oyster *Ostrea edulis* (Bivalvia). *Dis. Aquat. Org.* 12, 67–70.
- Hine, P.M., 1991. Ultrastructural observations on the annual infection pattern of *Bonamia* sp. in flat oysters *Tiostrea chilensis*. *Dis. Aquat. Org.* 11, 163–171.
- Hine, P.M., 1999. The inter-relationships of bivalve haemocytes. *Fish Shellfish Immun.* 9, 367–385.
- Hine, P.M., Cochenec-Laureau, N., Berthe, F.C.J., 2001. *Bonamia exitiosa* n. sp. (Haplosporidia) infecting flat oysters, *Ostrea chilensis*, in New Zealand. *Dis. Aquat. Org.* 47, 63–72.
- Hine, P.M., Wesney, B., Besant, P., 1998. Replication of a herpes-like virus in larvae of the flat oyster *Tiostrea chilensis* at ambient temperatures. *Dis. Aquat. Org.* 32, 161–171.
- Holt, R.D., Dobson, A.P., Begon, M., Bowers, R.G., Schaubert, E.M., 2003. Parasite establishment in host communities. *Ecol. Lett.* 6, 837–842.
- Honess, R.W., Roizman, B., 1974. Regulation of herpesvirus macromolecular synthesis: I. Cascade regulation of the synthesis of three groups of viral proteins. *J. Virol.* 14, 8–19.
- Hughes, F.M., Foster, B., Grewal, S., Sokolova, I.M., 2010. Apoptosis as a host defense mechanism in *Crassostrea virginica* and its modulation by *Perkinsus marinus*. *Fish Shellfish Immun.* 29, 247–257.
- Iwamoto, M., Ayers, T., Mahon, B.E., Swerdlow, D.L., 2010. Epidemiology of seafood-associated infections in the United States. *Clin. Microbiol. Rev.* 23, 399–411.
- Jensen, S., Samulsen, O.B., Andersen, K., Torkildsen, L., Lambert, C., Choquet, G., Paillard, C., Bergh, O., 2003. Characterization of strains of *Vibrio splendidus* and *V. tapetis* isolated from corkwing wrasse *Symphodus melops* suffering vibriosis. *Dis. Aquat. Org.* 2003, 25–31.

- Johnson, P.T.J., Dobson, A., Lafferty, K.D., Marcogliese, D.J., Memmott, J., Orlofske, S.A., Poulin, R., Thieltges, D.W., 2010. When parasites become prey: ecological and epidemiological significance of eating parasites. *Trends Ecol. Evol.* 25, 362–371.
- Johnson, P.T.J., Lund, P.J., Hartson, R.B., Yoshino, T.P., 2009. Community diversity reduces *Schistosoma mansoni* transmission, host pathology and human infection risk. *Proc. R. Soc. Lond., B* 276, 1657–1663.
- Johnson, P.T.J., Thieltges, D.W., 2010. Diversity, decoys and the dilution effect: how ecological communities affect disease risk. *J. Exp. Biol.* 213, 961–970.
- Jones, J., Scotti, P., Dearing, S., Wesney, B., 1996. Virus-like particles associated with marine mussel mortalities in New Zealand. *Dis. Aquat. Org.* 25, 143–149.
- Jorgensen, C.B., 1996. Bivalve filter feeding revisited. *Mar. Ecol. Prog. Ser.* 142, 287–302.
- Jouaux, A., Lafont, M., Blin, J.-L., Houssin, M., Mathieu, M., Lelong, C., 2013. Physiological change under OsHV-1 contamination in Pacific oyster *Crassostrea gigas* through massive mortality events on fields. *BMC Genomics* 14, 590.
- Kang, Y.-S., Kim, Y.-M., Park, K.-I., Kim Cho, S., Choi, K.S., Cho, M., 2006. Analysis of EST and lectin expressions in hemocytes of Manila clams (*Ruditapes philippinarum*) (Bivalvia: Mollusca) infected with *Perkinsus olseni*. *Dev. Comp. Immunol.* 30, 1119–1131.
- Kellogg, M.L., Cornwell, J.C., Owens, M.S., Paynter, K.T., 2013. Denitrification and nutrient assimilation on a restored oyster reef. *Mar. Ecol. Prog. Ser.* 480, 1–19.
- Kern, F., 1976. Sporulation of *Minchinia* sp. (Haplosporida, Haplosporidiidae) in the Pacific oyster *Crassostrea gigas* (Thunberg) from the Republic of Korea. *J. Protozool.* 23, 498–500.
- Kim, J.Y., Adhya, M., Cho, S.K., Choi, K.S., Cho, M., 2008. Characterization, tissue expression, and immunohistochemical localization of MCL3, a C-type lectin produced by *Perkinsus olseni*-infected Manila clams (*Ruditapes philippinarum*). *Fish Shellfish Immun.* 25, 598–603.
- Kiss, T., 2010. Apoptosis and its functional significance in molluscs. *Apoptosis* 15, 313–321.
- Kleman, S.N., Adlard, R.D., Lester, R.J.G., 2002. Detection of the initial infective stages of the protozoan parasite *Marteilia sydneyi* in *Saccostrea glomerata* and their development through to sporogenesis. *Int. J. Parasitol.* 32, 767–784.
- Kleinschuster, S.J., Smolowitz, R., Parent, J., 1998. In vitro life cycle and propagation of Quahog Parasite Unknown. *J. Shellfish Res.* 17, 75–78.
- Kach, D.J., Ward, J.E., 2008. The role of marine aggregates in the ingestion of picoplankton-size particles by suspension-feeding molluscs. *Mar. Biol.* 153, 797–805.
- Kramer, A.M., Lyons, M.M., Dobbs, F.C., Drake, J.M., 2013. Bacterial colonization and extinction on marine aggregates: stochastic model of species presence and abundance. *Ecol. Evol.* 3, 4300–4309.

- La Peyre, J.F., Chu, F.-L.E., Meyers, J.M., 1995. Haemocytic and humoral activities of eastern and Pacific oysters following challenge by the protozoan *Perkinsus marinus*. *Fish Shellfish Immun.* 5, 179–190.
- La Peyre, J.F., Xue, Q.-G., Itoh, N., Li, Y., Cooper, R.K., 2010a. Serine protease inhibitor cvSI-1 potential role in the eastern oyster host defense against the protozoan parasite *Perkinsus marinus*. *Dev. Comp. Immunol.* 34, 84–92.
- La Peyre, M.K., Casas, S.M., Gayle, W., La Peyre, J.F., 2010b. The combined influence of sub-optimal temperature and salinity on the in vitro viability of *Perkinsus marinus*, a protistan parasite of the eastern oyster *Crassostrea virginica*. *J. Invertebr. Pathol.* 105, 176–181.
- La Peyre, M.K., Casas, S.M., La Peyre, J.F., 2006. Salinity effects on viability, metabolic activity and proliferation of three *Perkinsus* species. *Dis. Aquat. Org.* 71, 59–74.
- La Peyre, M.K., Casas, S.M., Villalba, A., La Peyre, J.F., 2008. Determination of the effects of temperature on viability, metabolic activity and proliferation of two *Perkinsus* species, and its significance to understanding seasonal cycles of perkinsosis. *Parasitology* 135, 505–519.
- Labreuche, Y., Soudant, P., Gonclaves, M., Lambert, C., Nicolas, J.-L., 2006. Effects of extracellular products from the pathogenic *Vibrio aestuarianus* strain 01/32 on lethality and cellular immune responses of the oyster *Crassostrea gigas*. *Dev. Comp. Immunol.* 30, 367–379.
- Lacoste, A., Jalabert, F., Cueff, A., Gelebart, F., Cordevant, C., Lange, M., Poulet, S.A., 2001. A *Vibrio splendidus* strain is associated with summer mortality of juvenile oysters *Crassostrea gigas* in the Bay of Morlaix (North Brittany, France). *Dis. Aquat. Org.* 46, 139–145.
- Lacoste, A., Malham, S.K., Gelebart, F., Cueff, A., Poulet, S.A., 2002. Stress-induced immune changes in the oyster *Crassostrea gigas*. *Dev. Comp. Immunol.* 26, 181–188.
- Lafferty, K.D., Dobson, A.P., Kuris, A.M., 2006. Parasites dominate food web links. *Proc. Natl. Acad. Sci. USA* 103, 11211–11216.
- Lallias, D., Arzul, I., Heurtebise, S., Ferrand, S., Chollet, B., Robert, M., Beaumont, A.R., Boudry, P., Morga, B., Lapègue, S., 2008. *Bonamia ostreae*-induced mortalities in one-year old European flat oysters *Ostrea edulis*: experimental infection by cohabitation challenge. *Aquat. Living Resour.* 21, 423–439.
- Lambert, C., Nicolas, J.-L., Bultel, V., 2001. Toxicity to bivalve hemocytes of pathogenic *Vibrio* cytoplasmic extract. *J. Invertebr. Pathol.* 77, 165–172.
- Lambert, H., Barragan, A., 2010. Modelling parasite dissemination: host cell subversion and immune evasion by *Toxoplasma gondii*. *Cell. Microbiol.* 12, 292–300.
- Lane, E., Birkbeck, T.H., 2000. Species specificity of some bacterial pathogens of bivalve molluscs is correlated with their interaction with bivalve haemocytes. *J. Fish Dis.* 23, 275–279.

- Langdon, C.J., Newell, R.I.E., 1990. Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia demissa*. *Mar. Ecol. Prog. Ser.* 58, 299–310.
- Le Guyader, F.S., Loisy, F., Atmar, R.L., Hutson, A.M., Estes, M.K., Ruvoen-Clouet, N., Pommepey, M., Le Pendu, J., 2006. Norwalk virus-specific binding to oyster digestive tissues. *Emerg. Infect. Dis.* 12, 931–936.
- Le Roux, F., Lorenzo, G., Peyret, P., Audemard, C., Figueras, A., Vivares, C., Gouy, M., Berthe, F.C.J., 2001. Molecular evidence for the existence of two species of *Marteilia* in Europe. *J. Eukaryot. Microbiol.* 48, 449–454.
- Lees, D., 2000. Viruses and bivalve shellfish. *Int. J. Food Microbiol.* 59, 81–116.
- Lewis, E.J., Farley, C.A., Small, E.B., Baya, A.M., 1996. A synopsis of juvenile oyster disease (JOD) experimental studies in *Crassostrea virginica*. *Aquat. Living Resour.* 9, 169–178.
- LoGiudice, K., Ostfeld, R.S., Schmidt, K.A., Keesing, F., 2003. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. *Proc. Natl. Acad. Sci. USA* 100, 567–571.
- Lopez-Cortes, L., Castro, D., Navas, J.I., Borrego, J.J., 1999a. Phagocytic and chemotactic responses of manila and carpet shell clam haemocytes against *Vibrio tapetis*, the causative agent of brown ring disease. *Fish Shellfish Immun.* 1999, 543–555.
- Lopez-Cortes, L., Luque, A., Martinez-Manzanares, E., Castro, D., Borrego, J.J., 1999b. Adhesion of *Vibrio tapetis* to clam shells. *J. Shellfish Res.* 18, 91–97.
- Lund, E.D., Chu, F.-L.E., Harvey, E., 2004. In vitro effects of temperature and salinity on fatty acid synthesis in the oyster protozoan parasite *Perkinsus marinus*. *J. Exp. Mar. Biol. Ecol.* 307, 111–126.
- Lynch, S.A., Carlsson, J., Reilly, A.O., Cotter, E., Culloty, S.C., 2012. A previously undescribed ostreid herpes virus 1 (OsHV-1) genotype detected in the pacific oyster, *Crassostrea gigas*, in Ireland. *Parasitology* 139, 1526–1532.
- Lyons, M.M., Lau, Y.-T., Carden, W.E., Ward, J.E., Roberts, S.B., Smolowitz, R., Vallino, J., Allam, B., 2007a. Characteristics of marine aggregates in shallow-water ecosystems: implications for disease ecology. *EcoHealth* 4, 406–420.
- Lyons, M.M., Smolowitz, R., Gomez-Chiarri, M., Ward, J.E., 2007b. Epizootiology of Quahog Parasite Unknown (QPX) disease in northern quahogs (=hard clams) *Mercenaria mercenaria*. *J. Shellfish Res.* 26, 371–381.

- Lyons, M.M., Ward, J.E., Gaff, H., Hicks, R.E., Drake, J.M., Dobbs, F.C., 2010. Theory of island biogeography on a microscopic scale: organic aggregates as islands for aquatic pathogens. *Aquat. Microb. Ecol.* 60, 1–13.
- Lyons, M.M., Ward, J.E., Smolowitz, R., Uhlinger, K.R., Gast, R.J., 2005. Lethal marine snow: pathogen of bivalve mollusc concealed in marine aggregates. *Limnol. Oceanogr.* 50, 1983–1988.
- Lucas, M.I., Newell, R.C., Shumway, S.E., Seiderer, L.J., Bally, R., 1987. Particle clearance and yield in relation to bacterioplankton and suspended particulate availability in estuarine and open coast population of the mussel *Mytilus edulis*. *Mar. Ecol. Prog. Ser.* 36, 215–224.
- MacCready, P., Geyer, W.R., 2010. Advances in estuarine physics. *Ann. Rev. Mar. Sci.* 2, 35–58.
- Maes, P., 1992. Pathologie bactérienne chez deux invertébrés marins d'intérêt commercial, *Ruditapes philippinarum* et *Paracentrotus lividus*. These de Doctorat, Université de Brest (n.d.).
- Maloy, A.P., Barber, B.J., Boettcher, K.J., 2007a. Use of the 16S–23S rDNA internal transcribed spacer of *Roseovarius crassostreae* for epizootiological studies of juvenile oyster disease (JOD). *Dis. Aquat. Org.* 76, 151–161.
- Maloy, A.P., Ford, S.E., Karney, R.C., Boettcher, K.J., 2007b. *Roseovarius crassostreae*, the etiological agent of Juvenile Oyster Disease (now to be known as *Roseovarius* Oyster Disease) in *Crassostrea virginica*. *Aquaculture* 269, 71–83.
- Mann, R., Harding, J.M., Southworth, M.J., 2009. Reconstructing pre-colonial oyster demographics in the Chesapeake Bay, USA. *Estuar. Coast. Shelf Sci.* 85, 217–222.
- Mann, R., Powell, E.N., 2007. Why oyster restoration goals in the Chesapeake Bay are not and probably cannot be achieved. *J. Shellfish Res.* 26, 905–917.
- Maranger, R., Bird, D.F., 1995. Viral abundance in aquatic systems: a comparison between marine and fresh waters. *Mar. Ecol. Prog. Ser.* 121, 217–226.
- Martenot, C., Fourour, S., Oden, E., Jouaux, A., Travaillé, E., Malas, J.P., Houssin, M., 2012. Detection of the OsHV-1 IVar in the Pacific oyster *Crassostrea gigas* before 2008 in France and description of two new microvariants of the Ostreid Herpesvirus 1 (OsHV-1). *Aquaculture* 338–341, 293–296.
- Martenot, C., Oden, E., Travaillé, E., Malas, J.-P., Houssin, M., 2011. Detection of different variants of Ostreid Herpesvirus 1 in the Pacific oyster, *Crassostrea gigas* between 2008 and 2010. *Virus Res.* 160, 25–31.
- Martin, L.B., Weil, Z.M., Nelson, R.J., 2006. Refining approaches and diversifying directions in ecoimmunology. *Integr. Comp. Biol.* 46, 1030–1039.

- Martinez-Manzanares, E., Castro, D., Navas, J.I., Lopez-Cortes, L., Borrego, J.J., 1998. Transmission routes and treatment of brown ring disease affecting Manila clams (*Tapes philippinarum*). *J. Shellfish Res.* 17, 1051–1056.
- Mathias, C.B., Kirschner, A.K.T., Velimirov, B., 1995. Seasonal variations of virus abundance and viral control of the bacterial production in a backwater system of the Danube River. *Appl. Environ. Microbiol.* 61, 3734–3740.
- McCollough, C.B., Albright, B.W., Abbe, G.R., Barker, L.S., Dungan, C.F., 2007. Acquisition and progression of *Perkinsus marinus* infections by specific pathogen-free juvenile oysters (*Crassostrea virginica* Gmelin) in a mesohaline Chesapeake Bay tributary. *J. Shellfish Res.* 26, 465–477.
- McHenry, J., Allen, J., Birkbeck, T.H., 1986. Distribution of lysozyme-like activity in 30 bivalve species. *Comp. Biochem. Phys. B* 85, 581–584.
- McLeod, C., Hay, B., Grant, C., Greening, G., Day, D., 2009. Localization of norovirus and poliovirus in Pacific oysters. *J. Appl. Microbiol.* 106, 1220–1230.
- Mele, A., Rastelli, M., Gill, O., Di Biscelglie, D., Rosmini, F., Pardelli, G., Valtriani, C., Patriarchi, P., 1989. Recurrent epidemic hepatitis A associated with consumption of raw shellfish, probably controlled through public health measures. *Am. J. Epidemiol.* 130, 540–546.
- Meyers, T.R., 1979. A reo-like virus isolated from juvenile American oysters (*Crassostrea virginica*). *J. Gen. Virol.* 43, 203–212.
- Meyers, T.R., 1984. Marine bivalve mollusks as reservoirs of viral finfish pathogens: significance to marine and anadromous finfish aquaculture. *Mar. Fish Rev.* 46, 14–17.
- Montes, J., Anadon, R., Azevedo, C., 1994. A possible life cycle for *Bonamia ostreae* on the basis of electron microscopy studies. *J. Invertebr. Pathol.* 63, 1–6.
- Morse, D.L., Guzewich, J.J., Hanrahan, J.P., Stricof, R., Shayegani, M., Deibel, R., Grabau, J.C., Nowak, N.A., Hermann, J.E., Cukor, G., Blacklow, N.R., 1986. Widespread outbreaks of clam- and oyster-associated gastroenteritis: role of Norwalk virus. *New Engl. J. Med.* 314, 678–681.
- Mouritsen, K.N., Poulin, R., 2002. Parasitism, community structure and biodiversity in intertidal ecosystems. *Parasitology* 124, S101–S117.
- Mouritsen, K.N., Poulin, R., 2003. The mud flat anemone-cockle association: mutualism in the intertidal zone? *Oecologia* 135, 131–137.
- Muehlstein, L.K., Porter, D.T.S.F., 1988. *Labyrinthula* sp., a marine slime mold producing the symptoms of wasting diseases in eelgrass, *Zostera marina*. *Mar. Biol.* 99, 465–472.

- Munoz, P., Vance, K., Gomez-Chiari, M., 2003. Protease activity in the plasma of American oysters, *Crassostrea virginica*, experimentally infected with the protozoan parasite *Perkinsus marinus*. *J. Parasitol.* 89, 941–951.
- Narváez, D.A., Klinck, J.M., Powell, E.N., Hofmann, E.E., Wilkin, J., Haidvogel, D.B., 2012. Circulation and behavior controls on dispersal of eastern oyster *Crassostrea virginica* larvae in Delaware Bay. *J. Mar. Res.* 70, 411–440.
- Newell, R.I.E., 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: a review. *J. Shellfish Res.* 23, 51–61.
- Nguyen, L., Pieters, J., 2005. The Trojan horse: survival tactics of pathogenic mycobacteria in macrophages. *Trends Cell Biol.* 15, 269–276.
- Novoa, B., Figueras, A., 2000. Virus-like particles associated with mortalities of the carpet-shell clam *Ruditapes decussatus*. *Dis. Aquat. Org.* 39, 147–149.
- Nyholm, S.V., Stabb, E.V., Ruby, E.G., McFall-Ngai, M.J., 2000. Establishment of an animal–bacterial association: recruiting symbiotic vibrios from the environment. *Proc. Natl. Acad. Sci. USA* 97, 10231–10235.
- Oprandy, J.J., Chang, P.W., Pronovost, A.D., Cooper, K.R., Brown, R.S., Yates, V.J., 1981. Isolation of a viral agent causing hematopoietic neoplasia in the soft shell clam *Mya arenaria*. *J. Invertebr. Pathol.* 38, 45–51.
- Paillard, C., 2004. A short-review of brown ring disease, a vibriosis affecting clams, *Ruditapes philippinarum* and *Ruditapes decussatus*. *Aquat. Living Resour.* 17, 467–475.
- Paillard, C., Jean, F., Ford, S.E., Powell, E.N., Klinck, J.M., Hofmann, E.E., Flye-SainteMarie, J., 2014. A theoretical individual-based model of Brown Ring Disease in Manila clams, *Venerupis philippinarum*. *J. Sea Res.* 91, 15–34.
- Paillard, C., Le Roux, F., Borrego, J.J., 2004. Bacterial disease in marine bivalves, a review of recent studies: trends and evolution. *Aquat. Living Resour.* 17, 477–498.
- Paillard, C., Maes, P., 1994. Brown ring disease in the Manila clam *Ruditapes philippinarum*: establishment of a classification system. *Dis. Aquat. Org.* 19, 137–146.
- Paillard, C., Maes, P., Oubella, R., 1994. Brown ring disease in clams. *Annu. Rev. Fish Dis.* 4, 219–240.
- Pales Espinosa, E., Winnicki, S., Allam, B., 2013. Early host–pathogen interactions in a marine bivalve: *Crassostrea virginica* pallial mucus modulates *Perkinsus marinus* growth and virulence. *Dis. Aquat. Org.* 104, 237–247.
- Pedrós-Alió, C., 2006. Marine microbial diversity: can it be determined? *Trends Microbiol.* 14, 257–263.

- Perkins, F., 1988. Structure of protistan parasites found in bivalve molluscs. *Am. Fish. Soc. Spec. Publ.* 18, 93–111.
- Perkins, F., 1996. The structure of *Perkinsus marinus* (Mackin, Owen and Collier, 1950) Levinem, 1978 with comments on taxonomy and phylogeny of *Perkinsus* spp. *J. Shellfish Res.* 15, 67–87.
- Pernet, F., Barret, J., Le Gall, P., Corporeau, C., Dégremont, L., Lagarde, F., Pepin, J.F., Keck, N., 2012. Mass mortalities of Pacific oysters *Crassostrea gigas* reflect infectious diseases and vary with farming practices in the Mediterranean Thau lagoon, France. *Aquacult. Environ. Interact.* 2, 215–237.
- Pernet, F., Lagarde, F., Le Gall, P., Roque D'Orbcastel, E., 2014. Associations between farming practices and disease mortality of Pacific oyster *Crassostrea gigas* in a Mediterranean lagoon. *Aquacult. Environ. Interact.* 5, 99–106.
- Pernet, F., Tamayo, D., Petton, B., 2015. Influence of low temperature on the survival of the Pacific oyster (*Crassostrea gigas*) infected with Ostreid herpes virus type 1. *Aquaculture* 445, 57–62.
- Perrigault, M., Buggé, D.M., Allam, B., 2010. Effect of environmental factors on survival and growth of quahog parasite unknown (QPX) in vitro. *J. Invertebr. Pathol.* 104, 83–89.
- Perrigault, M., Dahl, S.F., Espinosa, E.P., Gambino, L., Allam, B., 2011. Effects of temperature on hard clam (*Mercenaria mercenaria*) immunity and QPX (Quahog Parasite Unknown) disease development: II. Defense parameters. *J. Invertebr. Pathol.* 106, 322–332.
- Petton, B., Pernet, F., Robert, R., Boudry, P., 2013. Temperature influence on pathogen transmission and subsequent mortalities in juvenile Pacific oysters *Crassostrea gigas*. *Aquac. Environ. Interact.* 3, 257–273.
- Petton, B., Boudry, P., Alunno-Bruscia, M., Pernet, F., 2015. Factors influencing disease-induced mortality of Pacific oysters *Crassostrea gigas*. *Aquac. Environ. Interact.* 6, 205–222.
- Poranen, M.M., Daugelavičius, R., Bamford, D.H., 2002. Common principles in viral entry. *Annu. Rev. Microbiol.* 56, 521–538.
- Potasman, I., Paz, A., Odeh, M., 2002. Infectious outbreaks associated with bivalve shellfish consumption: a worldwide perspective. *Clin. Infect. Dis.* 35, 921–928.
- Poulin, R., Morand, S., 2000. The diversity of parasites. *Q. Rev. Biol.* 75, 277–293.
- Powell, A., Baker-Austin, C., Wagley, S., Bayley, A., Hartnell, R., 2013. Isolation of pandemic *Vibrio parahaemolyticus* from UK Water and shellfish produce. *Microb. Ecol.* 65, 924–927.
- Power, U.F., Collins, J.K., 1989. Differential depuration of poliovirus, *Escherichia coli*, and a coliphage by the common mussel, *Mytilus edulis*. *Appl. Environ. Microbiol.* 55, 1386–1390.
- Power, U.F., Collins, J.K., 1990. Tissue distribution of a coliphage and *Escherichia coli* in mussels after contamination and depuration. *Appl. Environ. Microbiol.* 56, 803–807.

- Prins, T.C., Smaal, A.C., Dame, R.F., 1998. A review of the feedbacks between bivalve grazing and ecosystem processes. *Aquat. Ecol.* 31, 349–359.
- Provost, K., Dancho, B.A., Ozbay, G., Anderson, R.S., Richards, G.P., Kingsley, D.H., 2011. Hemocytes are sites of enteric virus persistence within oysters. *Appl. Environ. Microbiol.* 77, 8360–8369.
- Pruzzo, C., Gallo, G., Canesi, L., 2005. Persistence of vibrios in marine bivalves: the role of interactions with haemolymph components. *Environ. Microbiol.* 7, 761–772.
- Pujalte, M.J., Ortigosa, M., Macian, M.C., Garay, E., 1999. Aerobic and facultative anaerobic heterotrophic bacteria associated to Mediterranean oysters and seawater. *Internatl. Microbiol.* 2, 259–266.
- Ragan, M.A., MacCallum, G.S., Murphy, C.A., Cannone, J.J., Gutell, R.R., McGladdery, S.E., 2000. Protistan parasite QPX of hard-shell clam *Mercenaria mercenaria* is a member of Labyrinthulomycota. *Dis. Aquat. Org.* 42, 185–190.
- Raghukumar, S., Damare, V.S., 2011. Increasing evidence for the important role of Labyrinthulomycetes in marine ecosystems. *Bot. Mar.* 54, 3–11.
- Ragone Calvo, L.M., Dungan, C.F., Robertson, B.S., Burreson, E.M., 2003. Systematic evaluation of factors controlling *Perkinsus marinus* transmission dynamics in lower Chesapeake Bay. *Dis. Aquat. Org.* 56, 75–86.
- Randolph, S.E., Dobson, A., 2012. Pangloss revisited: a critique of the dilution effect and the biodiversity-buffers-disease paradigm. *Parasitology* 139, 847–863.
- Reid, H.I., Duncan, H.L., Laidler, L.A., Hunter, D., Birkbeck, T.H., 2003. Isolation of *Vibrio tapetis* from cultivated Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture* 221, 65–74.
- Renault, T., Bouquet, A.L., Maurice, J.T., Lupo, C., Blachier, P., 2014. Ostreid herpesvirus 1 Infection among Pacific oyster (*Crassostrea gigas*) spat: relevance of water temperature to virus replication and circulation prior to the onset of mortality. *Appl. Environ. Microbiol.* 80, 5419–5426.
- Renault, T., Lipart, C., Arzul, I., 2001. A herpes-like virus infects a non-ostreid bivalve species: virus replication in *Ruditapes philippinarum* larvae. *Dis. Aquat. Org.* 45, 1–7.
- Renault, T., Novoa, B., 2004. Viruses infecting bivalve molluscs. *Aquat. Living Resour.* 17, 397–409.
- Renault, T.C., Arzul, I., 2001. Herpes-like virus infections in hatchery-reared bivalve larvae in Europe: specific viral detection by PCR. *J. Fish Dis.* 24, 161–167.
- Richards, G.P., McLeod, C., Le Guyader, F.S., 2010. Processing strategies to inactivate enteric viruses in shellfish. *Food Environ. Virol.* 2, 183–193.
- Riisgard, H.U., Larsen, P.S., 1995. Filter-feeding in marine macro-invertebrates: pump characteristics, modeling and energy cost. *Biol. Rev.* 70, 67–106.

- Rippey, S.R., 1994. Infectious diseases associated with molluscan shellfish consumption. *Clin. Microbiol. Rev.* 7, 419–425.
- Robertson, L.J., 2007. The potential for marine bivalve shellfish to act as transmission vehicles for outbreaks of protozoan infections in humans: a review. *Int. J. Food Microbiol.* 120, 201–216.
- Rodrigues, S., Paillard, C., Dufour, A., Bazire, A., 2014. Antibiofilm activity of the marine bacterium *Pseudoalteromonas* sp. 3J6 against *Vibrio tapetis*, the causative Agent of Brown Ring Disease. *Probiotics Antimicrob. Prot.* 7, 45–51.
- Rodriguez, F., Navas, J.I., 1995. A comparison of gill and hemolymph assays for the thioglycollate diagnosis of *Perkinsis atlanticus* (Apicomplexa, Perkinsea) in clams, *Ruditapes decussatus* (L.) and *Ruditapes philippinarum* (Adams et Reeve). *Aquaculture* 132, 145–152.
- Romalde, J.L., Luz Vilariño, M., Beaz, R., Rodríguez, J.M., Díaz, S., Villalba, A., Carballal, M.J., 2007. Evidence of retroviral etiology for disseminated neoplasia in cockles (*Cerastoderma edule*). *J. Invertebr. Pathol.* 94, 95–101.
- Romero, J., Garcia-Varela, M., Laclette, J.P., Espejo, R.T., 2002. Bacterial 16S rRNA gene analysis revealed that bacteria related to *Arcobacter* spp. constitute an Abundant and common component of the oyster microbiota (*Tiostrea chilensis*). *Microb. Ecol.* 44, 365–371.
- Roubal, F.R., Masel, J., Lester, R.J.G., 1989. Studies on *Marteilia sydneyi*, agent of QX disease in the Sydney rock oyster, *Saccostrea commercialis*, with implications for its life cycle. *Aus. J. Mar. Fresh Res.* 40, 155–167.
- Roy, S., Wainberg, M.A., 1988. Role of the mononuclear phagocyte system in the development of acquired immunodeficiency syndrome (AIC). *J. Leukocyte Biol.* 43, 91–97.
- Rubenstein, D.I., Koehl, M.A.R., 1977. Mechanisms of filter-feeding: some theoretical considerations. *Am. Nat.* 111, 981–994.
- Sainz-Hernandez, J.C., Maeda-Martinez, A.N., 2005. Sources of *Vibrio* bacteria in mollusc hatcheries and control methods: a case study. *Aquac. Res.* 36, 1611–1618.
- Sandifer, P.A., Sutton-Grier, A.E., 2014. Connecting stressors, ocean ecosystem services, and human health. *Nat. Resour. Forum* 38, 157–167.
- Sauvage, C., Pépin, J.-F., Lapègue, S., Boudry, P., Renault, T., 2009. Ostreid herpes virus 1 infection in families of the Pacific oyster, *Crassostrea gigas*, during a summer mortality outbreak: differences in viral DNA detection and quantification using real-time PCR. *Virus Res.* 142, 181–187.

- Savin, K.W., Cocks, B.G., Wong, F., Sawbridge, T., Cogan, N., Savage, D., Warner, S., 2010. A neurotropic herpesvirus infecting the gastropod, abalone, shares ancestry with oyster herpesvirus and a herpesvirus associated with the amphioxus genome. *Virology* 7, 308.
- Scanlon, C., Ragone Calvo, L.M., Burreson, E.M., 1997. The potential for transmission of *Perkinsus marinus* by faecal matter from the eastern oyster *Crassostrea virginica*. *J. Shellfish Res.* 16, 332.
- Schikorski, D., Faury, N., Pepin, J.F., Saulnier, D., Tourbiez, D., Renault, T., 2011. Experimental ostreid herpesvirus 1 infection of the Pacific oyster *Crassostrea gigas*: kinetics of virus DNA detection by q-PCR in seawater and in oyster samples. *Virus Res.* 155, 28–34.
- Schmader, K., 2001. Herpes zoster in older adults. *Clin. Infect. Dis.* 32, 1481–1486.
- Schmidt, K.A., Ostfeld, R.S., 2001. Biodiversity and the dilution effect in disease ecology. *Ecology* 82, 609–619.
- Schott, E.J., Vasta, G.R., 2003. The PmSOD1 gene of the protistan parasite *Perkinsus marinus* complements the sod2D mutant of *Saccharomyces cerevisiae*, and directs an iron superoxide dismutase to mitochondria. *Mol. Biochem. Parasitol.* 126, 81–92.
- Segarra, A., Pépin, J.F., Arzul, I., Morga, B., Faury, N., Renault, T., 2010. Detection and description of a particular Ostreid herpesvirus 1 genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas*, in France in 2008. *Virus Res.* 153, 92–99.
- Segarra, A., Faury, N., Pépin, J.F., Renault, T., 2014a. Transcriptomic study of 39 Ostreid herpesvirus 1 genes during an experimental infection. *J. Invertebr. Pathol.* 119, 5–11.
- Segarra, A., Mauduit, F., Faury, N., Trancart, S., Dégremont, L., Toubiez, D., Haffner, P., Barbosa-Solomieu, V., Pépin, J.F., Travers, M.A., Renault, T., 2014b. Dual transcriptomics of virus-host interactions: comparing two Pacific oyster families presenting contrasting susceptibility to Ostreid herpesvirus 1. *BMC Genomics* 15, 580.
- Shimeta, J., Jumars, P.A., 1991. Physical mechanisms and rates of particle capture by suspension-feeders. *Oceanogr. Mar. Biol. Annu. Rev.* 29, 191–257.
- Shumway, S.E., 1985. Particle selection, ingestion, and adsorption in filter-feeding bivalves. *J. Exp. Mar. Biol. Ecol.* 91, 77–92.
- Silverman, H., Achberger, E.C., Lynn, J.W., Dietz, T.H., 1995. Filtration and utilization of laboratory-cultured bacteria by *Dreissena polymorpha*, *Corbicula fluminea*, and *Carunculina texasensis*. *Biol. Bull.* 189, 308–319.
- Simon, M., Grossart, H.-P., Schweitzer, B., Ploug, H., 2002. Microbial ecology of organic aggregates in aquatic ecosystems. *Aquat. Microb. Ecol.* 28, 175–211.

- Sindermann, C.J., Rosenfield, A., 1967. Principal diseases of commercially important marine bivalve mollusca and crustacea. *Fish B-NOAA* 66, 335–385.
- Smolowitz, R., Leavitt, D., Perkins, F., 1998. Observations of a protistan disease similar to QPX in *Mercenaria mercenaria* (hard clams) from the coast of Massachusetts. *J. Invertebr. Pathol.* 71, 9–25.
- Smyth, A.R., Thompson, S.P., Siporin, K.N., Gardner, W.S., McCarthy, M.J., Piehler, M.F., 2012. Assessing nitrogen dynamics throughout the estuarine landscape. *Estuar. Coast.* 36, 44–55.
- Soniat, T., 1996. Epizootiology of *Perkinsus marinus* disease of eastern oysters in the Gulf of Mexico. *J. Shellfish Res.* 15, 35–43.
- Soudant, P., Chu, F.-L.E., 2001. Lipid class and fatty acid composition of the protozoan parasite of oysters, *Perkinsus marinus* cultivated in two different media. *J. Eukaryot. Microbiol.* 48, 309–319.
- Soudant, P., Chu, F.-L.E., Volety, A.K., 2013. Host-parasite interactions: marine bivalve molluscs and protozoan parasites, *Perkinsus* species. *J. Invertebr. Pathol.* 114, 196–216.
- Stokes, N.A., Burreson, E.M., 2001. Differential diagnosis of mixed *Haplosporidium nelsoni* infections in the western oyster, *Crassostrea virginica*, using DNA probes. *J. Shellfish Res.* 20, 207–213.
- Stokes, N.A., Ragone Calvo, L.M., Reece, K.S., Burreson, E.M., 2002. Molecular diagnostics, field validation, and phylogenetic analysis of Quahog Parasite Unknown (QPX), a pathogen of the hard clam *Mercenaria mercenaria*. *Dis. Aquat. Org.* 52, 233–247.
- Sunila, I., Hamilton, R.M., Dungan, C.F., 2001. Ultrastructural characteristics of the in vitro cell cycle of the protozoan pathogen of oysters *Perkinsus marinus*. *J. Eukaryot. Microbiol.* 48, 348–361.
- Sunila, I., Karolus, J., Lang, E.P., Mroczka, M.E., Volk, J., 2000. Transmission of the haplosporidian parasite MSX *Haplosporidium nelsoni* to the eastern oyster *Crassostrea virginica* in an upweller system. *Dis. Aquat. Org.* 42, 152–155.
- Sunila, I., LaBanca, J., 2003. Apoptosis in the pathogenesis of infectious diseases of the eastern oyster *Crassostrea virginica*. *Dis. Aquat. Org.* 56, 163–170.
- Suttle, C.A., 2005. Viruses in the sea. *Nature* 437, 356–361.
- Suttle, C.A., 2007. Marine viruses—major players in the global ecosystem. *Nat. Rev. Microbiol.* 5, 801–812.
- Suttle, C.A., Chen, F., 1992. Mechanisms and rates of decay of marine viruses in seawater. *Appl. Environ. Microbiol.* 58, 3721–3729.
- Swaddle, J.P., Calos, S.E., 2008. Increased avian diversity is associated with lower incidence of human West Nile infection: observation of the dilution effect. *PLoS One* 3, e2488.

- Tasumi, S., Vasta, G.R., 2007. A galectin of unique domain organization from hemocytes of the eastern oyster (*Crassostrea virginica*) Is a receptor for the protistan parasite *Perkinsus marinus*. *J. Immunol.* 179, 3086–3098.
- Thieltges, D.W., Amundsen, P.-A., Hechinger, R.F., Johnson, P.T.J., Lafferty, K.D., Mouritsen, K.N., Preston, D.L., Reise, K., Zander, C.D., Poulin, R., 2013. Parasites as prey in aquatic food webs: implications for predator infection and parasite transmission. *Oikos* 122, 1473–1482.
- Thieltges, D.W., Bordalo, M.D., Caballero Hernandez, A., Prinz, K., Jensen, K.T., 2008. Ambient fauna impairs parasite transmission in a marine parasite-host system. *Parasitology* 135, 1111–1116.
- Thompson, R.M., Mouritsen, K.N., Poulin, R., 2005. Importance of parasites and their life cycle characteristics in determining the structure of a large marine food web. *J. Anim. Ecol.* 74, 77–85.
- Thongchan, J., Bhoopong, P., Yingkajorn, M., Nishibuchi, M., Vuddhakul, V., 2013. Total number, virulence genes, and heterogeneity of *Vibrio parahaemolyticus* in a single shellfish. *Sci. Asia* 39, 230–235.
- Tsui, C.K.M., Marshall, W., Yokoyama, R., Honda, D., Lippmeier, J.C., Craven, K.D., Peterson, P.D., Berbee, M.L., 2009. Labyrinthulomycetes phylogeny and its implications for the evolutionary loss of chloroplasts and gain of ectoplasmic gliding. *Mol. Phylogenet. Evol.* 50, 129–140.
- Ueki, Y., Shoji, M., Suto, A., Tanabe, T., Okimura, Y., Kikuchi, Y., Saito, N., Sano, D., Omura, T., 2007. Persistence of caliciviruses in artificially contaminated oysters during depuration. *Appl. Environ. Microbiol.* 73, 5698–5701.
- Valle Levinson, A., Wong, K., 2000. Fortnightly variability in the transverse dynamics of a coastal plain estuary. *J. Geophys. Res.*, 3413–3423
- Vigneron, V., Sollic, G., Montanie, H., Renault, T., 2004. Detection of Ostreid Herpesvirus 1 (OsHV-1) DNA in seawater by PCR: influence of water parameters in bioassays. *Dis. Aquat. Org.* 62, 35–44.
- Villalba, A., Casas, S.M., Lopez, C., Carballal, M.J., 2005. Study of perkinsosis in the carpet shell clam *Tapes decussatus* in Galicia (NW Spain). II. Temporal pattern of disease dynamics and association with clam mortality. *Dis. Aquat. Org.* 65, 257–267.
- Villalba, A., Reece, K.S., Camino Ordás, M., Casas, S.M., Figueras, A., 2004. Perkinsosis in molluscs: a review. *Aquat. Living Resour.* 17, 411–432.
- Volety, A.K., Chu, F.-L.E., 1995. Suppression of chemiluminescence of eastern oyster (*Crassostrea virginica*) hemocytes by the protozoan parasite *Perkinsus marinus*. *Dev. Comp. Immunol.* 19, 135–142.
- Wagner-Dobler, I., Biebl, H., 2006. Environmental biology of the marine *Reseobacter* lineage. *Annu. Rev. Microbiol.* 60, 255–280.
- Ward, J.E., 1996. Biodynamics of suspension-feeding in adult bivalve molluscs: particle capture, processing, and fate. *Invertebr. Biol.* 115, 218–231.

- Ward, J.E., Levinton, J.S., Shumway, S.E., Cucci, T., 1998. Particle sorting in bivalves: in vivo determination of the pallial organs of selection. *Mar. Biol.* 131, 283–292.
- Ward, J.E., Shumway, S.E., 2004. Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. *J. Exp. Mar. Biol. Ecol.* 300, 83–130.
- Weinbauer, M.G., Bettarel, Y., Cattaneo, R., Luef, B., Maier, C., Motegi, C., Peduzzi, P., Mari, X., 2009. Viral ecology of organic and inorganic particles in aquatic systems: avenues for further research. *Aquat. Microb. Ecol.* 57, 321–341.
- Wesche, S.J., Adlard, R.D., Lester, R.J.G., 1999. Survival of spores of the oyster pathogen *Marteilia sydneyi* (Protozoa, Paramyxia) as assessed using fluorogenic dyes. *Dis. Aquat. Org.* 36, 221–226.
- White, M.E., Powell, E.N., Ray, S.M., Wilson, E.A., 1987. Host-to-host transmission of *Perkinsus marinus* in oyster *Crassostrea virginica* populations by the ectoparasitic snail *Boonea impressa* (Pyramidellidae). *J. Shellfish Res.* 6, 1–5.
- Whittington, R.J., Hick, P.M., Evans, O., Rubio, A., Alford, B., Dhand, N., Paul-Pont, I., 2015a. Protection of Pacific oyster (*Crassostrea gigas*) spat from mortality due to Ostreid herpesvirus 1 (OsHV-1 Ivar) using simple treatments of incoming seawater in land-based upwellers. *Aquaculture* 437, 10–20.
- Whittington, R.J., Dhand, N.K., Evans, O., Paul-Pont, I., 2015b. Further observations on the influence of husbandry practices on OsHV-1 IVar mortality in Pacific oysters *Crassostrea gigas*: age, cultivation structures and growing height. *Aquaculture* 438, 82–97.
- Wilcox, R.M., Fuhrman, J.A., 1994. Bacterial viruses in coastal seawater: lytic rather than lysogenic production. *Mar. Ecol. Prog. Ser.* 114, 35–45.
- Wilhelm, S.W., Weinbauer, M.G., Suttle, C.A., Pledger, R.J., Mitchell, D.L., 1998. Measurements of DNA damage and photoreactivation imply that most viruses in marine surface waters are infective. *Aquat. Microb. Ecol.* 14, 215–222.
- Wolf, K., Darlington, R.W., 1971. Channel catfish virus: a new herpesvirus of ictaurid fish. *J. Virol.* 8, 525–533.
- Wommack, K.E., Colwell, R.R., 2000. Virioplankton: viruses in aquatic ecosystems. *Microbiol. Mol. Biol. R.* 46, 69–114.
- Wong, K.-C., Garvine, R.W., 1984. Observations of wind-induced subtidal variability in the Delaware estuary. *J. Geophys. Res.* 89, 10589–10597.
- Wong, K.C., 1994. On the nature of transverse variability in a coastal plain estuary. *J. Geophys. Res.* 99, 14209–14222.
- Wood, C.L., Lafferty, K.D., 2013. Biodiversity and disease: a synthesis of ecological perspectives on Lyme disease transmission. *Trends Ecol. Evol.* 28, 239–247.

- Wood, J.L., Andrews, J.D., 1962. Haplosporidium costale (Sporozoa) associated with a disease of Virginia oysters. *Science* 136, 710–711.
- Wootton, E.C., Dyrinda, E.A., Ratcliffe, N.A., 2003. Bivalve immunity: comparisons between the marine mussel (*Mytilus edulis*), the edible cockle (*Cerastoderma edule*) and the razor-shell (*Ensis siliqua*). *Fish Shellfish Immun.* 15, 195–210.
- Wright, A.C., Ahmed, H., Gauthier, J.D., Silva, A.M., Vasta, G.R., 2002. CDNA cloning and characterization of two iron superoxide dismutases from the oyster parasite *Perkinsus marinus*. *Mol. Biochem. Parasit.* 123, 73–77.
- Wyatt, R., Sodroski, J., 1998. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. *Science* 280, 1884–1888.
- Yildiz, F.H., Visick, K.L., 2009. *Vibrio* biofilms: so much the same yet so different. *Trends Microbiol.* 17, 109–118.
- Zhang, L., Li, L., Zhu, Y., Zhang, G., Guo, X., 2014. Transcriptome analysis reveals a rich gene set related to innate immunity in the eastern oyster (*Crassostrea virginica*). *Mar. Biotechnol.* 16, 17–33.
- Zhang, L., Li, L., Guo, X., Litman, G.W., Dishaw, L.J., Zhang, G., 2015. Massive expansion and functional divergence of innate immune genes in a protostome. *Sci. Rep.* 5, 8693.
- Zlotkin, A., Chilmonczyk, S., Eynigor, M., Hurvitz, A., Ghittino, C., Eldar, A., 2003. Trojan Horse Effect: phagocyte-mediated *Streptococcus iniae* Infection of fish. *Infect. Immun.* 71, 2318–2325.