Environmental Research 161 (2018) 425–438

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Alteration of host-pathogen interactions in the wake of climate change – Increasing risk for shellfish associated infections?

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ARTICLE INFO

Keywords:
Climate change
Ocean acidification
Sea surface temperature
Manganese
Hypoxia
Marine invertebrates
Human pathogens

ABSTRACT

The potential for climate-related spread of infectious diseases through marine systems has been highlighted in several reports. With this review we want to draw attention to less recognized mechanisms behind vector-borne transmission pathways to humans. We have focused on how the immune systems of edible marine shellfish, the blue mussels and Norway lobsters, are affected by climate related environmental stressors. Future ocean acidification (OA) and warming due to climate change constitute a gradually increasing persistent stress with negative trade-off for many organisms. In addition, the stress of recurrent hypoxia, inducing high levels of bioavailable manganese (Mn) is likely to increase in line with climate change. We summarized that OA, hypoxia and elevated levels of Mn did have an overall negative effect on immunity, in some cases also with synergistic effects. On the other hand, moderate increase in temperature seems to have a stimulating effect on antimicrobial activity and may in a future warming scenario counteract the negative effects. However, rising sea surface temperature and climate events causing high land run-off promote the abundance of naturally occurring pathogenic *Vibrio* and will in addition, bring enteric pathogens which are circulating in society into coastal waters. Moreover, the observed impairments of the immune defense enhance the persistence and occurrence of pathogens in shellfish. This may increase the risk for direct transmission of pathogens to consumers. It is thus essential that in the wake of climate change, sanitary control of coastal waters and seafood must recognize and adapt to the expected alteration of host-pathogen interactions.

1. Introduction

The ongoing global climate changes are predicted to proceed in the next hundred years (IPCC, 2014). The green-house effect, mainly caused by increasing anthropogenic emission of carbon dioxide (CO_2), will gradually increase the average temperature of the Earth´s atmosphere by 1.8–4.0 °C by 2100 and also elevate sea surface temperature (SST). Moreover, absorption of CO_2 by the oceans is influencing seawater chemistry, with a subsequent decrease in pH values and the calcium carbonate ($CaCO_3$) saturation state (Orr et al., 2005; Doney et al., 2009). Ocean acidification (OA), has already caused a reduction in ocean pH values of about 0.1 units in comparison to pre-industrial levels. A further reduction of approximately 0.4 pH units is predicted for the end of this century (Caldeira and Wickett, 2003; Raven et al., 2005; IPCC, 2014).

Global warming is expected to occur heterogeneously, with high latitudes warming faster than mid-latitudes, and winters warming more than summers (IPCC, 2014). In the Northern hemisphere, changes in the hydrological cycle are assumed to increase precipitation, which will affect coastal salinity and inputs of terrestrial-derived pollutants and nutrients. The surplus of nutrients generates an overload of primary production, exacerbated by increasing water temperatures as a consequence of global warming (IPCC, 2014). This combined with overfishing that causes cascade effects between trophic levels (Frank et al., 2005; Casini et al., 2009; Carstensen et al., 2014) will, in turn, increase the biomass decomposed below the pycnocline. The degradation process creates periods of hypoxia, which is an expanding problem all over the Northern hemisphere (Diaz and Rosenberg, 2008), especially as climate change most likely will strengthen stratification of water masses (Rabalais et al., 2010). As the oxygen saturation of water decreases with increasing temperature, the lower saturation becomes an aggravating stressor for the organisms.

Moreover, during hypoxic periods manganese (Mn) which normally stays oxidized in the bottom sediments, becomes reduced and released into more bioavailable forms. Mn is one of the most abundant metals in soft bottoms of the oceans (Post, 1999) and predominantly bound to the

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https://doi.org/10.1016/j.envres.2017.11.032
Received 3 July 2017; Received in revised form 19 November 2017; Accepted 20 November 2017

sediment in a four-valent colloid state, MnO_2. During hypoxic periods, lower than 20% of air saturation, the concentration of redox mobilized Mn can increase by a factor of 1000 and reach $\sim 20\ mg\ L^{-1}$ (Trefry et al., 1984; Magnusson et al., 1996) in contrast to e.g. Cd, Cu, Pb and Zn, which get more strongly bound during hypoxia (Gerringa, 1991). In 1990, the first observation of Mn precipitation after hypoxic events was reported on gills of Norway lobsters (Baden et al., 1990). After that several investigations have verified bioavailability and accumulation of Mn in marine invertebrates during hypoxia (Baden et al., 1994; Nordahl-Hansen and Bjerregaard, 1995; Baden and Neil, 1998, 2003; Draxler et al., 2005; Magel et al., 2009).

Manganese plays essential roles in many metabolic functions, cellular protection, bone and skeleton mineralization processes and reproductive mechanisms (ATSDR, 2008; Santamaria, 2008). However, it has long been known that humans inhaling high concentrations of Mn-dust can get a central nervous disruption called manganism, with similarities to Parkinson´s disease (Iregren, 1990; Verity, 1999). Marine invertebrates exposed to Mn-concentrations likely to be found in hypoxic bottom waters have manifested significant neurotoxic (Baden and Neil, 1998; Holmes et al., 1999; Sköld et al., 2015), behavioural (Krång and Rosenqvist, 2006) and immunotoxic effects (Hernroth et al., 2004, 2015; Oweson et al., 2006, 2008; Oweson and Hernroth, 2009) as well as disruption of embryonal morphogenesis (Pinsino et al., 2010, 2011).

The future scenario of stressors, directly caused by climate change (increasing SST, decreasing pH; hypercapnia, reduced saturation of carbonate minerals) has shown to be of great importance for the well-being of marine organisms and ecosystems (Kroeker et al., 2010, 2013). Meta-analysis of impact of OA and warming revealed decreased survival, calcification, growth, development and abundance, in response to acidification and with enhanced tendency at higher temperatures, but with great differences between taxa (Kroeker et al., 2013). Several additional stress factors will be more common in coastal waters due to indirect effects of climate change such as increased precipitation causing more land run-off (Trenberth, 2011), leading to lower salinity, increased load of colored dissolved organic matter and changes in phytoplankton composition (Harvey et al., 2015). Obviously, the stressors one by one or in combination could impact biota differently depending e.g. on intensity and timing of each stressor, the structure of habitats and adaptation or the life stage of the organisms (Byrne and Przeslawski, 2013; Gunderson et al., 2016), making interpretation of climate impact on ecosystems in future scenarios complex.

For a very large part of the human population the oceans provide the resources needed to have an income and getting the protein needed to stay healthy. As humans are “fishing down the food web” (Pauly et al., 1998; Jackson et al., 2001) the consumption of invertebrates is globally increasing. Impaired immunity of the invertebrates could decrease their fitness and biomass and as well prolong the residence time of pathogens with enhanced risk for transmission to consumers. However, vulnerability and response of marine organisms to climate change are highly variable and there is a considerable lack of knowledge about the impacts on host-pathogen relationships (Burge et al., 2014). During the last decade our research group has gathered data on how different environmental stressors related to climate change (temperature, OA, hypoxia, excess of Mn) affect the immunity of marine bivalves and crustaceans (as illustrated in Fig. 1). In addition, we have conducted both field and laboratory studies of environmental impact (focusing particularly on land run-off and temperature) on prevalence and survival of human pathogens in marine environment. However, subsequent consequences of these climate related stressors on alteration of host-pathogen interactions, and ultimately the risk for transmission of pathogens to human consumers have so far not been compiled. Being aware of all limitations, the present review, based on our own and literature data, will focus on how these direct and indirect stressors caused by climate change, may exacerbate the incidence of human infectious diseases.

2. Impact of environmental stressors on marine invertebrate immunity

2.1. Model organisms

This review has focused on the potential impact of climate change on immunological functions of crustaceans and bivalve mollusks, used for human consumption. The crustacean is here represented by Norway lobster, *Nephrops norvegicus* (L.), which is distributed all along the eastern coastline of the Atlantic, from Iceland to, and including the Mediterranean Sea. It is a stationary inhabitant of soft bottom sediments where it occupies borrows at depths below the pycnocline. Natural variations in pH of seawater occur with temperature and season but is in borrows even more variable. However, *N. norvegicus* is known to be relatively pH tolerant and can counteract low pH through stirring activities increasing water circulation (Zhu et al., 2006). The studies on bivalve mollusks have mainly focused on the blue mussel, *Mytilus edulis* (L.). It is common on hard and sandy bottoms at a broad range of depths (1–10 m) in Northern Atlantic, in the Pacific around Japan but also in the estuarine Baltic Sea. Bivalves are sessile filter feeders and can utilize microorganisms as food resource. Therefore, they constitute a particular risk for transmission of pathogens to human consumers (Wilson and Moore, 1996; Lees, 2000; Potasman et al., 2002; Rehnstam-Holm and Hernroth, 2005).

2.1.1. Invertebrate immunity

With the current discoveries of the wide variety of genes, which are essential in immunity and non-self-recognition (as reviewed by Ghosh et al. (2011)), there is no doubt that invertebrates, although lacking adaptive immunity, are well prepared to face invading pathogens. They are particularly dependent on the immune response (briefly summarized in Table 1) of immunocytes, which in crustaceans and bivalves are called hemocytes. These are developed from proliferating stem cells in the hematopoietic tissue (Hpt), localized as described in Table 1. The Hpt progenitors are further differentiated to different categories of circulating hemocytes. Granulae of lobster hemocytes contain the so called pro-phenol oxidase activating system (ProPO-AS) that through degranulation can be released to act extracellularly. Activation of this zymogen package induces a cascade of reactions including the bactericidal enzyme phenoloxidase, which further catalases the production of toxic quinone intermediates. The end product is melanin which encapsulates the invaders to prevent spreading of the infection (Söderhäll and Cerenius, 1998; Johansson et al., 2000; Cerenius and Söderhäll, 2004; Cerenius et al., 2010). In contrast to the crustaceans the immune defense of bivalve mollusks is not very dependent on ProPO-AS but mainly based on the high capacity of phagocytosis and encapsulation, including central bactericidal mechanisms such as reactive oxygen radicals and lysosomal enzymes (Cheng, 1983; Leippe and Renwrantz, 1988; Pipe, 1990, 1992; Song et al., 2010).

An increasing number of constitutive and inducible antimicrobial peptides (AMPs) have been demonstrated important for immune defense in all phyla (reviewed by e.g. Zasloff, 2002; Yeaman and Yount, 2003). The most prominent groups of crustacean AMPs are the crustins and penaeidins (reviewed by Smith et al. (2008) and Cuthbertson et al. (2002), respectively). However, according to Cerenius et al. (2010) the upregulation of these in response to microbial challenge is limited. In contrast, AMPs seem to play a particular role in the defense of *M. edulis* (Wotton et al., 2003). *M. edulis* has shown to express three different kinds of AMPs; mytilin, mytimicin and defensins (reviewed by Tincu and Taylor (2004)). Recently, myticin A that previously has been reported from the closely related mussel, *M. galloprovincialis* (Mitta et al., 1999) was found upregulated in gill epithelium of *M. edulis* in response to LPS (Hernroth et al., 2016; Hörnaeus et al., 2016).

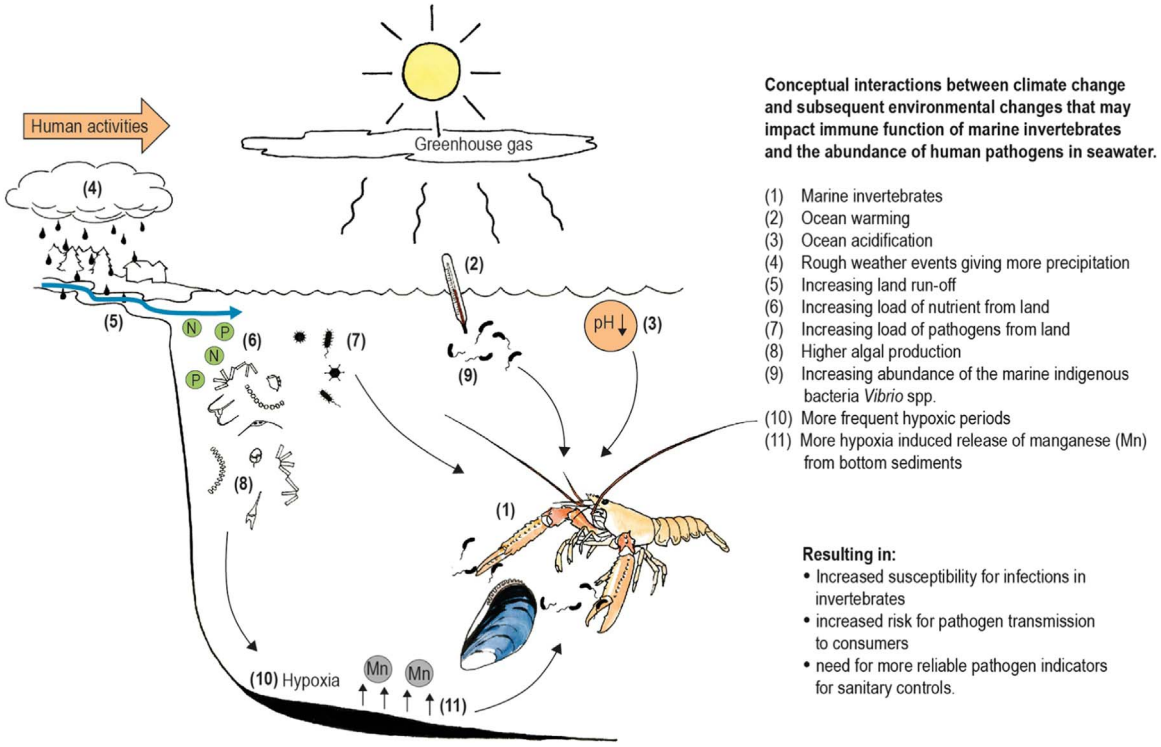

Fig. 1. Illustration of the links between climate change related parameters (increasing sea surface temperature, land run-off, hypoxia, bioavailable Mn) that affect host-parasite interaction and are discussed in this review. *Illustration Maj Persson*.

2.2. Direct and indirect effects of climate change on immunity

Ocean acidification and elevated temperature in the footsteps of climate change are gradually progressing stressors in coming decades. Giving the wide distribution of Mn in bottoms of the oceans, as part of the biogeochemical diagenetic cycle, periodic occurrence of Mn is supposed to become more frequent (Canfield et al., 1993) along with the global increase of hypoxic events (Diaz and Rosenberg, 2008; Rabalais et al., 2010). In the studies on adult *N. norvegicus* and *M. edulis* we have focused on species relevant, mostly functional, defense mechanisms to explore direct (OA and temperature) and indirect (hypoxia and Mn) sub lethal effects on immunity (briefly summarized in Table 1). Although using relatively long-term exposure (up to 4 months) our experiments have great limitations in terms of the possibility to mimic these scenarios in a dynamic, multi stressed environment under future long-term climate change. However, our results on effects on immunity, could when used in a careful way, be valuable for interpretations of future host-pathogen alterations.

2.2.1. Elevated temperature

Marine invertebrates are thermo-conformers known to maintain energy balance within the temperature zone of their distribution. In temperate regions, they exhibit a seasonal cycle in activities such as feeding, growth and reproduction. During winter temperatures, the metabolic activities are low and increase with the seasonal rise of temperature. However, when a high critical temperature threshold is reached, the oxygen levels in the organisms will be insufficient, depressing physiological activities (Pörtner, 2002). Thus, both decreasing and increasing temperature may cause stress. Experimental studies on *N. norvegicus* carried out after gradual adaptation to 5, 10, 12, 14, 16, and 18 °C demonstrated after 4 months exposure that the temperature within this range did not affect lobster immune responses (Hernroth et al., 2012).

In the Pacific oyster, *Crassostrea gigas,* temperature affected the expression of genes involved in antiviral response, which were inhibited at 12 °C but activated at 22 °C (Green et al., 2014). Moreover, experimental studies of *M. edulis* have demonstrated a significantly higher antimicrobial capacity of hemocytes at 20 °C compared to that of 6 °C. This was revealed both through investigation of hemocyte activity against *Salmonella enterica* serotype *Typhimurium* (*S. enterica*) (Hernroth, 2003a) and the activity of AMP (extracted from hemocytes) against *Escherichia coli* (Hernroth, 2003b). However, the outcome of temperature stimulated antimicrobial activity was shown negatively affected by increasing virulence of the bacteria (Hernroth, 2003a). There, hemocytes were tested against four different mutants of *S. enterica*, which to different degree were deficient in the synthesis of their *O*-antigen polysaccharide chains and core sugars of LPS (the mutants have previously been described by Stendahl and Edebo (1972)), and it was obvious that the hemocytes were most efficient in degrading the mutant with most reduced LPS. The endotoxic effects of the lipid A component of LPS and its polysaccharide chains are known to provide gram negative bacteria with resistance to defense mechanisms such as opsonization, phagocytosis and intracellular degradation (Lindberg et al., 1991). Accordingly, when inoculated in the hemolymph of *M. edulis* it was shown that intact LPS protected *S. enterica* from being degraded and thereby gave them the advantage at the higher temperature to replicate with lethal effect on the mussels (Hernroth, 2003a).

In addition, the number of pathogens which the immune system has to fight is of course of great importance for the degree of success. Filter feeders take up microbes from the surrounding water via the gills, which retain particles mainly based on their size and densities (Mölenberg and Riisgård, 1978; Riisgård, 1988; Brilliant and Mac Donald, 2000) but probably as well based on aspects such as shape, motility, and chemical cues (Newell et al., 1989; Allison et al., 1998; Hernroth et al., 2002a). Similar factors may be involved also during the rejection process on the labial palps. When labeling *S. enterica* with SnF_2 to reduce the negative charge of their cell surface (Hernroth et al., 2000), the mussel's uptake of the bacteria increased significantly. Also, when offering different strains of *V. cholera* to *M. edulis* considerable

Table 1
Experimental studies on marine shellfish response and host-pathogen interactions, in relation to the climate change stressors: Temperature (**Temp**), Ocean acidification (**OA**) with pH decrease of ~0.4, **Hypoxia** (< 20%), and Manganese (**Mn**; 10–20 mg/L). As described in Section 2.2 the experiments intend to mimic OA as a persistent stressor while Hypoxia and Mn are periodically occurring stressors. Chosen temperatures mostly reflect the known limit of upper tolerance of the species. L-T = Long-term exposure (months); M-T = Medium-term (one month); S-T = Short-term exposure (days/weeks). To exemplify alteration of host-pathogen interactions *Vibrio* spp serve as a model-pathogen.

Invertebrate class	External barrier against pathogens and predators	Immune active hemocytes	Main immune active mechanism	Immune response relative to control under **the climate change stressors**.	Examples of altered host capacity to reduce *Vibrio* under **the climate change stressors**
Crustaceans	Calcite $CaCO_3$ with trigonal symmetry[1]	Hemocytes (THC ~15 × 10^6 ml^{-1})[2–4]: Granular cells, semigranular cells, hyalinocytes[5]. HPT on dorsal side of stomach[5]	Degranulation and activation of the ProPO cascade producing bacteriocidal components and melanization (granular cells). Phagocytosis (hyaline cells)[13–15]	**Temp (L-T):** No effect on immune parameters within the range of 5–18 °C in *N. norvegicus*[4]. **OA (L-T):** Acidosis and protein damage in *N. norvegicus*[4] **Hypoxia (S-T):** Decreased ProPO-activity in the Atlantic blue crab, *Callinectes sapidus*[23] **Mn (S-T):** Inhibition of degranulation, ProPo-AS, proliferation of hematopoietic precursors[2], and induction of apoptosis in hematopoietic cells[3] of *N. norvegicus* **OA (L-T) followed by Mn (S-T):** THC, ProPO-AS and phagocytosis decreased in *N. norvegicus*[24]. **OA (L-T) followed by hypoxia (S-T):** Synergistic effects on THC reduction in *N. norvegicus*[24].	**Hypoxia (S-T):** Reduced bacteriostatic capacity against *V. campbellii* in the Pacific white shrimp *Litopenaeus vannamei*[32] and in *C. sapidus*[33]. Increased susceptibility for *V. alginolyticus* in the Blue shrimp, *Penaeus stylirostris* [34] **Mn (S-T):** *V. parahaemolyticus* able to multiply in hepatopancreas of *N. norvegicus*[8]. **OA (L-T) followed by Hypoxia or Mn (S-T):** Reduced bacteriostatic effect on inoculated *V. parahaemolyticus* in *N. norvegicus*[24]
Mollusks	Aragonite $CaCO_3$ with orthorhombic symmetry, which is more soluble than calcite[1]	Hemocytes (THC ~2 × 10^6 ml^{-1})[6–9]: Granular cells (eosinophilic and basophilic)[10–11]. HPT in gills of oysters[12]	Phagocytosis, encapsulation, production of bactericidal oxygen radicals, lysosomal enzymes[16-18] and AMPs[19-22]	**Temp (S-T):** Higher bacteriostatic capacity[25] and AMP-activity at 20 °C compared to 6° in *M. edulis*[7] **OA (L-T):** Acidosis, thickness and structure of shells affected[9], inhibition of AMP activity on gills[21], THC unaffected [9,26,27], phagocytic activity reduced[26] in *M. edulis* **Hypoxia (S-T)**: Reduced THC and lysosomal content in the Asian green mussel *Perna viridis*[28]. No significant differences of THC in the abalone *Haliotis diversicolor* [29] nor in the oyster *Crassostrea virginica* [30]. Reduced ROS-activity in *C. virginica*[31] **Mn (S-T):** THC reduced in *M. edulis*[8].	**Temp (S-T):** *V. vulnificus* able to multiply in *C. virginica* stored at high temperature[35] **OA (L-T):** Reduced capacity of *M. edulis* to eliminate *V. tubiashii*[9] **Hypoxia (S-T):** Reduced clearance capacity of *V. parahaemolyticus* by *H. diversicolor*[29] and of *V. campellii* by *C. virginica*[30] **Mn (S-T):** Reduced clearance capacity of *V. parahaemolyticus* by *M. edulis*[8].

THC = Total hemocyte counts; HPT = Hematopoetic tissue; ProPO-AS = Prophenoloxidase-activating system, AMP = Antimicrobial peptide, ROS = reactive oxygen species.
Marine invertebrate classes are in this review represented by the model organisms (*Nephrops norvegicus* and *Mytilus edulis,* respectively) described in Section 2.1 and closely related species.
References: [1]Mucci, 1983; [2]Hernroth et al., 2004; [3]Oweson et al., 2006; [4]Hernroth et al., 2012; [5]Chaga et al., 1995; [6]St-Jean et al., 2002; [7]Hernroth, 2003b; [8]Oweson and Hernroth, 2009; [9]Asplund et al., 2013; [10]Pipe et al., 1997; [11]Dyrynda et al., 1997; [12]Jemaà et al., 2014; [13]Söderhäll and Cerenius, 1992; [14]Söderhäll and Cerenius, 1998; [15]Johansson et al., 2000; [16]Cheng, 1983; [17]Leippe and Renwrantz, 1988; [18]Pipe, 1992; [19]Tincu and Taylor, 2004; [20]Wotton et al., 2003; [21]Hernroth et al., 2016; [22]Hörnaeus et al., 2016; [23]Tanner et al., 2006; [24]Hernroth et al., 2015; [25]Hernroth 2003a; [26]Bibby et al., 2008; [27]Mackenzie et al., 2014; [28]Wang et al., 2012; [29]Cheng et al., 2004; [30]Macey et al., 2008; [31] Boyd and Burnett, 1999; [32]Burgents et al., 2005; [33]Holman et al., 2004; [34]Le Moullac et al., 1998; [35]Kaspar and Tamplin, 1993

differences were observed. The uptake of the human virulent pandemic strain El Tor was barely detectable while less virulent clinical strains and strains isolated from marine estuaries were accumulated in the mussels. On the other hand, the more virulent strains persisted longer in the tissue of *M. edulis* (Collin et al., 2012).

Altogether, these experimental studies indicate that a temperature considered moderately high at latitudes of distribution stimulates both the immune response of the mussels and the immune resistance of the bacteria. The outcome of the host-pathogen interactions is therefore probably determined by the amount of ingested or invading pathogens but even more decisive is their virulence. Studies on coral reefs make known that if pathogens are virulent enough to establish an infection before the temperature-boosted immune systems of the corals are able to act, warmer conditions could have significant implications on host survival (Ward et al., 2007; Mydlarz et al., 2009). Accordingly, infectious diseases causing mass mortality among diverse marine invertebrates have been linked to heat waves (Farley et al., 1972; Le Deuff et al., 1996; Bates et al., 2009; Burge et al., 2014; Sweet et al., 2016). In cases when host and pathogen thermal optima are particularly divergent the effect of temperature may be more pronounced (Elliot et al., 2002). Thus, studies on temperature effect on invertebrate immunity and susceptibility for infections have to include investigations of the temperature effect on the dynamics of the particular pathogens.

2.2.2. Ocean acidification

In our experiments, we exposed the animals to CO_2 manipulated seawater in order to reduce pH by approximately 0.4 units, which is expected by the end of this century (Caldeira and Wickett, 2003). Measurements of pH_{nbs} of cell free hemolymph of both *M. edulis* (Asplund et al., 2013) and *N. norvegicus* (Hernroth et al., 2012) indicated that OA induced acidosis relative to the control animals. Hypercapnia, caused by increased pCO_2 giving decreased hemolymph pH, occurs regularly in e.g. intertidal mussels (Lindinger et al., 1984) and is metabolically costly to overcome (Gazeau et al., 2007). However, the tidal rhythm allows recovery while the predicted chronical exposure to OA constitutes a major threat to many calcifying organisms as acidosis could be counteracted through dissolution of $CaCO_3$ from the shells (Guinotte and Fabry, 2008). Exposure to OA (pH 7.7) for 4 months was enough to generate significant disruption in the shell structures and inhibited growth of *M. edulis* (Asplund et al., 2013). This was in accordance with the registered malformation and/or dissolution of the growing edge of the shells of the pearl oyster *Pinctada fucata* after being exposed to OA (pH 7.6) for 28 days (Welladsen et al., 2010).

Indeed, intact shells are needed for protection against invading pathogens but also the gills constitute a barrier, of particular importance for filter feeding organisms. Both in the studies on uptake of virus (Hernroth et al., 2000) and of bacteria (λ) a large proportion of the microbes were found accumulated along the gills of *M. edulis*. Most degrading mechanisms of immunity are acting in acidic compartments of hemocytes (Beaven and Paynter, 1999). Gill epithelium is in direct contact with the pH of ambient seawater and thus of particular interest when exploring impact of OA on immunity. In the study by Hernroth et al. (2016) AMPs were extracted from gill tissue of *M. edulis* and the antimicrobial capacity against *V. parahaemolyticus*, *V. tubiashii*, *V. splendidus*, *V. alginolyticus* and *E. coli* was tested *in vitro*. The bacteria were exposed to the AMP-extracts diluted in PBS at pH 8.1 (Control) and 7.7 (OA), respectively. Already after one hour of incubation, the metabolic activity of the bacteria was reduced by ~ 65–90%, depending on bacterial species, but the reduction was in no case affected by pH. However, when pre-exposing *M. edulis* to these pH regimes for 4 months before extracting the AMPs, their capacity to inhibit bacterial growth was significantly reduced. This was tested against a strain of *V. parahaemolyticus* which in a recentt study showed unaffected growth rate, survival and hemolytic capacity in the lowered pH (Hernroth et al., 2015). Therefore, the reduction of the antimicrobial capacity was most probably due to alteration of AMPs´ activity or modulation of the composition of AMPs, which has been seen induced in OA-exposed *M. chilensis* (Castillo et al., 2017). However, it might be that oxidative stress caused by hypercapnic acidosis, following OA, disrupts not only transcription, but also translation and completion of protein synthesis (Pörtner et al., 2011; Tomanek et al., 2011).

In a study of potential impact of OA on immunity of *M. edulis,* Bibby et al. (2008) demonstrated that total hemocyte counts (THC) was unaffected after 16 and 32 days of exposure to pH 7.7 at approximately 16.5 °C, but the phagocytic activity was significantly reduced. Mackenzie et al. (2014) did not see any pH effect on THC or phagocytic capacity when *M. edulis* was exposed to pH 7.65 at 12 °C for six months and the same results were revealed in the study by Asplund et al. (2013) where the mussels were exposed to OA (pH 7.7) for 4 months at 14 °C. However, when OA exposed mussels were inoculated with a sub-lethal dose of *V. tubiashii* in the adductor muscle, the bacteria managed to multiply, in both the hemolymph and the hepatopancreas within 24 h post injection. The number of bacteria in the control mussels, kept in ambient sea water (pH 8.1), was on the contrary suppressed (Asplund et al., 2013). Similar affected bacteriostatic impairment against inoculated *V. harveyi* was demonstrated in the blood clam *Tegillarca granosa*. (Zha et al., 2017) after 30 days of exposure to OA (pH 7.7). The declined ability to fight the bacteria indicates that exposure to OA will increase bivalve susceptibility for infections. It also points out that other defense mechanisms in addition to that of phagocytic activity of mussel hemocytes are affected by OA. Previous studies have shown that humoral components, such as lysozyme activity in cell free hemolymph of *M. galloprovincialis*, are inhibited after exposure to pH 7.7 for seven days (Matozzo et al., 2012) and as mentioned above, reduced AMP activity was noted in *M. edulis* after OA exposure (Hernroth et al., 2016).

Under circumstances where the antioxidant systems are insufficient in meeting oxidative stress, ROS products will react with proteins, lipids and DNA and impair their functions (Benzie, 2000). After 28 days exposure to OA (pH 7.55) such reactions with increased level of ROS production and number of apoptotic hemocytes were demonstrated in the oyster *C. gigas* (Wang et al., 2016). In *N. norvegicus* OA (pH 7.7) exposure for 4 months caused an oxidative damage of proteins. This could be noted within the temperature range of 10–18 °C but not at 5 °C (Hernroth et al., 2012). It appears that when lobsters are under pressure of OA their oxidative defense is unable to counteract the level of ROS-products, created by increased metabolic rate at higher temperature. It should be pointed out that damaged proteins did not increase within the temperature range of 10–18 °C, despite the fact that the highest temperature corresponds to the maximum for the species' distribution. Moreover, at these temperatures OA caused reduction of THC by 50% and the phagocytic capacity of remaining hemocytes by 60%. Such an effect of the activation of pro-phenoloxidase was not demonstrated, but given the reduced number of hemocytes their total capacity to utilize ProPO-AS should be considerably impaired by OA. In another study (Hernroth et al., 2015) where *N. norvegicus* was exposed to OA (pH 7.7) for a shorter period (8 weeks at 12 °C), the bacteriostatic capacity of the lobsters was significantly reduced compared to that of lobsters from control treatment (kept in ambient seawater), although no effect on THC was observed.

2.2.3. Hypoxia

Sessile organisms inhabiting coastal tidal areas often encounter hypoxia, accompanied by hypercapnia and subsequent decrease in pH (Levin et al., 2009; Zhang et al., 2010). In this situation they have to compensate for the decrease in oxygen by initiating higher ventilation, lowering their metabolism and the demand for energy, or switch to anaerobic pathways to produce energy (Burnett, 1997). Several studies illustrate that disease-related mortality is associated to hypoxia (Boyd and Burnett, 1999). Significant reduction of THC and the lysosomal content have been recorded in the Asian green mussel *Perna viridis* exposed to hypoxia (Wang et al., 2012) and especially so when exposed

to hypoxia and low salinity in combination. This is a common scenario of many estuarine habitats and might increase mussels´ susceptibility to infections as the immunomodulation was not restored after 24 h of recovery. Furthermore, Dwyer and Burnett (1996) have proposed that hypercapnic hypoxia favors the growth of the parasite *Perkinsus marinus* in the oyster *Crassostrea virginica*. Observed reduction of ROI-products in the oysters when exposed to this condition may explain the success of the parasite (Boyd and Burnett, 1999).

Immunity of crustaceans is seemingly not well adapted to hypoxic conditions as experimental studies have displayed inhibited bacteriostatic capacity in e.g. the Atlantic blue crab, *Callinectes sapidus,* (Holman et al., 2004) and the Pacific white shrimp, *Litopenaeus vannamei,* (Burgents et al., 2005). This is probably due to decreased activation of prophenoloxidase under hypoxic conditions with a further suppression appearing with decreasing pH (Tanner et al., 2006). Moreover, an increased prevalence for infections has shown associated to hypoxic conditions in the shrimp *Penaeus stylirostris* (Le Moullac et al., 1998).

Along the Swedish west coast, *N. norvegicus*, living below the halocline, is frequently exposed to natural or eutrophication-induced hypoxic periods with oxygen < 20% of air saturation during 4–10 weeks (Baden et al., 1990; Rosenberg and Loo, 1988). Then, oxidative reduction of organic matter in bottom sediments gives rise to hypercapnic hypoxia. Several sub-lethal ecophysiological effects of hypoxia, such as feeding reduction, moderation of hemocyanin, increase of lactate concentration and shift to anaerobic metabolism, have been demonstrated in *N. norvegicus* as reviewed by Eriksson et al. (2013). However, only few studies have explored effects on immunity. An experimental study on *N. norvegicus* (Hernroth et al., 2015) revealed slightly negative effects on clearance capacity of *V. parahaemolyticus* being injected in lobsters kept in hypoxia (23% of air saturation) for two weeks, although no effect on THC was recorded. However, the same study showed that when lobsters were pre-exposed to OA for 6 weeks and then subsequently subjected to hypoxia for an additional two weeks, THC became significantly reduced and the bacteria were also able to multiply in the hepatopancreas. Exposure to only the OA condition as stressor did not reduce THC while it was concluded that OA and hypoxia in combination resulted in synergistic effects. It should be pointed out that bacteria of the genus *Vibrio* are facultative anaerobic, able to use fermentative and respiratory metabolism simultaneously, depending on the extent of oxygen limitation (Gottschal and Szewzyk, 1985). The growth rate of the strain of *V. parahaemolyticus* used for the infectivity study was neither affected by the hypoxic- nor by OA conditions (Hernroth et al., 2015).

2.2.4. Surplus of manganese

Manganese at concentrations that are realistic to find in bottom waters (~10–20 mg L^{-1}) during hypoxic events (< 20% of air saturation) have shown toxic effects on immunity of invertebrates. The degree of impact is seemingly linked to the rate of accumulation of Mn in the organisms. A comparison between Mn-exposed *M. edulis*, *N. norvegicus* and the sea star *Asterias rubens*, (15 mg L^{-1} for 5 days) showed that the concentration in their digestive glands differed between species. In *A. rubens* the accumulation factor was ~1.2, in *M. edulis* ~1.6 and in *N. norvegicus* ~3.3. Clearance of inoculated *V. parahaemolyticus* was followed 8, 24 and 48 h post injection. Within this time period *A. rubens* managed to reduce the number of bacteria (CFU mg^{-1}) to the same level as for control animals. This was significantly more efficient compared to that of *M. edulis* and *N. norvegicus*, which showed increasing number of bacteria in hepatopancreas. A recovery period of three days without Mn additive was enough to fully restore the clearance capacity of *M. edulis* but not that of *N. norvegicus* (Oweson and Hernroth, 2009).

Surplus of Mn in *N. norvegicus* creates a dose dependent hemocytopenia (Oweson et al., 2006). Generally, loss of hemocytes should be compensated for by increasing renewal. However, the proliferation of hematopoietic stem cells did not respond to Mn-induced hemocytopenia (Hernroth et al., 2004). Moreover, a significant increase in apoptotic cells was registered in the hematopoietic tissue (Oweson et al., 2006), indicating that surplus of Mn significantly affected the renewal of hemocytes. Immune functions of the hemocytes were also affected by surplus of Mn. Exposure to 20 mg L^{-1} for 10 days significantly inhibited the degranulation process and thereby the possibility to release the zymogen package of ProPO-AS. In addition, Mn severely inhibited the production of the bactericidal enzyme prophenoloxidase (Hernroth et al., 2004). Reduced expression of these important immune mechanisms in *Daphnia* has shown to increase its susceptibility to the bacterial pathogen *Pasteuria ramose* (Mucklow et al., 2004). In *N. norvegicus*, exposed to 9 mg Mn L^{-1} for two weeks, a clear reduction of their clearance capacity was noted, 24 h after inoculation with *V. parahaemolyticus*. The number of culturable bacteria (CFU mg^{-1}) was reduced by 34% in control lobsters (kept in ambient seawater) while the number slightly increased in hepatopancreas of Mn-exposed lobsters. The same pattern was found also for lobsters that were pre-exposed to OA (pH 7.7) for six weeks before encountering Mn, as an additional stressor for two weeks. Microcosm experiments showed that these stressors did not affect growth, survival or hemolytic capacity of *V. parahaemolyticus*, why it was concluded that the different fate of the bacteria in hepatopancreas was due to the affected immune capacity of the lobsters. Also, the reduction of THC was similar for those lobsters exposed to OA as a simple stressor and those that experienced Mn in combination with OA (Hernroth et al., 2015). The lack of synergistic effect might be explained by the fact that Mn (Oweson et al., 2006) but not OA (Hernroth et al., 2012) has shown to induce apoptosis of hematopoietic cells of lobsters. Similar to the Mn-induced hemocytopenia observed in *N. norvegicus* (Hernroth et al., 2004) the THC in *M. edulis* was reduced by approximately 50% after exposure to 15 mg Mn L^{-1} (Oweson and Hernroth, 2009).

3. Environmental impact on distribution and persistence of human pathogens in coastal waters

A brief summary of different environmental drivers affecting human pathogens in seawater is presented in Table 2.

3.1. Climate effect on naturally occurring pathogens

Bacteria of the genus *Vibrio,* of which several can resist great ranges in salinity and temperature, occur naturally in the marine environment and are of particular interest since they include species which are able to infect humans as well as fish and shellfish (Chen et al., 1992; Jackson et al., 1997). As sea surface temperatures increase, *Vibrio* has been pointed out as the most emergent risk for increasing waterborne infections in Europe (Lindgren et al., 2012). At high storage temperature *V. vulnificus* has shown able to multiply in oysters (Kaspar and Tamplin, 1993). Since 2006, severe and even lethal vibrioses, have been reported from the Baltic Sea (Andersson and Ekdahl, 2006), where high summer temperature correlates with waterborne *Vibrio* infections (Baker-Austin et al., 2013). It has been pointed out that an increase in maximum SST seems more important for *Vibrio* outbreaks in moderately warm waters, e.g. continental Europe and southern USA, whereas higher minimum SST increases the risk in moderately cold waters, e.g. northern Europe and USA (Parmesan and Attrill, 2016). The relevance of climatic events, such as more intensive monsoons e.g. along the Bay of Bengal, as causative factors for the cholera outbreaks, has long been known (Colwell, 1996). Other climate oscillations, such as El Niño, have also shown to influence the distribution of *Vibrio* (Martinez-Urtaza et al., 2008). Recently, it was reported that the last 50 years of climate change have significantly increased the abundance of *Vibrio* spp, and associated human diseases, along the North Atlantic coast (Vezzulli et al., 2016).

Vibrio parahaemolyticus is of particular interest since it is the most common cause of gastroenteritis transmitted via seafood and is

Table 2
Effect of environmental factors on investigated human pathogens in bivalves. Summarize of field studies showing effects of weather events with high land run off and sea surface temperature (SST). Experimental studies showing effects of changing temperature, ocean acidification with pH decrease of ~ 0.4 (OA); hypoxia (~ 23% of oxygen saturation); bioavailable manganese (Mn ~ 9 mg L^{-1}).

Pathogen origin	Human pathogens	Effects of SST and OA	Other environmental effects	Additional observations of relevance for transmission
Naturally occurring marine pathogens	*Vibrio* spp. e.g. *V. cholerae* non-01, *V. parahaemolyticus*, *V. vulnificus*	Increasing abundance[1-4] and distribution[5-8] with increasing SST	Occurrence and distribution benefit from extreme weather events such as high land run off and flood[17-18]	When blue mussels are offered different *V. cholera* strains they discriminate the uptake of the most virulent but elimination of these take longer time compare to non-virulent strains[32]
		Virulence has shown upregulated by rising SST[9-10]	Growth is promoted by coexistence with copepods[19-21] and phytoplankton[22-23]	Bacteria can form viable but non culturable (VBNC) state to resist harsh conditions such as e.g. low temperature[33]
		Growth, survival and hemolytic capacity of *V. parahaemolyticus* was shown un-affected by OA[11]	Growth, survival and hemolytic capacity of *V. parahaemolyticus* was shown un-affected by hypoxia and Mn[11]	Heterotrophic bacteria have shown increased expression of protease[34] and glycosidase[35] in response to OA, indicating increased virulence[36]
Fecal contaminants with human-animal origin	Virus, e.g. Norwalk-(Calici)viruses Enterovirus Hepatit A virus Adenovirus	Adenovirus showed higher resistance in bivalves at 4°C compared to 18°C and remained infectivity for several weeks at the low temperature[12]	Higher frequencies of virus contaminated bivalves during rainy periods[24-26]	Seasonal occurrences are reflecting outbreaks in society[22,26,37]. Genetic markers of different strains of Norwalk virus confirmed a link between society and contamination of bivalves[38]
		Effects of OA has not yet been reported,	Norwalk-virus, Enterovirus and Adenovirus more frequently detected during winter months[26-27]	Adenovirus is the most frequently found human viruses in field samples of seawater and bivalves [25,39]
	Bacteria, e.g. *Salmonella* spp. ETEC *Escherichia coli*	Bacterial growth is favored by increasing SST[13]. Virulent *Salmonella* is even able to multiply in bivalves at 20°C[14]	Higher frequencies of *Salmonella* and *E. coli* contaminated bivalves during rainy periods[28]	VBNC state of ETEC in seawater remained virulence expressions for several months[40]
		Effects of OA has not yet been reported,		Survival of the pathogens is dependent on e.g. UV-exposure[13,41] and efficiency of host immune defense[11,42]
				Low seawater temperature preserves a stable but low level of culturability of ETEC, *Salmonella* and *E. coli*[13,43-45]
	Protozoan, e.g. *Giardia duodenalis* *Cryptosporidium* spp *Toxoplasma gondii*	Infectivity remains better at low temperature[15,16]	Elevated concentrations after rainfall[15,29-30]	Limit survival of *Giardia* cysts in seawater, but infections in marine mammals suggest they can resist at least in low salinities[29]
		Effects of OA has not yet been reported,	Survival increases with lowered salinity[15,31]	

References: [1]Lindgren et al., 2012; [2]Baker-Austin, 2013; [3]Parmesan and Attrill, 2016; [4]Vezzulli, et al., 2016; [5]DePaola et al.,1990; [6]Daniels et al., 2000; [7]Vezzulli, 2013; [8]McLaughlin et al., 2005; [9]Oh et al., 2009; [10]Kimes et al., 2012; [11]Hernroth et al., 2015; [12]Hernroth and Allard, 2007; [13]Hernroth et al., 2010a; [14]Hernroth, 2003a; [15]Fayer, 2004; [16]Lindsay and Dubey, 2009; [17]Colwell, 1996; [18]Martinez-Urtaza et al., 2008; [19]Huq et al., 1983; [20]Pruzzo et al., 2008; [21]Vezzulli et al., 2013; [22]Turner et al., 2009; [23]Asplund et al., 2011; [24]Lipp et al., 2001a; [25]Lipp et al., 2001b; [26]Hernroth et al., 2002a; [27]Formiga-Cruz et al., 2002; [28]Collin et al., 2008; [29]Fayer et al., 2002; [30]Miller et al., 2008; [31]Fayer et al., 1998; [32]Collin et al., 2012; [33]Coutard et al., 2007; [34]Grossart et al., 2006; [35]Piontek et al., 2010; [36]Asplund et al., 2013; [37]Nenonen et al., 2006; [38]Nenonen et al., 2008; [39]Pina et al., 1998; [40]Lothigius et al., 2010; [41]Sinton et al., 1999; [42]Oweson and Hernroth, 2009; [43]Carlucci and Pramer, 1960; [44]Vasoncelos and Swartz, 1976; [45]Craig et al., 2004;

observed to increase worldwide. Its spreading in coastal waters may alter with growing international traveling and tourism but elevated temperature is also suggested as an important factor affecting its geographical distribution (DePaola et al., 1990; Daniels et al., 2000; Vezzulli et al., 2013). For example, a severe outbreak (> 400 confirmed cases) among consumers of Alaskan oysters has for the first time been experienced at latitudes higher than 60°N (McLaughlin et al., 2005).

As heterotroph *Vibrio* can utilize biotic carbon sources and are frequently found attached to the chitinous carapaces of copepods (Huq et al., 1983; Pruzzo et al., 2008). Warm temperature has shown ideal for the attachment to copepods (Vezzulli et al., 2013), promoting vibrio growth. *Vibrio* spp. are also attracted to phytoplankton blooms (Turner et al., 2009; Asplund et al., 2011) and have shown more prevalent in bottom sediments with high chlorophyll *a* content (Johnsson et al., 2010). However, as noted by Vezzulli et al. (2016) natural coincidence of phytoplankton abundance and high temperature makes it difficult to distinguish which of the parameters that affects the presence of *Vibrio* the most. When using *V. parahaemolyticus* as a model organism in microcosm studies the bacteria stayed culturable in sterile filtered seawater for a period of at least eight weeks at 18 °C (Hernroth et al., 2010). Here, temperature was particularly important as the culturability was greatly reduced already after one week at 8 °C. During harsh conditions such as at low temperature this bacterium can enter a viable but non-culturable (VBNC) state but has shown able to recover culturability when temperature rises (Coutard et al., 2007).

Some studies indicate that water temperature may also play a more direct role in the regulation of *Vibrio* pathogenicity. This includes up-regulation of virulence factors involved in e.g. motility, secretion, and antimicrobial resistance at higher temperature (Oh et al., 2009; Kimes et al., 2012). As a consequence, outbreaks of vibriosis causing mass mortality in bivalve aquaculture have often been linked to warm water events (Lee et al., 2001; Elston et al., 2008). In addition, marine bacterioplankton communities are supposed to increase production of protease (Grossart et al., 2006) and glycosidase (Piontek et al., 2010) in response to OA. These enzymes are important for heterotrophic bacteria, such as *Vibrio,* as tools to degrade biomass for generating energy and are thus associated with the pathogenicity against other organisms (Ridgway et al., 2008). The study by Asplund et al. (2013) indicated such an induced pathogenicity of *V. tubiashii,* in terms of enhanced hemolytic and protease activity when pH was reduced from that of today (8.1) to that predicted for 2100 (7.7). In contrast, an experimental study conducted on *V. parahaemolyticus* showed that such a reduction of pH did not affect the hemolytic properties of the bacteria (Hernroth et al., 2015). Although potential effects on virulence have not yet been clarified, it was obvious that OA did not generate negative effects on growth and culturability (Asplund et al., 2013; Hernroth et al., 2015), which indicates that both species are well adapted to the future climate scenario. To get an equitable picture of possible changes of virulence expression in different *Vibrio* species, further studies should include combinations of other parameters that are influenced by climate change, such as e.g. nutrient availability, temperature and salinity.

3.2. Pathogens from human-animal reservoirs

3.2.1. Changing distribution of human pathogens in marine environments

Some pathogens are discharged through waste water treatment plants or through diffuse outlets from private households, shipping and agriculture activities but also as a result of increase in global travels. Thus, most non-*Vibrio* human pathogens found in seawater are those transmitted through the fecal-oral route and are reflecting the prevalence in the surrounding human-animal reservoirs (Griffin et al., 2003; Kelsey et al., 2004).

Few studies have considered how climate change affects these human pathogens in the marine environment. However, field studies in different geographical areas have pointed out seasonal rain and land run-off as significant for the distribution of pathogenic protozoans (Fayer et al., 2002; Miller et al., 2008), viruses and bacteria into coastal waters (Lipp et al., 2001a, 2001b; Formiga-Cruz et al., 2002; Hernroth et al., 2002b; Collin et al., 2008; Burge et al., 2014). For example, *Salmonella* spp. were present in high densities in 100% of clam samples collected during the rainy season in shallow areas of Maputo Bay, Mozambique, whereas only in low numbers in 30% of the samples during the dry season (Collin et al., 2008). Outbreaks of *Salmonella* in the population occur more frequently during the rainy season which is also the warmest period. Enteric viruses, Adenovirus (AdV), and Norovirus (NoVs; or Norwalk-like viruses) were found more frequently in blue mussels collected at the Swedish west coast during periods of elevated land run-off, in contrast, this coincided with winter temperatures (Hernroth et al., 2002b). In Sweden, particularly NoVs, which is a group of closely related RNA-viruses belonging to the genus *Calicidae*, is causing one of the most common outbreaks of gastroenteritis, the so-called winter vomiting disease. Hepatitis A virus (HAV) was not found in any of these samples. However, in Mediterranean countries and in Mozambique where infection of Hepatitis A is considered endemic the virus was detected in bivalves (Formiga-Cruz et al., 2002; Nenonen et al., 2006). Another study on source tracking confirmed such a society-circulating theory. By tracing genetic markers of NoVs in blue mussels, collected downstream the wastewater treatment plant of Gothenburg, a city at the west coast of Sweden, almost 100% similarity with patient strains, isolated from local outbreaks, were found (Nenonen et al., 2008). This indicates an increasing load of pathogens from land to sea along with the predicted increase of extreme events of precipitation (Trenberth, 2011).

3.2.2. Factors affecting persistence of human pathogens in marine environments

For enteric pathogens, coastal waters with e.g. exposure to UV-radiation, higher salinity, lower levels of nutrients and temperature form an adverse environment compared to that of their intestinal habitat. Thus, not all of them survive or retain their infectivity. However, oocytes from the human and animal parasites *Cryptosporidium* and *Toxoplasma gondii* can survive for weeks or months in a wide range of salinities and temperatures (Fayer et al., 2004) and are able to accumulate in bivalves (Freire-Santos et al., 2000; Ladeiro et al., 2014). Although salinities above 20 PSU have shown to limit the infection ability of *Cryptosporidium* (Fayer et al., 1998) an experimental study, conducted at 35 PSU, revealed that 75% of *C. parvum* oocytes retained viable when recovered from clams and were also able to infect neonatal CD-1 Swiss mice (Freire-Santos et al., 2001). Another long-term study in seawater (15 PSU) showed that low temperature (4 °C) better preserved infectivity of *T. gondii* sporulated oocytes compared to that at room temperature (Lindsay and Dubey, 2009). Also when *T. gondii* oocytes were ingested by the oyster *C. virginica* their infection capacity remained when tested on mice (Lindsay et al., 2001).

Most enteric bacteria can under harsh conditions form VBNC states in order to decrease metabolic activity as an energy saving strategy (reviewed by e.g. Rozen and Belkin, 2001). Although being inhibited, low water temperature preserves a stable but low level of culturability of *E. coli* (Carlucci and Pramer, 1960; Vasconcelos and Swartz, 1976; Craig et al., 2004). The culturability of enterotoxigenic *E. coli* (ETEC) was also shown more constant in seawater microcosms at 8 °C compared to that of 18 °C (Hernroth et al., 2010). Lothigius et al. (2010) incubated six different clinical strains of ETEC in seawater for 3 months, after which only two strains were still culturable. However, all strains expressed the different genes encoding ETEC toxins (STh and LT), colonization factors (CS7 and CS17), *gapA* and 16 S RNA, indicating that their VBNC state has the potential to be infectious after long-term incubation in seawater. In the study of Hernroth et al. (2010), incubation of *S. enterica* in seawater microcosms demonstrated quite similar and stable culturability both at 8 °C and 18 °C. However, on the last sampling occasion, conducted after 8 weeks, the culturability dropped

significantly at the lower temperature. Moreover, when inoculated into hemolymph of the blue mussel, *Mytilus edulis* kept at 20 °C, *S. enterica* has shown able to multiply in the mussels (Hernroth, 2003a). As the culturability of the bacteria is highly affected by UV-radiation (e.g. Sinton et al., 1999; Hernroth et al., 2010) bivalves may constitute a protective site for the pathogens in sheltered areas.

Altogether, these findings indicate that enteric human pathogens discharged in seawater may persist for several months and still keep their pathogenicity. During favorable temperature they have the potential to increase replication when finding a host. This has particular relevance in a changing climate and already now in many low-income countries, such as Mozambique. There, bivalves are often collected in contaminated areas, stored and sold in markets without any refrigerating facilities (Collin et al., 2008). High temperature may in this way greatly increase the risk for transmission of pathogens from contaminated coastal waters back to humans through shellfish consumption.

As obligate intracellular parasites, viruses rely on specific host cells to provide genetic tools for replication. However, human viruses could stay intact without replication in bivalve tissues for long. Adenovirus has shown to be one of the most commonly found human viruses in field samples of seawater and bivalves (Girones et al., 1995; Pina et al., 1998; Hernroth et al., 2002b). Persistence of this virus, when accumulated in bivalves is seemingly favored by cold temperature. In an experimental study, where uptake and elimination of AdV35 were examined in blue mussels, *M. edulis,* and oysters, *Ostrea edulis*, bioaccumulation of the virus was shown obvious in both species. It was most pronounced in oysters where the virus was also detectable during 6–10 weeks post ingestion (p.i.) and more so at 4 °C compared to 18 °C. Remarkably, viruses from the oysters at 4 °C retained their infectivity when extracted from the tissue 6 weeks p.i. This was indicated as cytopathic effect on cell cultures (A549 cells) and the effect was sustained double the time compared to that of viruses extracted from blue mussels (Hernroth and Allard, 2007). The bivalve immune response is more active at the higher temperature (see Section 2.2.1) which may explain the faster decay at 18 °C. Among shellfish, oysters are the most common vector of e.g. NoVs and it has been assumed that it is because oysters are mostly eaten raw. However, Le Guyader et al. (2012) have demonstrated a selective accumulation of NoVs due to specific binding to carbohydrate ligands. Such special host-pathogen relationship may expand the capacity of certain human viruses to stay intact in bivalves at low temperatures.

Briefly, it could be concluded that low temperature seems to favor the persistence of human pathogenic viruses and protozoans whereas those pathogenic bacteria that are able to persist in marine environments, better retain culturability and infectivity at higher temperature. Other may enter a VBNC state but may resuscitate and maintain pathogenicity. Thus, regional pathogen reservoirs that may influence the seasonality of waterborne infectious diseases should impact risk assessment models and guidelines for monitoring shellfish safety.

4. Alteration of the Red Queen's Race between hosts and pathogens

The overall consequences of climate change for the immune suppressed invertebrates depend on their ability to adapt to the stressors and their resilience to pathogenic threats. As pointed out by Browman (2016) in a summary of recent OA-research, experimental animals often display great individual variability; a phenomenon that should be considered. Those that better resist stressors are likely to force the population towards genetic adaptation. In addition, certain bottlenecks, such as larvae being more vulnerable than adults, might be overcome by individuals, making future adaptation possible (Ross et al., 2016). A meta-analysis by Seebacher et al. (2014) concluded that the more stable environments generate greater capacity for acclimation, giving increased thermal acclimation with decreasing latitude. However, as suggested by Calosi et al. (2016) current knowledge points to that plasticity and adaptation might not overcome the evolutionary challenge of the multi stressed environment under pressure of climate change.

Microorganisms, with short generation time, can rapidly adapt when facing environmental changes. Even in a constant environment they are better suited due to beneficial mutations and through transfer of resistance genes between bacteria (Ochman et al., 2000). Metatranscriptome analyses of marine bacteria indicate that physiological acclimation to OA is possible but the required export of protons across cell membrane is energy demanding (Bunse et al., 2016), making these bacteria more vulnerable in oligotrophic waters (Sala et al., 2016). Thus, energy negative trade-off may influence microbial growth and future adaptation to multiple stressed environments. However, along with increasing load of nutrients into coastal waters the overall hypothesis is that most human pathogens will benefit from global warming (Parmesan and Attrill, 2016). Since abundance and survival of pathogens are increasing while OA, hypoxia and surplus of Mn suppress the immunity of the invertebrates, the residence time of pathogens in the tissue will extend with increased risk of infections to consumers as a consequence. In Table 1 some examples with *Vibrio* spp as pathogens are given, illustrating alteration of host-pathogen interactions under the pressure of these stressors.

5. Improvement of monitoring shellfish safety

Fecal coliforms, enterococcus and *E. coli* are commonly used as indicator organisms for sanitary controls of seawater and seafood. Based on decay studies of these indicators, it was for long believed that the residence time for enteric bacteria in sea water and in marine bivalves was short. Thus, mussels from harvesting areas, classified as category B, according to the legal framework of European Food Hygiene Regulation (Sections 5, 6) only require 42 h of depuration. However, as reviewed by Rozen and Belkin (2001) most enteric bacteria form VBNC-states when meeting severe conditions. This is overlooked in sanitary controls based on bacterial culture techniques. Depending on the previous history of the bacteria (Gauthier, 2000) they may retain pathogenicity or resuscitate when getting into a more favorable environment (Roszak et al., 1984; Roth et al., 1988). Moreover, survival of *E. coli* in the salinity and pH range of seawater is limited (Carlucci and Pramer, 1960; Andersson et al., 1979) making them less reliable as fecal indicator in marine environments.

It has often been demonstrated that the common use of fecal indicators (*E. coli*, fecal coliforms, enterococci) for monitoring coastal waters and the enforcement of shellfish harvesting regulation is inadequate to protect shellfish consumers from viral or vibrio infections (Desenclos et al., 1991; Lipp and Rose, 1997; Formiga-Cruz et al., 2003; Myrmel et al., 2004; Fleming et al., 2006) as well as from human protozoan parasites (Gómez-Couso et al., 2003; Nasser et al., 2003; Graczyk et al., 2005). The emerging science of Microbial Source Tracking (MST) has directed great efforts to introduce more reliable indicators for routine monitoring of viral fecal pollution. Selected bacteriophage groups, such as e.g. F-specific RNA bacteriophages and phages infecting *Bacteroides fragilis* have been evaluated as indicators for viral contamination, as these are infecting human enteric bacteria (Formiga-Cruz et al., 2003; Myrmel et al., 2004; Jofre et al., 2014). However, in some studied areas, there was low phage recovery also when human viruses were detected. Specific viral pathogens can be quantified with real-time PCR technique but with certain limitations, such as difficulty to concentrate samples and absence of host specificity among some human and animal-associated microbial markers (Roslev and Bukh, 2011). However, as reviewed by Fong and Lipp (2005) this technique has been successfully used for detecting both HEV and human AdV in aquatic environments and a promising study has recently been conducted using molecular markers able to distinguish between human and animal (porcine and bovine) AdV and pylomavirus. This

multi-laboratory study demonstrated the possibility to identify different sources of fecal contamination in river catchments of diverse geographical areas (Rusiñol et al., 2014). Moreover, as AdV has been reported the most prevalent human viral finding in shellfish and also the study by Formiga-Cruz et al. (2002) discovered a significant relationship with the presence of other human viruses, AdV seems to be as a strong candidate to ensure a more reliable control.

As reviewed by Robertson (2007) the infectious stages of protozoan parasites *Giardia duodenalis, Cryptosporidium* spp., and *Toxoplasma gondii* are frequently detected in bivalves. Although both *Cryptosporidium* spp., and *T. gondii* apparently retain their infectivity for prolonged periods in shellfish there is so far only minimal evidence of infection transmission, probably due to lack of investigations. Studies on mollusk diseases reveal that parasite infections commonly cause concurrent infections by bacteria and/or viruses (Montes et al., 2001), which in immunosuppressed animals may become even more apparent. It is now possible to meet the need for quality control through molecular tools that recently have been developed for wastewater and mussels, such as multiplex PCR simultaneously covering detection of *Giardia, Cryptosporidium* and *Toxoplasma* (Marangi et al., 2015).

6. Synopsis and concluding remarks

Experimental studies have shown that OA at a level predicted to occur at the end of this century, highly affected immunity of both blue mussels and Norway lobsters. The animals were not able to keep homeostasis and acidosis was developed. Total counts of immunocytes (hemocytes) of lobsters were significantly reduced and antimicrobial peptides in gills of mussels showed reduced capacity to degrade bacteria. Altogether, this indicates that OA makes the animals more susceptible to infections. This was shown as reduced capacity to eliminate inoculated *Vibrio*. In lobsters, hypoxia in combination with OA gave synergistic effects with further reduction of their bacteriostatic capacity. It has been pointed out that OA creates high maintenance cost to keep homeostasis which significantly impacts physiological functioning (Pan et al., 2015).

The most prominent effect on immunity was induced by elevated levels of Mn, at concentrations that are realistic to find in bottom waters during hypoxic conditions. A significant reduction of hemocytes was observed, probably due to the inhibition of renewal of the cells as was demonstrated in lobsters. This was further reflected by reduced capacity to eliminate inoculated bacteria, and in lobsters the bacteria were even able to multiply in hepatopancreas. Excess of Mn can interfere with calcium (Ca) cell trafficking (reviewed by e.g. Van Baelen et al., 2004) and may cause a more direct toxic effect on immunity compared to the energy trade-off due to OA. In multi-stressed marine environments there are several stressors, such as biocides, copper, cadmium and other pollutants that have not been taken into consideration in this review, despite being known as immune modulators (reviewed by e.g. Mydlarz et al., 2006; Ellis et al., 2011). These may of course contribute even more to weakened immune defense in marine invertebrates.

Studies on the impacts of climate change on transmission of human diseases, mediated by marine systems, have mostly been demonstrated through correlations between SST and disease incidences, particularly concerning *Vibrio* infections. Climate events with high land run-off have also shown to promote *Vibrio* abundance and will in addition, bring enteric pathogens that are circulating in society into coastal waters. Thereby it could be assumed that the future climate change, with increasing rainfall in e.g. the Northern hemisphere, may raise the abundance of pathogens. Moreover, temperature has been recognized as a noticeable impact factor on persistence of survival and infectivity when these pollutants are reaching seawater. The human pathogen *S. enterica* has like *V. vulnificus* even seen able to multiply within mussels during warm conditions. On the other hand, moderately rising temperature has shown stimulating effect also on mussel immune response. Thereby, the success of temperature boosting immune defense is highly dependent on the virulence and concentration of the encountered bacteria.

Human viruses do not replicate in marine hosts but if staying intact in shellfish they can be brought back to humans through consumption. At low temperature, both human viruses and protozoans have shown to preserve their infectivity in bivalves for several weeks. Probably, the bivalves better manage to counteract these pathogens at summer temperatures, when the bivalves are metabolically more active. In a general aspect it means that global warming should be unfavorable for human viruses and protozoans accumulated in bivalves. However, OA, hypoxia and exposure to bioavailable Mn, which come along with the warming, counteract this phenomenon, since these stressors have shown negative impact on bivalve immunity.

We could conclude that the long-term, large scale environmental changes, here discussed, have the potential to alter the outcome of host-pathogen interactions by:

- Increasing the load of pathogens and facilitate their distribution in coastal waters.
- Impairing immune defense and thereby enhancing the persistence of pathogens in marine invertebrates.
- Increasing prevalence of infections of marine animals and transmission to consumers.

Marine invertebrates are of high ecological impact and negative effects on their health status are very worrying. As food resource they are of increasing importance also for humans. The attained knowledge points to a more flexible approach for sanitary control of recreational coastal waters and seafood for consumption, compared to that of the standard measurements of today and should also include viral and parasitological analyses. It is time to face the fact that risk factors are different for different geographical areas, depending on the kind of pathogens that are circulating in society, their seasonal distribution and their ability to cope with changes in marine environments. Recent analytical achievements open opportunities for easy and cost-effective molecular identification of persistent pathogens of interest. This should be the guiding principle for meeting the future challenge of protecting consumers. Particularly so since progression of climate change may further reduce immune competence of marine invertebrates and thus their ability to eliminate pathogens, which represent an increasing risk for centuries to come.

Acknowledgements

We gratefully acknowledge all our colleges who have been involved in the different projects which have formed the basis for this review. We also acknowledge the financial support mainly provided by Swedish International Development Cooperation Agency (Sida SWE-2003-108; SWE-2005-397; BH), The Swedish Research Council for Environmental, Agriculture and Special Planning (FORMAS 217-2004-947; BH and SB), European Communities, Agriculture and Fisheries (FAIR; project CT98-4039; BH), Birgit and Birger Wåhlström Memory Foundation (2013; 2016; BH), and Gothenburg University Research Platform for Integrative Physiology (GRIP; 2011; BH and SB). This review reflects only the author´s view.

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