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Environmental drivers of host-parasite interaction in the Baltic and Adriatic Sea under a global change perspective

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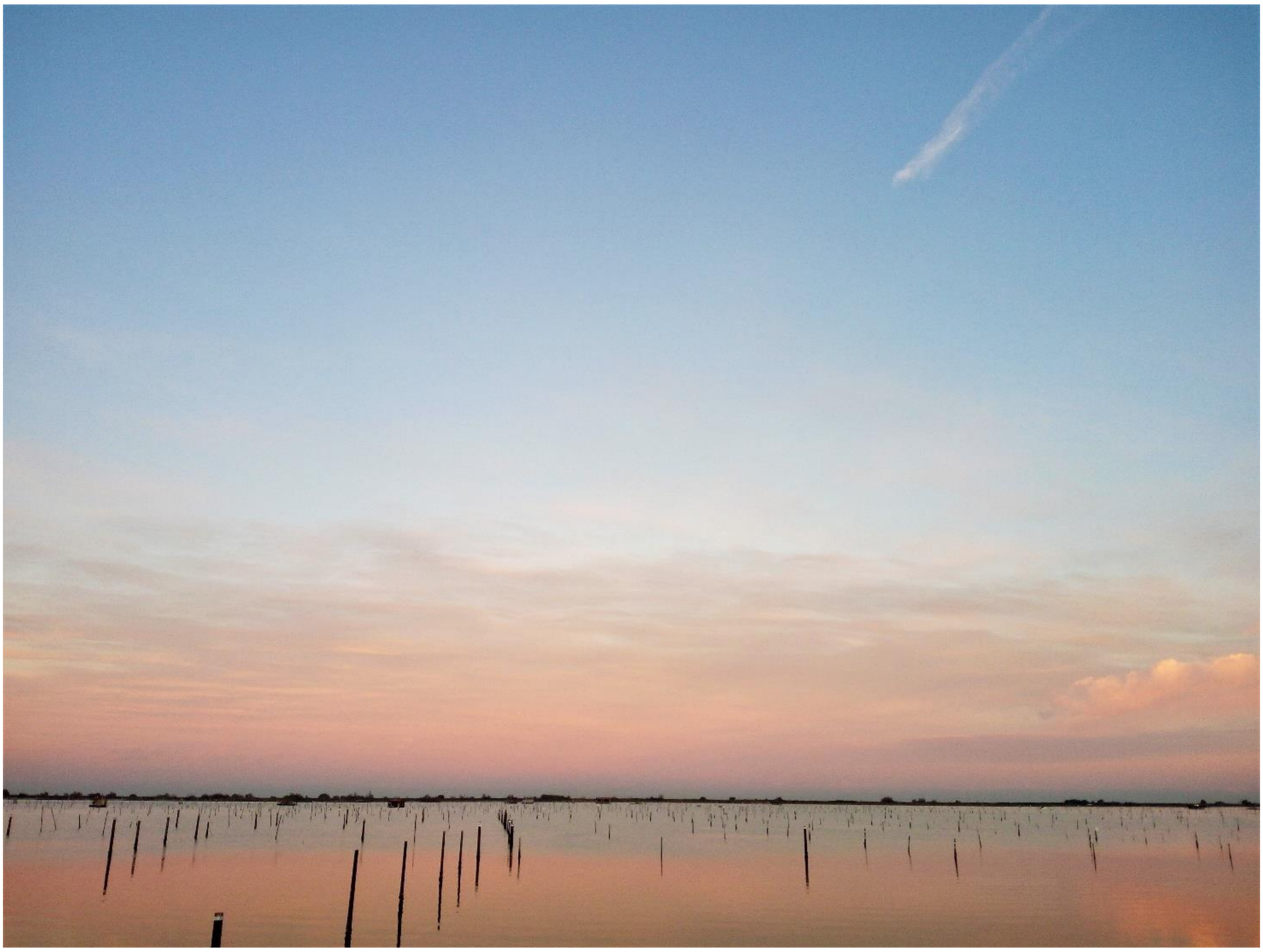


“Keep Ithaka always in your mind.
Arriving there is what you’re destined for.
But don’t hurry the journey at all.
Better if it lasts for years,
so you’re old by the time you reach the island,
wealthy with all you’ve gained on the way,
not expecting Ithaka to make you rich.
Ithaka gave you the marvellous journey.
Without her, you wouldn't have set out
What are you waiting for?”

ABSTRACT

Parasites have a crucial role in coastal marine communities. Their sensitivity to environmental conditions and the diversity of hosts involved in their cycle, make parasites fundamental organisms of the food web. In coastal areas experiencing fast changes, such as the Adriatic and the Baltic Sea, investigating on the possible effects of environmental drivers on host-parasite interaction becomes more urgent. The main goals of this study were 1) to describe and quantify the parasites communities of the North Adriatic and the South-Western Baltic Sea 2) to identify the main environmental factors (abiotic and biotic) driving parasites distribution 3) to experimentally assess the influence of salinity and temperature in the transmission between the first and the second intermediate host of a common digenean trematode (*Himasthla elongata*). The species investigated were *Mytilus galloprovincialis* in the Adriatic Sea, and *Littorina littorea* and *Mytilus edulis* in the Baltic Sea. Parasites prevalence, richness, abundance and intensity were evaluated in two regional-scale field samplings. Parasites prevalence, abundance and richness were then related to biotic and abiotic environmental drivers, such as salinity, eutrophication, host density and individual host traits (i.e. length). Results of the Adriatic sampling showed *Parvatrema timondavidi* as the dominant species infecting mussel all over the northern coast, followed by *Eugymnanthea inquilina*, more widespread in the north-eastern side. Eutrophication and salinity were the main drivers of parasite prevalence and abundance. Results of the Baltic sampling showed the trematodes *Renicola roscovita* and *Himasthla elongata* as the dominant parasite species. Statistical analysis showed salinity as the main factor driving digeneans distribution, followed by upstream host density and mussel size. The experimental study confirmed salinity as the most important driver, positively affecting cercarial emergence and transmission to the second intermediate host to infection. Surprisingly, temperature negatively affected only cercarial survival. Under a global change perspective, results of this study reveal a potential decrease in parasitism with freshening in the Baltic Sea. In the Adriatic Sea, the potential decrease of eutrophication might be detrimental for parasites as digenean trematodes and beneficial for the intermediate host as mussels. The effects of decreasing eutrophication might be, however, buffered by increasing salinity. The findings present in this thesis improved the knowledge of the different effects that abiotic and biotic factors on parasites distribution and transmission and, at the same time, the key role that parasites can have on their host population dynamics under global change. Insight from this thesis could lead to further field and experimental works, focusing mainly on the tight association among digeneans and their intermediate hosts.

INTRODUCTION

Importance of ecological interactions

Biotic interactions are often neglected but are crucial components of the ecosystem diversity and functioning (Tylianakis et al. 2008; Valiente-Banuet et al. 2015). The definition of ecological interactions includes a wide variety of links among species, which can have direct or indirect effects on the organisms. Direct effects can result from competition or predation (MacArthur and Levins 1964; Tilman 1982), in which presence or abundance of species 1 changes in relation to species 2. Others can result from mutualism and commensalism, where both interacting species benefit from the interaction or, at least, species 1 benefits and species 2 is not negatively affected (Hacker and Gaines 1997). Indirect effects occur when, interacting with each other, two species modify the distribution and abundance of another species (Menge 1995). Previous authors identify indirect effects in four main groups: trophic linkage, behavioural, environmental and chemical responses (Miller and Kerfoot 1987; Strauss 1991; Pennings 1994). Trophic linkage has been investigated worldwide by several studies (Lubchenko and Menge 1978; Kiekckbush et al. 2004; Rilov et al. 2006). The primary effect is the change in abundance of the preyed species, however, a multitude of other effects (i.e. change in the species structure or genotypic composition) have been detected. In general, indirect effects were assessed of primary importance to understand dynamics in biological communities (Strauss 1991; Wootton 1992).

Effects of parasites

Among the broad spectrum of ecological interactions, parasitism has been often neglected in the past and only recently it has been recognised its essential ecological role (Minchella and Scott 1991; Combes 2001; Marcogliese 2004; Hatcher et al. 2012). According to Price (1986), mediation by parasites involves 1) interfering in the competition between one species and another 2) naturally helping one species to avoid enemies 3) assisting consumers with their feeding. In many cases, the importance of parasites mediation is considered comparable to predation or competition. Parasites can have both direct and indirect effects on their host population. Direct effects are the result of the pathology transmitted during the infection. When the infection occurs, organs and tissues of the host are damaged and, even if parasites themselves are usually not lethal, this can result in the death of the host (Marcogliese 2004). Survival of the host is usually directly dependent on parasites intensity (number of parasites per host) (Minchella and Scott 1991).

Parasites directly affecting certain species can originate a cascade effect on other species, which make the formers keystone parasites (Marcogliese and Cone 1997; Mouritsen and Poulin 2009; Hatcher et al. 2012) (Fig.1). One clear example of the keystone role is the alteration of the community structure through traits-mediated effects on the host (Hatcher et al. 2014). Trematodes-infected individuals of the periwinkle *Littorina littorea*, for example, show a reduction of 40% in grazing activity compared to uninfected, which result in a change of the macroalgal community. By phenotypic modifications, behaviour or physiology of the host individual are also manipulated. This manipulation can lead to habitat transformation, in which parasites act as ecosystem engineers (Thomas and Poulin 1998; Thomas et al. 1998).

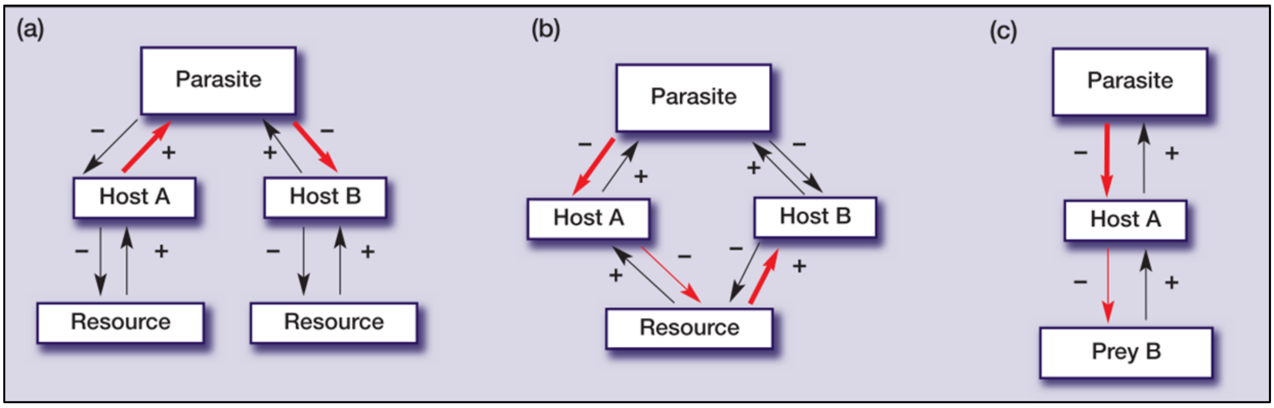


Fig.1 Relevant effects of parasitism (by Hatcher et al. 2012). Arrows indicate positive (+) and negative (–) direct effects on the density of a population derived from the impact of a consumer or a resource; arrow thickness indicates the strength of interaction; red arrows indicate relevant interactions, leading to: (a) apparent competition: higher densities of host A enhance higher parasite population densities, which then have a higher detrimental effect on host B (b) Parasite-mediated coexistence: regulation of a superior competitor A by a parasite enables B to inhabit that environment. (c) Parasite-mediated trophic cascade: regulation of herbivore (or predator) A by a parasite enhance a cascade, releasing the immediately lower trophic level B from herbivory or predation pressure

Because of their typical complex life cycle and the sensitiveness to changes in the environmental conditions, parasites have been recently targeted as biological indicators of their host and, in general, of the ecosystem diversity and health (MacKenzie 1993; Hudson et al. 2006; Marcogliese 2004 and 2005; Nachev and Sures 2015). Within the context of the food-web, parasites can alter the complexity of trophic links between organisms, often stimulating an increase in species richness (Lafferty et al. 2008; Sures et al. 2017).

Parasites can be divided into microparasites and macroparasites (Anderson and May 1979; Marcogliese 2004). Microparasites can be unicellular but also multicellular organisms with size < 50 µm (i.e. protists, viruses, bacteria, fungi), which typically multiply at a high rate in or on the host. The transmission is normally direct, but it can also occurs indirectly and involving alternate host or other vectors. Macroparasites (i.e. helminths, fleas) are of larger size and they typically reproduce sexually, but do not proliferate, in or on the host. An exception is represented by the cercariae in the intermediate host, which are produced asexually by proliferation (Marcogliese 2004). Macroparasites typically display a complex life cycle, constituted by one or more intermediate hosts and one final host. Among macroparasites, digenean trematodes provide an ideal example of a typical helminth complex life cycle. The digenean trematode life cycle involves (Poulin and Cribb, 2002; Cribb et al. 2003) (Fig.2): 1) eggs produced by the adult trematode into the final host, are released usually through the final host faeces and can hatch into 2) miracidium (free-living stage), a swimming larva which penetrates the first intermediate host, generally represented by a suitable mollusc. Inside the first intermediate host, the miracidium develops into 3) the sporocyst stage which develops a more mature sporocyst or in turn, in redia, which is mouth-provided. The sporocysts or the rediae then asexually produce 4) cercariae (free-living stage), all identical genetically, which are released by the first intermediate host and actively swim into the second intermediate host. When the cercariae infect the second intermediate host it develops and encysts in the 5) metacercaria stage, provided by a thick or thinner wall depending on the digenean species. When the final host ingests the second intermediate host, the metacercariae will become an adult, which then will sexually reproduce and complete the life cycle.

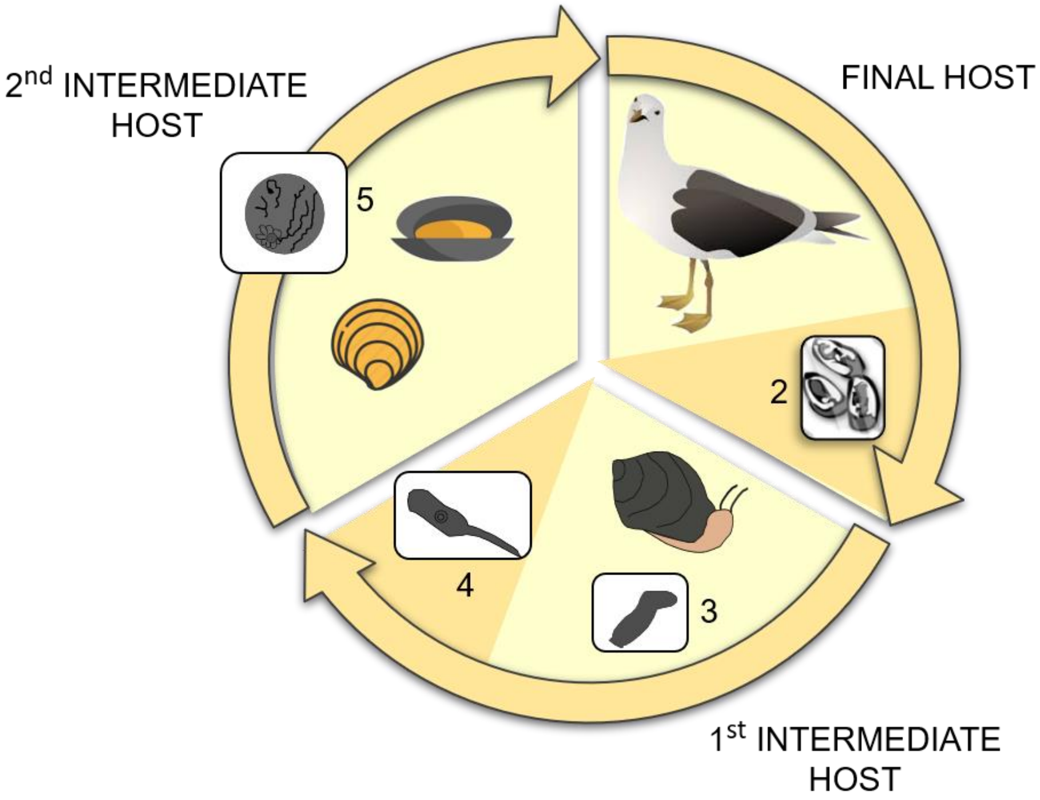


Fig.2 Digenean trematodes complex life cycle, with 2) miracidia 3) redia 4) cercaria 5) metacercaria and with rows representing the transmission from one host to the other host. See the main text for more details.

Effects of environmental variables on the host-parasite interactions

Common host of digeneans are molluscs, for example, gastropods which serve as first intermediate host, or bivalve which serve as first or second intermediate host. As mentioned before, molluscan first host shed free-living cercariae into the environment. Here, cercariae experience a survival-challenge. Abiotic variables can determine the activity and survival of cercariae and then modulate their infectivity to the second intermediate host (Pietrock and Marcogliese 2003). Temperature, salinity, oxygen depletion are generally known to influence cercarial emergence, survival and infectivity. Among them, the majority of studies focused on temperature, which showed to positively influence cercarial emergence, survival and infectivity (Thieltges and Rick 2006; Koprivnikar and Poulin 2009; Studer and Poulin 2013). Temperature, however, displays a species-specific threshold. When this threshold is exceeded, temperature influence on cercariae becomes negative, until the death (Pechenik and Fried 1995; McCarthy 1999; Thieltges and Rick 2006).

Nevertheless, many parasites species are characterized by a broad range of thermal tolerance (Morley 2011; Morley and Lewis 2013; Marcogliese 2016). For this reason, more recent studies attempted to encompass other factors, which may enhance or buffer the effect of warming (Marcogliese 2016). Reduced salinity is generally known to negatively affect free-living stages of marine species (Pietrock and Marcogliese 2003). Single-factor experimental studies, indeed, found cercarial emergence, survival infectivity of marine parasites decreasing with reduced salinity (Lei and Poulin 2011; Studer 2012). Multifactorial experimental studies detected cercarial infectivity increasing with increasing salinity and temperature (Moller 1978) and survival diminishing at supra-optimal salinity and temperature (Koprivnikar et al. 2010). Salinity gradient can represent the main driver in intertidal areas or estuaries, contributing to the parasite diversity and distribution, as observed by previous field studies (Thieltges et al. 2010; Blanar 2011). Another abiotic factor to consider, especially in estuarine and coastal environments characterized by a high-nutrients load, is hypoxia (Pietrock and Marcogliese 2003). Previous studies demonstrate that hypoxia can negatively affect the free-living stage of parasites and also provoke the decline of their host population (Thomas and Daldorph 1994; Marcogliese 2001). However, until a certain limit, high nutrients load can raise the host population density and consequently, the infected proportion of the population (Johnson et al. 2007). Abiotic factors can indeed enhance parasites distribution through mediated effects on their host populations. When the host is physiologically affected by changes in the environmental conditions, its susceptibility to infection can rise (Morley and Lewis 2013).

The spatial distribution of parasites is primarily associated with the density and distribution of their host, especially for parasites having complex life cycle (Thieltges and Reise 2007). While the positive influence of the final host on parasites prevalence and abundance is nowadays of public domain (Combes 2001; Hechinger and Lafferty 2005), fewer studies focused on the association among parasite and intermediate hosts distribution (Mouritsen et al. 2003; Thieltges and Reise 2007; Goedknegt et al. 2019). These studies generally observed a significant correlation between parasites prevalence and abundance with intermediate host density. The association among host and its parasites is also found at a smaller scale (Lauckner 1980; Thieltges and Reise 2007), with different traits among the host within the population resulted in the heterogeneity of their parasites prevalence and abundance.

In the last decades, abiotic and biotic environmental factors are experiencing fast shifts, due to anthropogenic pressure and predicted global change (IPCC 2019), which are likely to impact strength and directions of host-parasites interaction (Harvell et al. 2002). Global change is expected to have a deep impact not only in the parasite species but also in the host species distribution (Marcogliese 2001; Dobson and Carper 1992), conducting to new species introduction or, potentially, local extirpations (Marcogliese 2001; Schindler 2001). The majority of the studies on climate change effect on parasitism predicted an increase in pathogens with increasing temperature (Marcogliese 2008). However, the impact of global warming does not make temperature the only driver to focus on, and studies based solely on temperature often do not result in very realistic predictions. Direct effects of global warming are changes in salinity, increasing eutrophication or acidification, which are occurring all over the world. Changes in salinity are expected to lead to profound changes in the host-parasite interaction, especially in intertidal areas, estuarine or brackish water habitats. With increasing salinity, new marine parasites species, before restrained by freshwater, could spread in new environments and adapt to brackish water host (Kesting et al. 1996; Zander 1998). On the other side, freshening could have direct adverse effects on the free-living stages (Lei and Poulin 2011; Studer et al. 2012) and adverse indirect effects with impact on mollusc host osmoregulation (Berger and Kharazova 1997) and consequently limited host distribution.

In habitats affected by anthropogenic pressure, another crucial environmental driver is eutrophication. Eutrophic waters are generally associated with generalist and allogenic parasites, like digenean, while oligotrophic with more specialistic parasites. Eutrophication includes a wide spectrum of consequences, which make its effects difficult to predict (Budria 2017). Increase in nutrients could result in an increase in host population density and, in turn, in higher digeneans abundance (Johnson et al. 2007). Nevertheless, an improved feeding regime for the host could also result in higher host health and reduced parasites proliferation (Bize et al. 2008; Ponton et al. 2011). When eutrophication overtakes critical limits, hypoxia or anoxia events could also occur, leading to a decline in parasites and their host (Marcogliese 2001; Pietrock and Marcogliese 2003).

STUDY SYSTEMS

Study region: The Adriatic Sea

The Adriatic Sea is the most northern part of the Mediterranean Sea. Due to the presence of many rivers, this basin receives a large amount of fresh water, with an annual average of 5700 m^3/s. The majority of this freshwater mass originates from the Po river, on the Eastern and shallowest area of the basin (Cushman-Roisin et al. 2013). The Adriatic Sea is 783 km long and with an average width of around 170 km (Blake et al. 1996). This basin can be divided into three subsections: northern, central and southern Adriatic, displaying different depths. The northern section is very shallow, with an average depth of 35 m, the central section has an average depth of 240 m, and the southern section consists of a massive depression of around 1200 m (Artegiani et al. 1997a, 1997b). The eastern coast is rocky, while the western coast is shallower and sandy. During the winter season, the Northern Adriatic is characterized by heavy and dense waters, which lead to complete mixing, while during the spring and autumn seasons by frequent precipitations and river discharges (i.e. the Po river) (Russo et al. 2009). Wind-induced circulation of waters and the Po river discharge create a nutrients gradient, with more eutrophic waters in the western and more oligotrophic waters in the eastern side. The high fluctuations of environmental variables in the North Adriatic make predictions complex. However, some authors detected a trend towards oligotrophication of the entire North Adriatic (Rinaldi 1998; Harding 1999; Giani 2012). Furthermore, an increase in salinity is expected to occur due to reduced river discharge and more inflow of Levantine water (Solidoro et al. 2009).

Host model: *Mytilus galloprovincialis*

The common Mediterranean mussel, *Mytilus galloprovincialis* is an indigenous filter-feeder bivalve widely distributed in the Mediterranean Sea, especially in meso and eutrophic waters. From the biological perspective, the spawning season usually occurs from November to March (Renzoni 1973) and a settlement which in some cases is seasonal, in other cases take place all over the year, with a peak during spring (Ceccherelli and Rossi 1984). Mussels are forming dense beds in medio littoral and infralittoral areas, hosting a large variety of organisms, both mobile and sedentary (Çinar et al. 2008). Mussel beds formation is supported by various environmental factors such as shallow water, tidal currents and high load of suspended phytoplankton (Colombo et al. 1979; Checcherelli and Rossi 1984). Mussels are often used as sentinel organisms because of their high tolerance to environmental stressors, the accumulation of chemicals and the suitability for field experiments in situ (Andral et al. 2004; Fasulo et al. 2008 and 2012), playing an essential ecological role in coastal ecosystems. Mussels are relevant also from a commercial perspective, especially in the Mediterranean Sea, where the amount of harvested mussels reach more than 75% of the total shellfish production (Saroglia et al. 2000). In the Mediterranean Sea, mussels of the species *M. galloprovincialis* are frequent host of digeneans of the Gymnophallidae family. Beyond trematodes, hydromedusan polyps, such as *Eugymnanthea inquilina* (Fig.3), build both symbiotic and parasitic relationship with the host organism, with harmful effects on host tissue (Mattox and Crowell 1951; Piraino et al. 1994; Mladineo et al. 2012) (Fig. 3). Other parasites are turbellarians such as *Urastoma cyprinae,* usually occurring on the gills tissue (Mladineo et al. 2012), copepods as *Myticola* sp., and Polychaeta as *Polydora ciliata* (Özer and Güneydağ 2014).

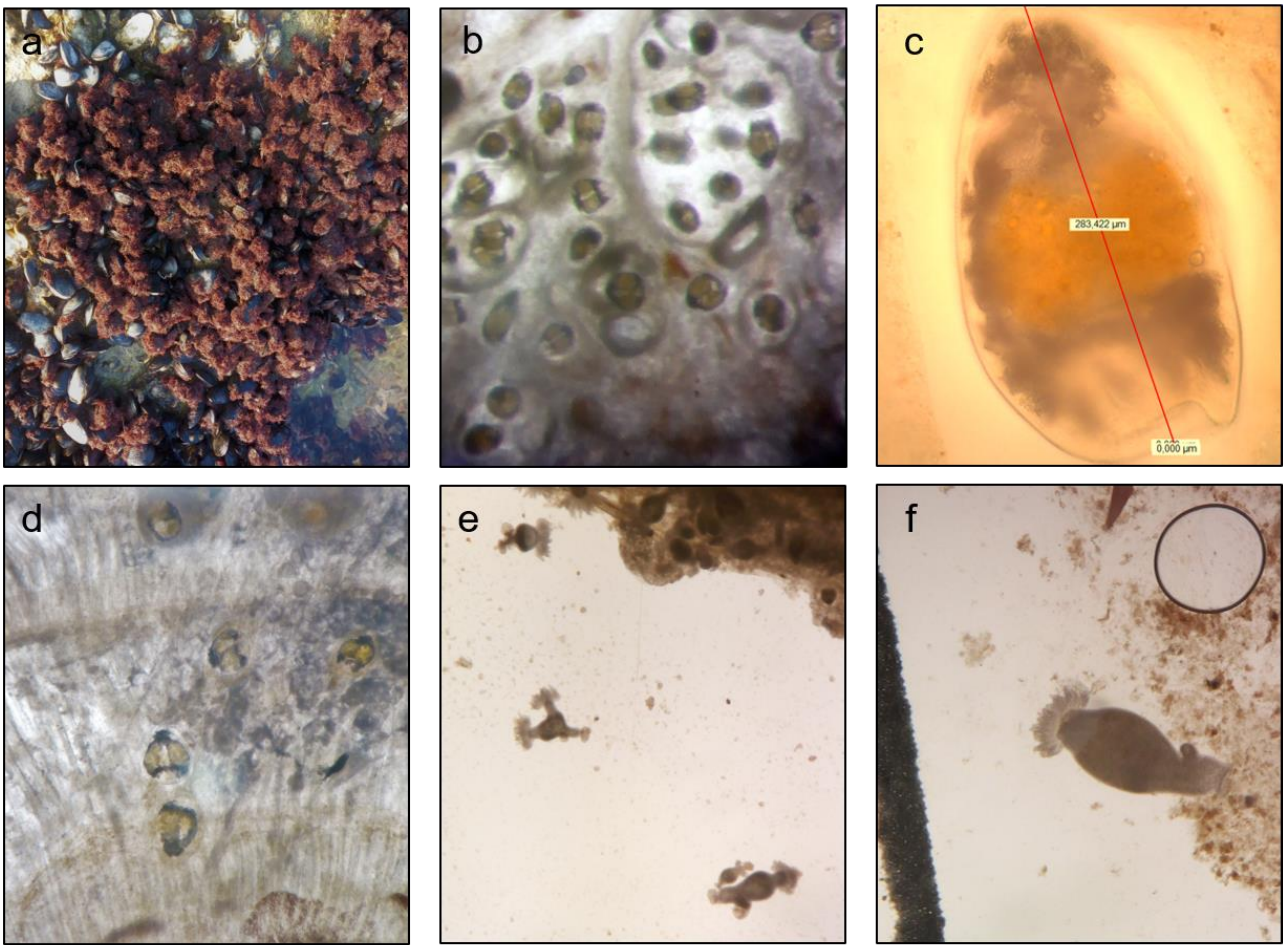


Fig.3 *Mytilus galloprovincialis* bed (a) in the North Adriatic Sea; metacerariae of *Parvatrema timondavidi* (b, c, d) and polyps of *Eugymnanthea inquilina* (e, f) in the sampled mussels.

Study region: the Baltic Sea

The Baltic Sea is a young water body originated with the last glaciation, which experienced many changes in the physicochemical composition over a geologically short period (Elken and Matthäus 2008; Telesh et al. 2010). The basin has a total area of 415000 km^2 and water exchange with the North Sea occur through the Kattegat strait. The entrance of seawater in the south-western area and the freshwater concentrated in the north-eastern area, create a steep salinity gradient which goes from around 30 (Kattegat and Skagerrak regions) to 3 psu (Gulf of Bothnia) (Müller et al. 2016). Along the salinity gradient, the benthic macrofauna decrease of 10 times, and species experience their limits of osmotic tolerance. Furthermore, due to both natural and anthropogenic pressure and related phenomena (i.e. eutrophication, oxygen depletion) some species frequently undergo through local extinction (Leppäkoski and Olenin 2001; Bonsdorff 2006; Zettler et al. 2014). The study by Zettler et al. (2014) reports that the highest marine macrozoobenthic species richness of the Baltic Sea is found in the Kattegat (1161 species), while in the Gulf of Finland the highest richness of freshwater species is found (410 species). Hence, the salinity gradient represents the main driver of the distribution patterns of organisms, with the south-western area displaying the highest species diversity. The multiple stressors characterizing this basin (i.e. fast warming of surface water, increasing nutrients load, oxygen depletion and shipping intensity) along with with a large amount of data available, make the Baltic Sea an excellent natural laboratory where to study global change consequences (Reusch et al. 2018).

Host models:

Littorina littorea

The periwinkle *Littorina littorea* is a common gastropod grazer spread all over the North European coasts, in the intertidal and subtidal zones. Periwinkles have a broad preference for different kinds of substrates (rocky, sandy, mud) and diet (they feed on diatoms, larvae, macroalgae), and are characterized by high tolerance to salinity (from 30 to 13 psu) (Lubchenco 1983; Taylor and Andrews 1988). Given these characteristics, periwinkles are easily suitable for experimental studies on the ecological interactions with other organisms, for instance, with epibionts (Wahl and Sönnichsen 1992), algae assemblages (Wihelmsen and Reise 1994; Anderson and Underwood 1997) or parasites. Periwinkles are indeed frequent host for many marine parasite species living the Baltic Sea, such as the digenean *Renicola roscovita*, *Himasthla elongata*, *Cryptocotyle* *lingua*, *Microphallus pygmaeus* and *Podocotyle atomon*, as reported by Lauckner (1984) and Kesting (1996). For these digenean species, periwinkles represent the first intermediate host, in which sporocysts, rediae and cercariae develop (Fig.4).

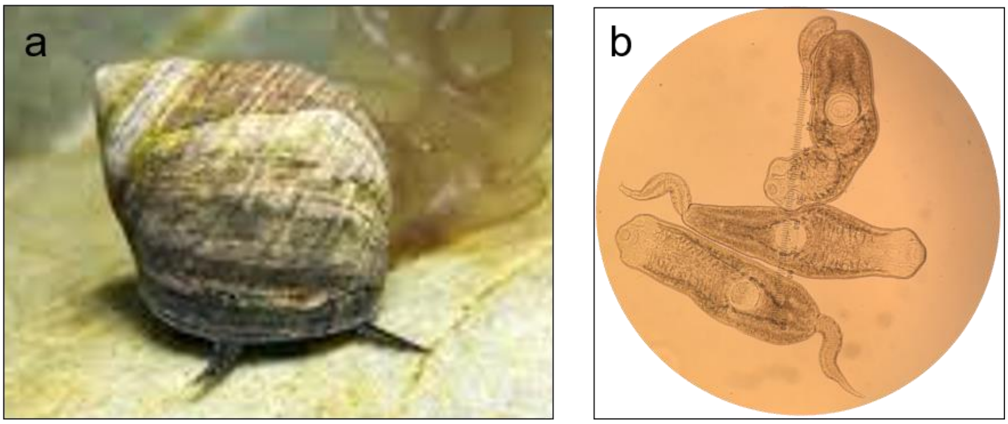


Fig.4 Individual of *Littorina littorea* (a) and cercariae of *Himasthla elongata* (b)

Mytilus edulis

The blue mussel *Mytilus edulis* is filter-feeder distributed all over the temperate European shores. Highly tolerant to environmental stressors, mussels dominate the rocky shores over other sessile organisms (Paine 1974). The role of mussels is however relevant due to the beds they form with their shells, which originates a unique habitat, suitable for many other organisms (Palomo et al. 2007; Krauvfelin and Diaz 2015). Furthermore, by filtering large amounts of water, mussels can alter plankton and seston compositions and convert them in benthic biomass for food web predators (Menge et al. 1997). Blue mussel represents the second intermediate host for many digeneans such as *Renicola roscovita* and *Himasthla elongata*, which infect the mussel at the cercaria stage and then, in the mussel tissue develop into metacercaria, waiting for the ingestion by the final host (Fig.5).

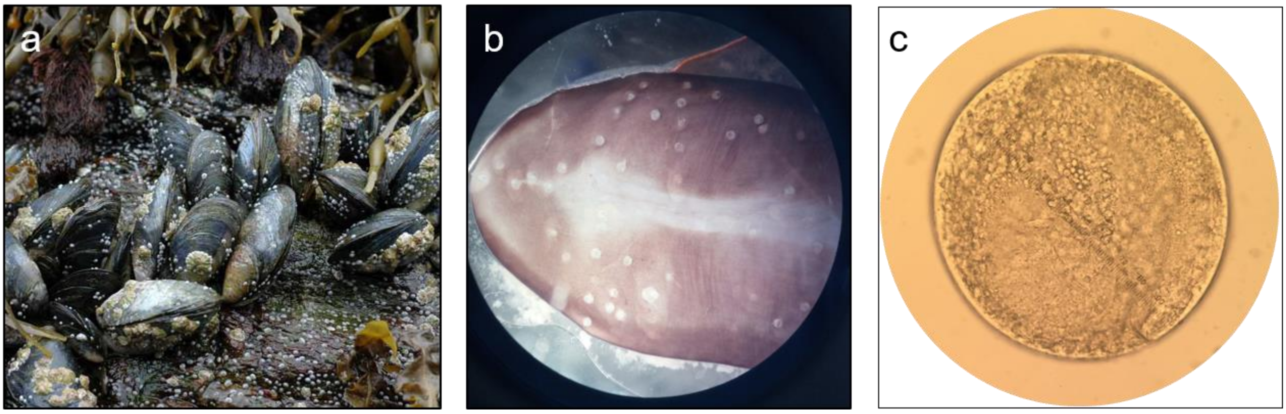


Fig.5 Individuals of *Mytilus edulis* (a), metacercariae of *Himasthla elongata* encysted in the mussel foot (b) and *Himasthla elongata* metacercaria.

THESIS OBJECTIVES

The Adriatic and the Baltic Sea display different features: the former is an old basin, characterized by high species diversity and subjected to a high load of new invasive species. The latter is a young basin, characterised by limnetic, brackish and marine habitats and by low species diversity. However, both basins are subjected to high anthropogenic pressure and are experiencing global change effects faster than other oceans. Studies on the host-parasite interaction in such different habitat are therefore important and can help to predict the effect of global change in a wider perspective. Furthermore, the host models used in the present study have all relevant roles in the marine communities of the Adriatic and the Baltic coasts. The main goals of this study were 1) to describe and quantify the parasites community of these two basins at a regional scale, particularly in the North Adriatic coast and in the south-western Baltic Sea 2) to identify the main environmental factors driving parasites distribution 3) to experimentally assess the influence of salinity and temperature in the transmission between the first and the second intermediate host of a common digenean species. The thesis will be divided in three chapters with the following aims:

- Chapter 1: “Correlation among parasite richness, prevalence and abundance in *Mytilus galloprovincialis* and abiotic and biotic factors along the coast of the North Adriatic Sea” which verify parasites prevalence, abundance and richness in the North Adriatic Sea, and their correlation with abiotic and biotic drivers
- Chapter 2: “Correlation among parasites infection, upstream host density and salinity in mussels of the South-Western Baltic Sea” which verify parasites prevalence and load in the south-western Baltic Sea and their correlation with abiotic and biotic drivers
- Chapter 3: “Freshening rather than warming affects trematode transmission from periwinkles to mussels” which assess the influence of salinity and temperature on cercarial emergence, activity and survival, infectivity and second intermediate host susceptibility to infection.

CHAPTER 1

Correlation among parasite richness, prevalence and abundance in *Mytilus galloprovincialis* and abiotic and biotic factors along the coast of the North Adriatic Sea

ABSTRACT

The spatial distribution and infection levels of parasites are known to be affected by various abiotic and biotic factors such as salinity, eutrophication and host density and size. The present field study investigated spatial patterns of occurrence, prevalence and abundance of parasites in *Mytilus galloprovincialis* along the coast of the northern Adriatic Sea, where the eastern side is characterized by oligotrophic and the western by eutrophic waters. Using GLMMs, the relationship between abiotic (salinity and eutrophication) and biotic (host size and density and local parasite richness) factors and prevalence, abundance and mean richness in individual mussels was analysed. Spatial heterogeneity among sampling locations was high, while at the regional scale the pattern was more pronounced. For all parasite species analysed, eutrophication was the factor contributing the most to prevalence, followed by salinity. Only for *Parvatrema timondavidi*, eutrophication was the factor contributing more to abundance, followed by salinity. Mussel density contributed to the distribution of *Eugymnanthea inquilina* only. Mean individual richness was positively correlated with local richness and mussel density. Furthermore, the abundance of *Parvatrema timondavidi* negatively affected mussels condition index. This study stresses the importance of parasites distribution monitoring, especially in areas subjected to high anthropogenic pressure and fast global changes like the Adriatic Sea.

INTRODUCTION

Parasites occur in all ecosystems and at all trophic levels. At the level of the individual, the impact of parasites is generally negative, altering host physiology and behaviour. Alterations of the host fitness can cause castration of the organism (Thieltges 2006; Carballal et al. 2001) or affect the susceptibility to predation and the competition with other species (Marcogliese 2004). The specificity of parasites for their hosts makes them excellent indicators of the community structure and diversity (Lafferty et al. 2006; Hechinger et al. 2007). In the food-web, for example, host-parasites links were recognized as more frequent than predator-prey links (Lafferty et al. 2006). Parasites richness and hosts richness and density generally correlate positively (Hechinger et al. 2007). Despite their relevance in the marine community, the role of parasites in ecology is still overlooked (Poulin 2016).

As for all other organisms, the various phases of parasite life-cycle are subjected to different environmental drivers such as temperature, salinity and eutrophication (Mouritsen et al. 2002; Studer et al. 2012; Johnson et al. 2007). Salinity gradients significantly affect parasites distribution and diversity, as reported by previous studies, which found parasites richness positively or negatively correlated with salinity (Schmidt et al. 2003; Blanar et al. 2011) depending on the habitat and on the host-parasites system investigated. Contrarily to richness, only a few studies investigated the effects of salinity on parasites abundance: Magalhães et al. (2018) detected no relation among salinity and abundance, and Rogowski et al. (2006) detected a negative effect of salinity on parasites abundance. However, the latest study was done in Salt Creek, an extreme environment where salinity goes up to 88.

Eutrophication is generally known to increase trematode abundance, by increasing the food availability for the intermediate hosts (Lafferty 1997; Marcogliese and Cone 2001). High availability of nutrients, for example, can enhance algal growth, which serves as first source of food for snails, common first intermediate host for many digenean species (McKenzie et al. 2007). This was experimentally confirmed by Johnson et al. (2007), who observed parasite transmission to be promoted by two different eutrophication-related mechanisms 1) an increase in host size leading to an increase in the production of trematode cercariae and 2) an increase of density of the host population promoting parasite prevalence. A higher density of first intermediate host can in turn favour higher prevalence and/or abundance of trematodes in the second intermediate host. For trematodes exploiting bivalves as single intermediate host, eutrophication could represent a two-sided coin. Bivalves condition index could increase with eutrophication (Carlsson et al. 2012). However, the additional nutrients could be competed for by parasites for growth and infection and by the host for resistance mechanisms (Aalto et al. 2015). Eutrophication could affect not only parasites prevalence and abundance but also richness. In eutrophic water few host species tend to dominate, limiting host diversity and therefore parasites diversity (Budria 2017).

Beside abiotic factors, host traits can affect parasite distribution and diversity (Poulin 1997; Thieltges 2007; Mouritsen et al. 2003; Magalhães et al. 2018). Host density represents one of the main drivers of parasites prevalence, abundance and richness (Poulin 1997; Arneberg et al. 1998; Galaktionov et al. 2015; Searle et al. 2017) but with contrasting, positive or negative, effects. A clear example is a recent study by Galaktionov et al. (2015) which reported that the effect of host density on infection levels depended on the parasite genera. Among biotic factors, an individual trait found positively associated with parasites abundance and richness is host size (Mouritsen et al. 2003; Thieltges and Reise 2007; Galaktionov et al. 2015). The underlying mechanisms could be either the host age, with mussels accumulating parasites over longer lifetime, or a higher quantity of available tissue to infect, and a greater diversity of niches within a host individual (Guegan et al. 1992; Nikolaev et al. 2006; de Montaudouin et al. 2009). With the present study, we will consider both second intermediate host population density and host size as main predictors of parasites prevalence, abundance and individual mean richness. Lastly, we will also include local parasite richness (total number of parasites species in one sampling location), as the pool of parasite species exploiting mussel per location. Parasite richness at the individual level should indeed positively correlates with the richness of the community in which they belong (Poulin 1997). However, except of some studies on snail intermediate host (Kuris and Lafferty 1994; Simberloff and Moore 1996) most other studies limited this correlation only to the final host.

The present study focuses on parasites of *Mytilus galloprovincialis*, as this species is ubiquitous in the Mediterranean Sea and important from both an ecological and commercial perspective. Hence, studies on environmental factors associated with parasitism can be even more relevant when involving commercially exploited species, not only for the economic value that these have but also for their ecological role in the marine communities. Bivalve species, for instance, are known as crucial filter feeders (Gosling 2003) and ecosystem engineers (Jones et al. 1994, Philippart et al. 2007), providing substrate for other species and thus increasing the habitat complexity (Ysebaert et al. 2009). Recently, mussel farming has also been proposed as a valid option to remove the overload of nutrients in the Baltic Sea (Kotta et al. 2020). Besides their key role for fisheries and ecosystem functioning, bivalves also represent suitable host for many parasites' species. The mussel of the genus *Mytilus*, for examples, serve as first or second intermediate host for a large number of digenean trematode species, such as Gymnophallidae or Renicolidae (Lauckner 1985; Galaktionov 1996). Not merely trematodes, but other multi-step life cycle organisms as the hydromedusan polyp *Eugymnanthea inquilina*, seem to appreciate mussel as host. This polyp in some cases acts as mutualistic symbiont, feeding on digenean parasitizing mussels (Piraino et al. 1994), and in other cases act as a parasite (Boero and Buillon 2005).

In the Adriatic Sea, bivalve aquaculture represents one of the major economic activities. The Adriatic basin can be divided in three subareas, southern, central and northern. The northern section is very shallow, with an average depth of 35 m (Artegiani et al. 1997). The western side of the northern section receives many river runoffs (i.e. the Po, the Adige and the Brenta rivers), which provoke a decrease in salinity, especially during spring and autumn (Russo et al. 2009). The eastern side is instead characterised by the warm and highly saline Eastern Adriatic current (Giani et al. 2012). Going from the east to the western side, salinity decrease, mainly in the surrounding area of the Po estuary. A wind-driven circulation of water masses (Kuzmić and Orlić 2006) together with the Po River discharge, originates a west-east gradient of nutrients (Solidoro et al. 2009). Hence, the western area is characterized by eutrophic waters, suitable for mussel farming (Rampazzo et al. 2013). The eastern area is instead characterized by oligotrophic waters (Hopkins 1999).

Until the 1980s, mainly due to anthropogenic pressure, an increase in eutrophication was detected all over the North Adriatic, which led to mass mortalities of benthic fauna due to hypoxia event (Stachowitsch 1984; Ott 1992; Orel et al. 1993). However, a review by Giani et al. (2012) showed that in the last decade, this trend is inverting, mainly due to a minor flow rate from the rivers. Both in the eastern and western sides of the North Adriatic Sea higher surface salinity and lower PO_4 and Chl a were registered (Djakovac et al. 2012). This inversion of trends, together with increasing shipping transport, led to changes in the macrobenthic community (Occhipinti-Ambrogi 2002). Native but also invasive species are now recovering in areas previously affected by eutrophication (Giani et al. 2012). Considering the complexity of all these combined factors, it becomes difficult to predict what could happen in the Northern Adriatic benthic community in the future. For this reason, field studies including relevant elements of the food-web such as parasites become even more urgent. So far, very few studies focused on host-parasite relation in the Adriatic Sea and the majority of them investigated parasites in fishes (Mladineo 2005; Smrzlic et al. 2012; Cavallero et al. 2015; Bušelić et al. 2018; Mladineo et al. 2020) and very few in bivalve molluscs (Piraino 1994; Mladineo et al. 2012). To our knowledge, this is the first study investigating the parasites community in mussel all over the Northern Adriatic coast.

The aims of the present study were to 1) study the spatial pattern of the prevalence and abundance of parasite community infecting *Mytilus galloprovincialis* from the eastern to the western coast of the northern Adriatic Sea 2) assess the temporal variability of prevalence and abundance of *P. timondavidi* in the single station of Lido (just outside the Venice lagoon, on the West side) 3) investigate the correlation between abiotic and biotic drivers (i.e. salinity, eutrophication, mussel density and length, local richness) with the prevalence, abundance and richness of parasites in individual mussels and 4) study whether the Condition Index (CI) of mussels is affected by *P. timondavidi* abundance.

METHODOLOGY

Sampling procedure

Spatial sampling

The sampling activity was carried out, between November and December 2018, in 16 stations spread from the north-western (Parco Nazionale del Delta del Po) to the north-eastern coast of the Adriatic Sea (northern Croatia) (Fig.1; Table 1). The stations were selected based on geographical distribution, along the coast and not inside the lagoons, and logistic feasibility. In each station, a total of 20 individuals of *M. galloprovincialis* were randomly collected. The mussels' density in each station was recorded by counting all individuals in three independent 50x50 cm plots, distanced by 25m other along a 50 m transect.

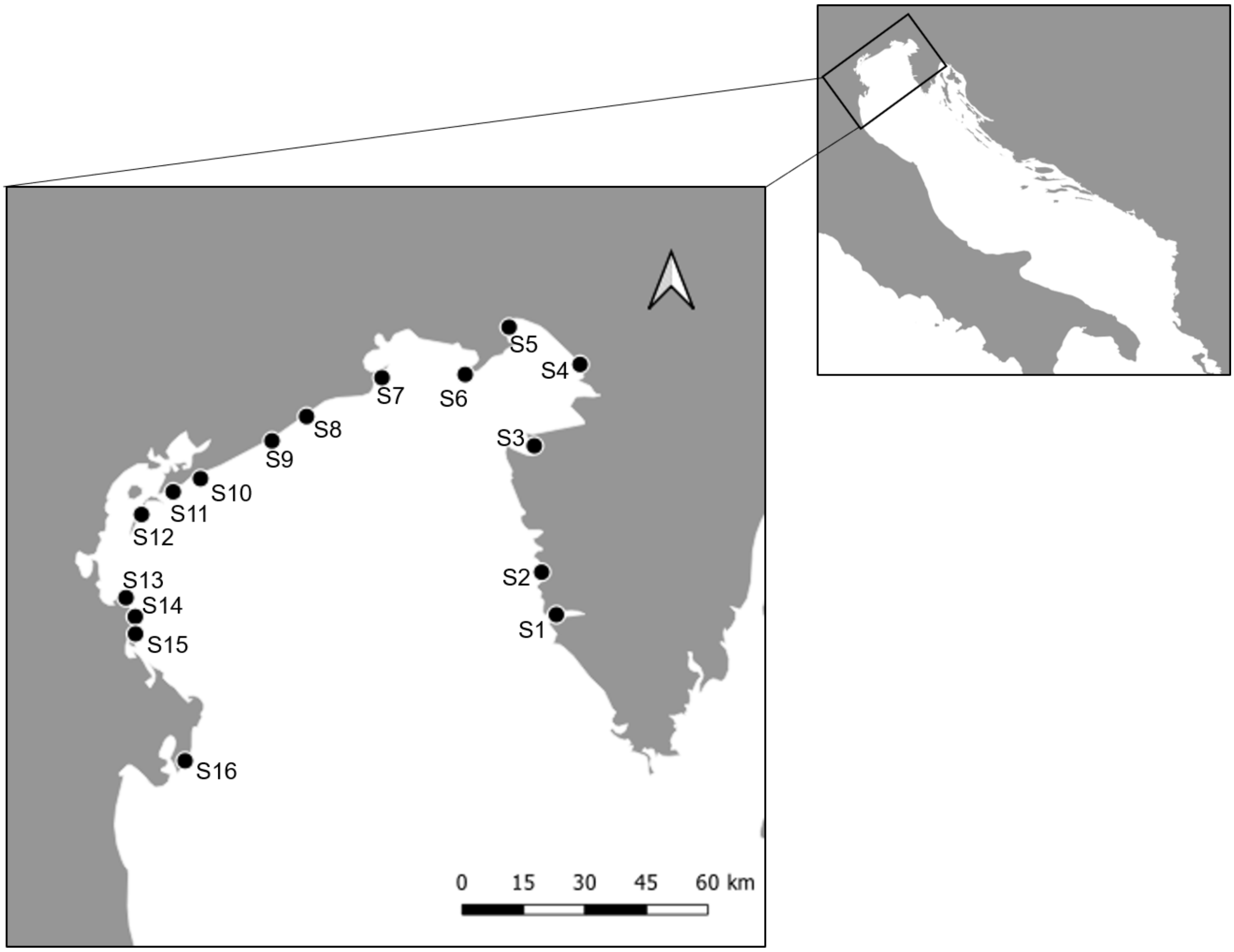
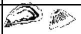
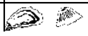


Fig. 1 Map of the sampling stations along the North Adriatic coast

Temporal sampling

In order to assess the temporal variation of parasites prevalence, abundance and richness, monthly samples have been collected from September 2018 to May 2019 (with the exception December and April) in the Lido station, located near the Venice lagoon inlet, on the West coast. A total of 20 individuals of *M. galloprovincialis* were randomly collected. The mussels’ density was recorded by counting all individuals in a 50x50 cm plot; three plots, equally distant each other along a 50 m transect.

Table 1 Station, salinity, temperature, TRIX, substrate, presence of other species, mussel mean density (±SE) and mean length (±SE) of mussels collected in each station along the North Adriatic Sea. Salinity is reported based on the annual mean of the 2018 and temperature on the average of November and December 2018 (Copernicus dataset). Other species present are visualised as symbols: oysters “🦪”, barnacles “ 🐚” , limpets “✺”, snails “ 🐌”, crabs “🦀”.

Station	*Salinity (psu)*	*Temperature (°C)*	*TRIX*	*Substrate*	*Mussels density (50x50cm)*	*Mean mussel length (mm)*	*Presence of other species*
S1	38.439	14.735	3.03	Mud	30±0	40.8±2.2	
S2	38.492	14.929	2.7	Rocky	14.6±6	44.5±0.8	
S3	38.143	14.119	2.37	Rocky/shingle	415±295	30.2±0.6	
S4	38.183	13.538	3.42	Rocky	156±97	42.9±0.9	
S5	38.066	13.382	4.47	Rocky/shingle	33.3±9	36.9±0.8	
S6	37.999	13.720	4.07	Sandy	26.6±7	36.6±0.9	
S7	37.852	13.437	4.35	Sandy	300±28	37.8±0.8	
S8	37.724	13.346	4.2	Sandy	466.6±33	35.4±0.9	
S9	37.523	13.493	4.7	Sandy	600±57	36.8±0.8	
S10	37.130	13.480	4.8	Sandy	1000±0	23.4±0.3	
S11	36.792	13.546	4.5	Sandy	500±0	30.9±0.9	
S12	36.697	13.651	4.7	sandy	523.3±14	36.1±1.2	
S13	36.498	13.389	4.3	Rocky/sandy	236.6±18	36.4±0.9	
S14	33.338	13.761	5.4	Sandy	800±57	34.1±0.8	
S15	33.338	14.458	5.5	Sandy	226.6±23	29.4±0.5	
S16	27.050	14.458	5.5	Sandy	5±0	30.0±1.9	

Parasites identification

After collection, samples were transferred in the laboratory, where the length and the total weight of each individual were registered, then mussels were dissected, and the tissue of each individual was squeezed between two slide glasses (compressors). Parasites identification was realized following previous studies descriptions (Bartoli 1965; Davey and Gee 1988; Mladineo et al. 2012). Prevalence, abundance, intensity and richness of each parasite species were registered.

After parasite identification, mussel tissue and shell were individually collected and located in numbered aluminium pots to measure the dry weight (24 h at 80°C). The condition index (CI) was then calculated, using the following formula: $CI=W/L^3$, where W is dry tissue weight (mg), and L is shell length (mm) following Riisgård (2001) and Stier et al. (2015).

Salinity and temperature measurements for all the stations were retrieved from the Adriatic Sea physics reanalysis product provided by Copernicus Marine Environment Monitoring Service (http://marine.copernicus.eu). The data were extracted for a depth of 1.5 m. Annual average salinity and the average temperature of November and December 2018 (period when the sampling was run) were calculated for each station.

To estimate eutrophication, TRIX index (Vollenweider et al. 1998) was used. This index is composed of four variables connected to primary production: chlorophyll-a, oxygen, dissolved inorganic nitrogen and total phosphorus. Annual average TRIX for each station was provided by the Agenzia Regionale per la Protezione dell'Ambiente del Veneto (ARPAV), by the Agenzia Regionale per la Protezione dell'Ambiente del Friuli-Venezia Giulia (ARPA FVG) and by the Institute of Oceanography and Fisheries, Croatia.

Data analysis

Prevalence, as the proportion of infected mussels, and richness were calculated for all parasites and symbionts found. Differences in prevalence of the most common species among the stations were analysed applying chi-square test and correcting the significance level for multiple testing using the Bonferroni approach.

To verify the correlation between abiotic (i.e. salinity, TRIX) and biotic predictors (i.e. mussel population density and mussel size) and the prevalence of *Parvatrema* timondavidi, *Myticola* sp., *Eugymnanthea inquilina* and *Urastoma cyprinae,* generalized linear mixed models (GLMMs) with binomial family (package lme4) were performed (Table 2). Since the aim was to focus on the individual level, prevalence was accounted as presence/absence of a parasite species per mussel individual. To verify the correlation between the same predictors and the abundance of *Parvatrema timondavidi* and *Eugymnanthea inquilina* generalized linear mixed models (GLMMs) with Negative Binomial family were performed (Table 2). Abundance was assessed as the mean number of parasites in infected and not infected hosts, at the individual level. Abundances of *Myticola* sp. and *U. cyprinae* were not included in the analysis since too much low. In all models, sampling location was included as a random factor. To linearize the relationships among environmental drivers, log-transformations were applied when necessary (See Table 2). The abundance of *E. inquilina* was also log transformed due to the high number of zero values. Temperature was not included due to the low variations recorded (13.3-14.9°C). Lastly, also substrate was not included due to unbalance in the dataset (only three sampling locations had rocky substrate, all the others sandy), and other species presence due to really low variability among locations (See Table 1).

All models were performed separately for each parasite species. The first model run included all predictors. Then, collinearity among predictors was checked through the "vif" (function packages "car" and "mctest"). In all models the vif value appeared < 4, thus no predictor was excluded. After testing collinearity, other models were run excluding one, two or all predictors (null model). All models, the one including all predictors and the simpler ones, were then compared using Akaike Information Criterion corrected for small sample sizes (AICc).

The model showing the lowest AICc value was chosen as the best explanatory model. Residual plots of the chosen models were inspected visually.

To test the correlation between mean individual richness and biotic and biotic predictors, a Generalised Linear Mixed Model with Gaussian family was applied, including location as a random factor. For this model, the same predictors mentioned above were used, plus local richness. The model selection followed the same procedure as for species prevalence and abundance.

To test the temporal variation of parasites prevalence and abundance in Lido, a GLMM with binomial family was applied for prevalence and GLMM with Gaussian family for abundance, with time (month) as fixed factor and individual identity as a random factor. For this analysis, only *P. timondavidi* was included, since prevalence and abundance of the other species were too low.

Lastly, to verify the effect of *P. timondavidi* abundance on mussels CI a GLMM with gaussian family was applied, using CI as dependent variable, abundance (log+1 transformed) as fixed factor and sampling locations as random factor. All analyses were performed using the statistical software R 3.5.0 (R Development Core Team 2018).

Table 2 Abiotic and abiotic predictors accounted as possible drivers for the prevalence (P) of *Parvatrema timondavidi* (P), *Myticola* sp. (M), *Eugymnanthea inquilina* (E) and *Urastoma cyprinae* (U), for the abundance (A) of *P. timondavidi* and *E. inquilina* and for the mean individual richness (IR). Range and transformation of each predictor is reported.

Type of factor	**Factor**	**Range**	**Transformation**	**Response variable**		
				P	**A**	**IR**
Abiotic	Salinity	38.43-27.05 psu	No	X	X	X
	TRIX	2.3-5.6	No	X	X	X
Biotic	Mussel density	5-1000 (50 cm^2)	Log	X	X	X
	Mussel size	23.4-44.5 cm	Log	X	X	X
	Local richness	2-7	No			X

RESULTS

Spatial pattern

During the sampling, a total of 320 mussels (n= 20 for each station) from the 16 locations were dissected. A total of 7 distinct taxonomic groups have been detected, out of these four species were determined: *Parvatrema timondavidi* (Mean Prevalence MP= 0.52±0.10), *Myticola* sp. (MP= 0.06±0.02), *Eugymnanthea inquilina* (MP= 0.14±0.07) and *Urastoma cyprinae* (MP= 0.03±0.01). Other species found were nematodes (MP= 0.04±0.02), ciliates (MP= 0.17±0.03) and other copepods (MP= 0.03±0.05). Among the species, *P. timondavidi* displayed the highest abundance, with an average of 68.6 metacercariae per individual in the total of mussels collected. The highest intensity observed per single infected mussel was of around 1200 metacercariae.

Among the most common parasites species, *P. timondavidi* and *Myticola* sp. showed high spatial heterogeneity both in terms of prevalence and abundance (Fig. 2a and b). The prevalence of *P. timondavidi* was significantly lower in stations S1, S2, S3, S7, S13 and S16 than all other stations (chi-square: p< 0.001), while the prevalence of *Myticola* sp. was significantly highest in S9 (chi-square: p< 0.001). Conversely, prevalences of *E. inquilina* and *U. cyprinae* were higher in the eastern stations, and significantly higher in S3 and S4 (*E. inquilina*) (chi-square: p< 0.001) and S2 (*U. cyprinae*) (chi-square: p< 0.001).

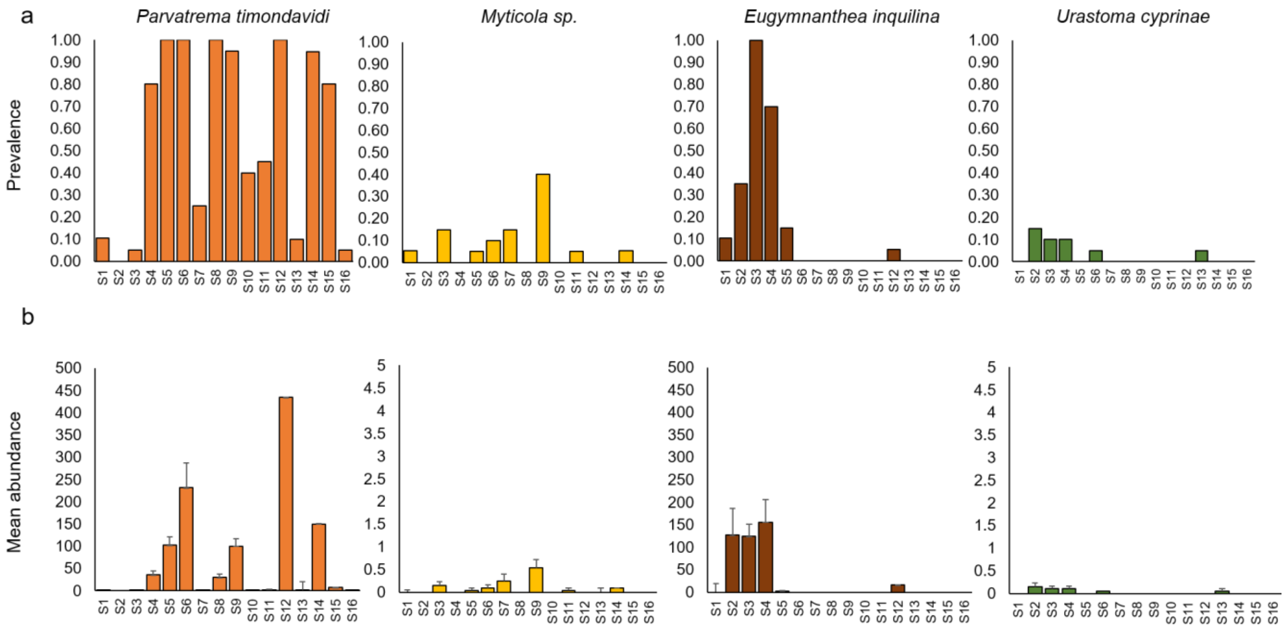


Fig. 2 Prevalence (a) and mean abundance (b) of the four most common parasite species found in the mussels collected during the sampling (n= 20 in each station). All plots are based on sampling station arranged from the eastern to the western side of the North Adriatic Sea. Error bars represent the standard error (st.err.) Notice the different scale for *Myticola* sp. and Urastoma *cyprinae abundance*

Temporal pattern

The temporal pattern of the prevalence and abundance of *P. timondavidi* - metacercariae showed no significant effects of time in prevalence (Table S1 Supplementary Material), while abundance significantly decreased from autumn 2018 to spring 2019, especially in May ($P<0.001$; Table S2 Supplementary Material; Fig. 3).

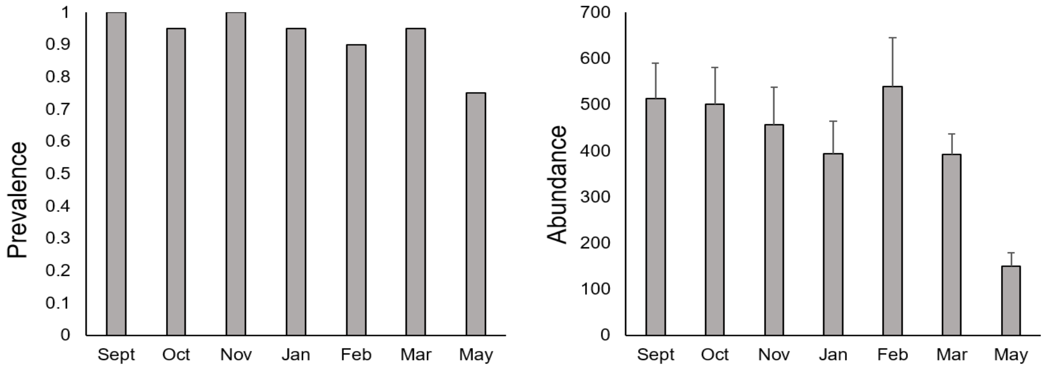


Fig. 3 Prevalence (a) and mean abundance (b) of *Parvatrema timondavidi* in mussels collected in Lido from September to May (n= 20 in each sampling). Error bars represent the standard error (SE)

Environmental drivers of parasites infection pattern

Effects on parasites/symbionts prevalence

The AICc selection showed the model including just TRIX and salinity as the best explanatory model for *P. timondavidi* prevalence (Table S3 Supplementary Material), with the two predictors explaining 24% of the variance in prevalence. The model including only TRIX was the best for *E. inquilina* prevalence (Table S3, Supplementary Material). However, the variance of prevalence explained by TRIX was almost zero (<0.1%). This may reflect the heterogenous of distribution of *E. inquilina,* with elevated prevalence (up to 100%) in five eastern locations and 0% prevalence in all other locations (except S12 were prevalence was low). This heterogeneity and the elevated number of zeros may blur the results of the model. The model including TRIX and mussel size was the best explanatory model for *U. cyprinae* (Table S3, Supplementary Material), with all predictors explaining the 36% of the variance in prevalence. Lastly, the results of GLMMs showed none significant interaction between the prevalence of *Myticola* sp. and abiotic and biotic predictors. Furthermore, AICc selection showed the null model as the best explanatory model (Table S3, Supplementary Material).

The eutrophication index, TRIX, was the factor fitting the best *P. timondavidi*, *E. inquilina* prevalences. However, while the effect of TRIX on prevalence of *P. timondavidi,* even if minor, was positive (GLMM, $P<0.05$; Table 4; Fig.S1 Supplementary Material), its effect on *E. inquilina* and *U. cyprinae* prevalence was negative (GLMM, P=0.002 and P=0.01 respectively; Table 4; Fig.S1 Supplementary Material). Salinity significantly affected only *P. timondavidi* prevalence, found higher at higher salinity locations (GLMM, P<0.05; Table 4; Fig.S1 Supplementary Material). Among biotic factors, mussel size displayed a slight positive correlation with *U. cyprinae* prevalence (GLMM, P=0.09; Table 4; Fig. S1 Supplementary Material).

Table 4 GLMM following binomial distribution on the effects of abiotic (TRIX, salinity) and abiotic (mussel size and density) factors on prevalence of *Parvatrema timondavidi, Eugymnanthea inquilina* and *Urastoma cyprinae* in mussels (*Mytilus galloprovincialis*). In the GLMM, sampling location was included as random factor. The symbols "." "*", "**" and "***" indicate p-values <0.1, <0.05, <0.01, <0.001, respectively.

Parasite species	**Predictors**	**Estimate**	**Std. Error**	**Z value**	**Pr(>|z|)**
Parvatrema timondavidi	Intercept	-36.903	16.710	-2.208	0.027*****
	TRIX	2.481	1.081	2.294	**0.021***
	Salinity	0.738	0.361	2.294	**0.041***

Eugymnanthea inquilina	Intercept	11.837	4.685	2.526	0.011*
	TRIX	-3.900	1.294	-3.014	**0.002****
Urastoma cyprinae	Intercept	-10.696	7.178	-1.490	0.136
	TRIX	-1.093	0.442	-2.472	**0.013***
	Mussel size	7.155	4.251	1.683	0.092.

Effects on parasites/symbionts abundance

The AICc selection showed the best explanatory model abundance the one including TRIX and Salinity (Table S4 Supplementary Material) for *P. timondavidi*, and the one including also mussel density for *E. inquilina* (Table S4 Supplementary Material). The chosen model explained 27% of the variance in abundance for *P. timondavidi* but only the 0.1% for *E. inquilina*.

TRIX appeared to be the most important driver for *P. timondavidi* abundance, followed by salinity. At higher TRIX and salinity, the latter with a minor effect, abundance increased (GLMM, P=0.007 and P=0.01 respectively; Table 5; Fig.S2 Supplementary Material). For *E. inquilina* abundance, the main driver was salinity, followed by mussel density and TRIX. While with the former two abundance positively increased, with the latter it decreased (GLMM, P<0.001, P=0.003 and P=0.08 respectively; Table 5; Fig. S3 Supplementary Material).

Table 5 GLMM with negative binomial distribution on the effects of abiotic (TRIX, salinity) and abiotic (mussel size, density and parasites local richness) factors on the abundance of *Parvatrema timondavidi* and *Eugymnanthea inquilina* in mussels (*Mytilus galloprovincialis*). The abundances of *Myticola intestinalis* and *Urastoma cyprinae* were not considered in this analysis because too low. In the GLMM, sampling location was included as random factor. The symbols "." "*", "**" and "***" indicate p-values <0.1, <0.05, <0.01, <0.001, respectively.

Parasite species	**Predictors**	**Estimate**	**Std. Error**	**Z value**	**Pr(>|z|)**
Parvatrema timondavidi	Intercept	-37.998	14.401	-2.638	0.008**
	TRIX	2.734	1.023	2.672	**0.007****
	Salinity	0.748	0.314	2.380	**0.017***
Eugymnanthea inquilina	Intercept	-202.676	7.047	-28.758	**<0.001*****
	TRIX	-0.7940	0.454	-1.746	**0.080.**
	Salinity	5.260	0.185	28..373	**<0.001*****
	Mussel density	1.709	0.590	2.895	**0.003****

Environmental drivers of mean individual richness

Mean species richness ranged from 0.15 to 1.95 (Fig. 3a), and individual richness per mussel ranged from 0 to 4 species. Among sampling locations, total richness ranged from two to seven species or phyla (for ciliates, copepods and nematodes), with higher total richness in the eastern stations (Fig. 4b). The GLMM analysis revealed a significant positive correlation between local richness, mussel density and mean individual richness (GLM, $P< 0.01$ and $P< 0.05$, respectively; Table 6). The two predictors explained 55% of the variance of mean individual richness.

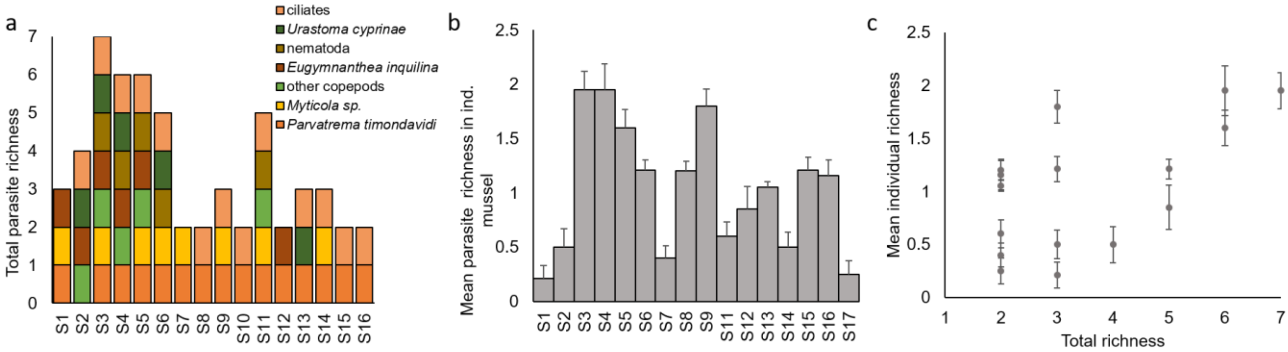


Fig.4 Maximum total richness (a) and mean richness per individual (b) based on sampling station arranged from the eastern to the western side of the North Adriatic Sea and mean individual richness versus total richness per location (c). In Fig. 3b the presence of one species is considered as 1, to show the species composition better. Error bars represent the standard error (st.err)

Table 6 GLMM with gaussian family on the correlation between local richness and mussel density (log-transformed) and mean individual richness. The symbols "." "*", "**" and "***" indicate p-values <0.1, <0.05, <0.01, <0.001, respectively.

Predictor	**Estimate**	**Std.Error**	**df**	**t value**	**Pr(>|t|)**
Intercept	-0.070	0.088	16.000	-0.799	0.435
Local richness	0.047	0.012	16.000	3.696	**0.001****
Mussels density	0.088	0.031	16.000	2.821	**0.012***

Effect of *P. timondavidi* abundance on mussels Condition Index (CI)

Results showed that abundance of *P. timondavidi* significantly but weakly affected mussels CI, which decreased with increasing abundance (GLMM, $P= 0.005$; Fig.5)

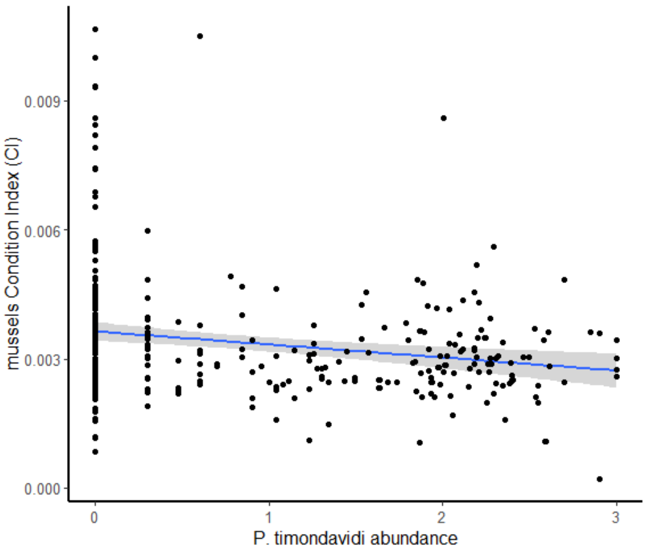


Fig.5 Condition index (CI) of mussels related to *Parvatrema timondavidi* abundance. In the GLMM analysis, sampling locations were used as a random factor.

DISCUSSION

Spatial patterns

Generally, the prevalence, abundance and richness of the parasite/symbiont species identified were highly variable among sampling locations and instead less variable on a regional scale. The species composition was very similar to the one observed by Ryyan et al. (2004) in the Aegean Sea, which suggests a broad range of distribution in the Eastern Mediterranean Sea of the species identified. The most dominant species all over the sampled area was *Parvatrema timondavidi*, which showed the highest prevalence and abundance in the majority of the locations. This is the first record of *P. timondavidi* in the North Adriatic waters, therefore no information is available on its spatial distribution and interaction with other species. The species was first detected in the Gulf of Marseilles, France, and described by Bartoli (1965) as *P. timondavidi* (Gymnophallidae family), but no other records followed. In general, metacercariae from the Gymnophallidae family are known to not encyst as other trematode species, and actively ingest their host tissue by the oral suckers (Irwin et al. 2003; Galaktionov et al. 2006). Regardless of conspecific traits, the massive levels of infection detected during our sampling are likely to have deleterious effects on mussels.

Other species found were *Eugymnanthea inquilina* and *Urastoma cyprinae*, however, while *P. timondavidi* appeared widespread all over the area, prevalences of the formers were mainly restricted to the eastern side of the North Adriatic. These two species were previously registered in the southern Adriatic Sea by Piraino et al. (1994) and Mladineo et al. (2012) and in the Aegean Sea by Ryyan et al. (2004), and their co-occurrence at the individual level is not rare. Their association with mussel ranges from mutualism, with *E. inquilina* ingesting trematodes sporocysts in mussel tissue, to parasitism (Piraino et al. 1994; Mladineo et al. 2012) with negative effects on host condition index (Rayyan et al. 2004). Lastly, also *Myticola* sp. was found, however, no patterns of distribution were identified, which is consistent with the observations by Rayyan et al. (2004).

Temporal patterns

At the temporal scale, the prevalence of *P. timondavidi* in Lido showed no variation from September to May, and abundance showed a significant decrease in May only. Mussels are sessile organisms, present all year long and *M. galloprovincialis* is particularly well-tolerant to environmental fluctuations (Skibinski et al. 1983; Kovačič et al. 2017), which may explain the absence of seasonality in prevalence. Another explanation might simply be that our temporal sampling lacks the summer months when the rate of digenean trematode infection is the highest (Thieltges and Rick 2006). Our results on abundance are coherent with the ones of Magalhães et al. (2018), who detected metacercariae abundance per cockle decreasing in summer. Reduced abundance may be triggered by variation of water surface temperature of the North Adriatic, characterised by a strong seasonal cycle, with winters of 9°C and late spring-summer from 23°C until 29°C (Russo et al. 2009). The effect of temperature is possibly more pronounced when related to the peculiar life-cycle of Gymnophallidae. Hence, some species belonging to this family are characterized by parthenogenic metacercariae with two generations, whit a second-generation developed inside the first. The proliferation of metacercariae could be affected by peaks in temperature. However, we can just speculate on this, since the entire life-cycle of *P. timondavidi* remains unknown.

Role of abiotic factors

The distribution of the species identified generally followed eutrophication and salinity gradients, the former increasing from the eastern to the western side of the North Adriatic and the latest decreasing. The gradients were mainly shaped by the distribution of *P. timondavidi* and *E. inquilina*. However, the drivers selected contributed more to the abundance than the prevalence of the species identified.

Salinity

Prevalence and abundance of *P. timondavidi* positively correlated with salinity in the range investigated in this study. There are no previous reports on the relation between *P. timondavidi* distribution and salinity, however, our results can be generalised to digenean trematodes transmission, which experimentally showed to be negatively affected by reduced salinity (Koprivnikar et al. 2010; Lei and Poulin 2011; Studer et al. 2012; Bommarito et al. 2020). Effects of reduced salinity in these studies were mainly attributed to the osmotic tolerance of free-living digeneans stages and of the intermediate mussel hosts, which susceptibility is reduced at suboptimal salinity (Bommarito et al. 2020). Our field study conducted in the Baltic Sea reported positive correlations among digeneans prevalence and abundance and salinity (Bommarito et al. in prep) in digeneans using mussels as second intermediate host (i.e. *Himasthla elongata* and *Renicola roscovita*). Others found no correlation among the former and parasites prevalence and abundance (Magalhães et al. 2018; Goedknegt et al. 2019) instead. All mentioned studies investigated on bivalve hosts, which are typically sessile organisms and which physiology strictly depends on environmental fluctuations occurring in their habitat. The gradient of salinity of our study area was not that steep, except for the location occurring in the proximity of the Po estuarine, where salinity falls from 33 up to 27. Such a strong decrease might indeed affect both mussel distribution and *P. timondavidi* free-living stages.

E. inquilina abundance increased with salinity. Our results are coherent with the ones of Zizec et al. (2012), who found a positive correlation between increasing salinity and *E. inquilina* infestation in a range of salinity between 27 and 37. They attributed this result to an increment of stress to the mussel host provoked by increasing salinity. We believe this is not our case since salinities up to 37 are normally experienced by organisms of the north-eastern Adriatic coast (Russo et al. 2009). The abundance of *E. inquilina* instead may be restricted to the eastern and saltier waters due to the fluctuations, with peaks of low salinities, that can occur in the north-eastern side. Furthermore, the variance of both prevalence and abundance explained by the best model were extremely low, which lead to argue that distribution of *E. inquilina* is also determined by other local factors characterising the sampling stations rather than the ones we analysed. This could be also the case of *Urastoma cyprinae* and *Myticola* sp., which showed extremely low significant or none correlation with the predictors included in our model.

Eutrophication

P. timondavidi abundance and, with a lesser extent, prevalence, increased with eutrophication. Higher abundance at higher TRIX values is coherent with what reported by Lafferty (1997) in a review, in which an increase of parasite abundance was in most of the case associated with an increase or resource for the intermediate host fitness. Hence, digeneans infections are always related to the presence of the hosts involved in their life cycle, and the first intermediate and final host are particularly sensitive to environmental factors (Hechinger and Lafferty 2005; Byers et al. 2008). The positive effect of eutrophication on prevalence and abundance of *P. timondavidi* in mussels could be then explained by an increase of first intermediate host population resulting from a higher nutrients load (Johnson et al. 2007). However, since the first intermediate host of *P. timondavidi* remains unknown, about this direct correlation, we can only speculate. As mentioned by Aalto et al. (2015), parasites fitness and infection also depend on the allocation of nutrients by the host. When higher nutrients are available, parasites could steal them before the host exploits them for immune defence. Furthermore, higher resource quantity may lead to higher host tolerance to the environment, and therefore beneficial effects for the parasites (Aalto et al. 2015). As opposite to *P. timondavidi*, we detected a negative effect of eutrophication on *E. inquilina* and *U.cyprinae* prevalence. Their life cycle is not strictly associated with the host as for trematodes, and nutrients load beyond certain limits could affect their fitness. In a study in North Western Ireland, the abundance of hydroids did not show any correlation with increasing eutrophication, through time (Little et al. 2018). Extremely low variance of *E. inquilina* prevalence explained by the chosen model it might indicate a similar pattern. To our knowledge, almost no studies investigated the response of turbellarians to eutrophication. One by Caires et al. (2013) run in the Lake Tahoe, showed negative correlations among turbellarians abundance and increasing eutrophication. However, more information should be gathered about these two overlooked, but considerably important (Kostenko et al. 2018), species and their relations with environmental drivers.

Role of biotic factors

Contrary to expectations, among the biotic factors only mussel size was included in the model explaining *U. cyprinae* prevalence, but with no statistically significant effect, and mussel density in the model explaining *E.*

inquilina abundance, with significant positive effect. For the latter, however, the chosen model scarcely explained the variance of abundance. Infection dependence on density and size was mainly detected in trematodes (Arneberg et al. 1998; Thieltges and Reise 2007; Galaktionov et al. 2015), which require obligatory hosts to complete the life-cycle. The lack of contribution of mussel density to *P. timondavidi* prevalence and abundance might simply be due to the fact that mussel density was high in the majority of the locations. Furthermore, in our sampling, only the density of the target host was verified, however for *P. timondavidi* the distributions of the final host may possibly represent more influential drivers (Hechinger and Lafferty 2005; Fredensborg et al. 2006; Song and Proctor 2020). For instance, Song and Proctor (2020) detected a positive significant interaction between the prevalence of parasites and the abundance of their final host, while the abundance of the intermediate host showed to be less important and even negatively correlated with the abundance of parasites. In their study, Hechinger and Lafferty (2005) detected a positive correlation among richness and abundance of parasites and the ones of the bird final hosts, and Fredensborg et al. (2006) a positive correlation among parasite prevalence and bird final host abundance. Another general explanation for the lack of correlation between parasites prevalence and abundance and mussel density could be that trematodes from the Gymnophallidae family as well as turbellarian and hydroids were previously reported in a broad variety of hosts (Fleming et al. 1981; Piraino 1994; Thieltges and Reise 2006; Galaktionov et al. 2015) which lead to argue a minor influence of a specific host presence and density. At the individual level, host size also did not show any effect on abundance and prevalence. Except site S10, with mean mussel length of 23.5 cm, the size range of the mussel populations was homogeneous among locations, which may explain the absence of an effect.

Drivers of individual richness

Individual richness showed spatial heterogeneity at the regional scale, decreasing from the eastern towards the western side of the study area. In the chosen model, both parasite local richness and mussel density positively affected richness at the individual basis, explaining 54% of its variance. Our findings are coherent with the review by Poulin (1997), which assessed infracommunities of parasites in the individual host representing subset of the species occurring in the component community. This might be determined by characteristics of the host population, for example homogeneity among individuals in the susceptibility to infection, and the environmental conditions they inhabit (Hartvigsen and Halvorsen 1994; Poulin 1997). Regarding the relation between parasites richness and mussel density, previous studies obtained different results, with positive, negative or none relationship found (Gregory et al. 1991; Watve and Sukumar 1995; Vitone et al. 2004). Mechanisms underlaying our result might be related to the benefit of a dense packaging of mussel beds, which may offer a suitable refugee for the parasites/symbionts themselves (i.e. hydroids) or for other intermediate hosts involved in digenean trematode's cycle.

Effect of *P. timondavidi* on mussels Condition Index

Finally, we observed a weak negative correlation between mussels Condition Index and *P. timondavidi* abundance, which is coherent with other studies showing detrimental effects of trematode metacercariae in second intermediate host (Thieltges 2006b; O'Connel-Milne et al. 2016).

CONCLUSIONS

In general, the drivers considered in our study significantly contributed to the distribution of the investigated parasites, especially the abiotic ones. However, the explained variance in the chosen models remained lower than the 40%, which may mean that other regional/local factors rather than the ones analysed have higher influence on prevalence and abundance of parasites. Eutrophication and salinity were the drivers most contributing to the analysis, affecting parasites prevalence and abundance. Studies on the future status of the North Adriatic Sea showed different outcomes, making accurate predictions on the effects of global change on parasitism difficult to develop (Giani et al. 2012). The potential decrease of eutrophication will may be detrimental for parasites as digeneans and beneficial for intermediate host. Negative effects of decreasing eutrophication could be however buffered by predicted increasing salinity. In the last years, under global warming effects, the North Adriatic Sea is serving as a sanctuary for temperate species (Lasram et al. 2010), including a wide variety of molluscs and marine birds, native and invasive. This could lead to new interactions between parasites and their host. Findings of our study should be filled with information about population and individual traits of the first intermediate and final host of *P. timondavidi*, as well as experimental studies on the effects of host traits, eutrophication and salinity on the most common species found.

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SUPPLEMENTARY MATERIAL

Table S1. GLMM with binomial family on the correlation between time (month) and prevalence of *Parvatrema timondavidi* metacercariae in the Lido station. The symbols "." "*", "**" and "***" indicate p-values <0.1, <0.05, <0.01, <0.001, respectively.

Predictor	**Estimate**	**Std. Error**	**Z value**	**Pr(>|z|)**
Intercept	2.197e+00	7.454e-01	2.948	0.0032**
January	7.472e-01	1.268e+01	0.589	0.555
March	7.472e-01	1.268e+01	0.589	0.555
May	-1.099e+00	9.068e-01	-1.212	0.225
November	3.242e+01	7.190e+06	0.000	1.000
October	3.893e+01	1.540e+07	0.000	1.000
September	4.394e+01	1.501e+07	0.000	1.000

Table S2. GLMM with Gaussian family on the correlation between time (month) and abundance of *Parvatrema timondavidi* metacercariae in the Lido station. The symbols "." "*", "**" and "***" indicate p-values <0.1, <0.05, <0.01, <0.001, respectively.

Predictor	**Estimate**	**Std. Error**	**df**	**t value**	**Pr(>|z|)**
Intercept	539.800	72.805	130.573	7.414	1.37e-11***
January	-144.275	100.743	112.858	-1.432	0.154
March	-147.300	100.743	112.858	-1.462	0.146
May	-390.250	100.743	112.858	-3.874	**<0.001*** **
November	-91.200	100.743	112.858	-0.905	0.367
October	-9.807	102.106	113.539	-0.096	0.923
September	-26.100	100.743	112.858	-0.259	0.796

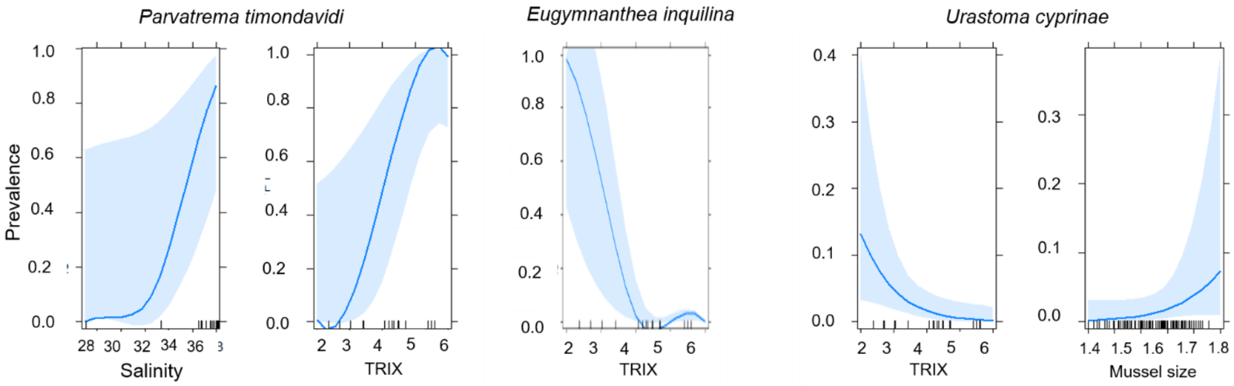

Fig.S1 Generalised linear mixed model (GLMM) on the effects of salinity and TRIX on *Parvatrema timondavidi* (a), TRIX on *Eugymnanthea inquilina* (b), and TRIX and length on *Urastoma cyprinae* (c) prevalence in mussels. The y-axes indicate the effects of salinity, TRIX and mussel length and are shown as solid lines. The 95% confidence intervals are presented as blue shaded areas.

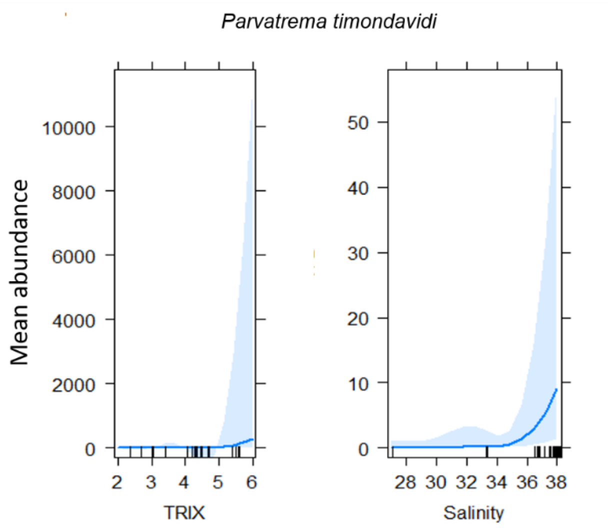

Fig. S2 Generalised linear mixed model (GLMM) on the effects of TRIX and salinity on *Parvatrema timondavidi* abundance. The y-axes indicate the effects of TRIX and salinity and are shown as solid lines. The 95% confidence intervals are presented as blue shaded areas.

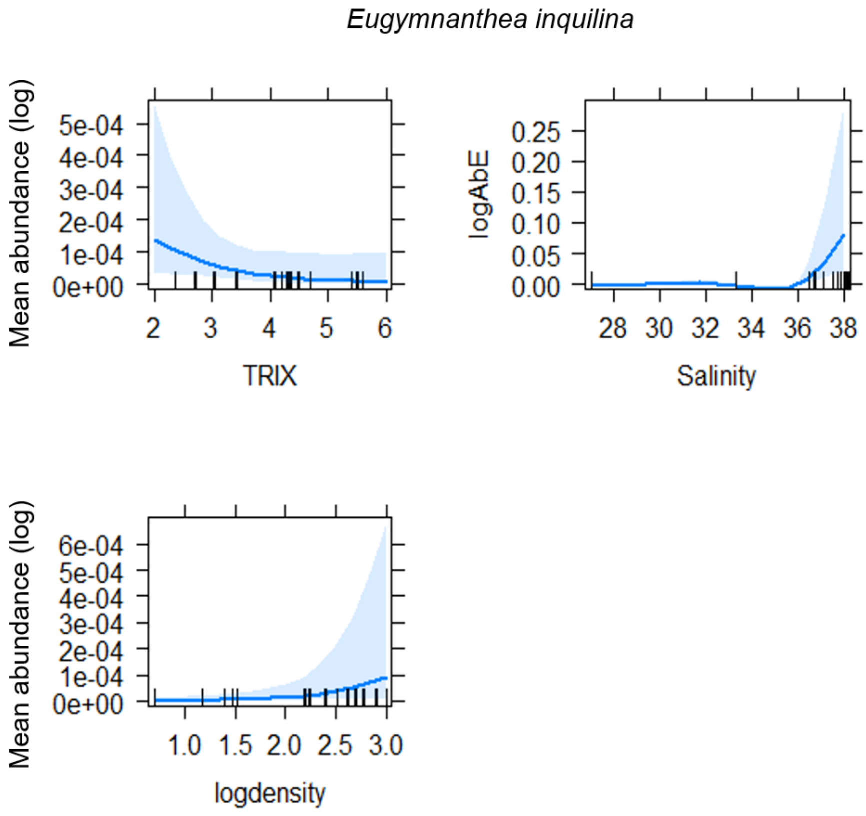

Fig.S3 Generalised linear mixed model (GLMM) on the effects of TRIX, salinity and mussel density on *Eugymnanthea inquilina* abundance. The y-axes indicate the effects of TRIX, salinity and mussel density and are shown as solid lines. The 95% confidence intervals are presented as blue shaded areas

Table S3. Model selection for the generalised linear models (GLMM, binomial family) applied in the analysis with *Parvatrema timondavidi, Myticola sp, Eugymnanthea inquilina and Urastoma cyprinae* prevalence. Predictors present in the model are indicated with "+" and predictors absent with "-". Model performance statistics shows the degrees of freedom (df), the transformation applied to the dependent variable and the corrected Akaike Information Criterion (AICc). The model with the lowest AICc is presented in bold.

Dependent Variable	**Model performance statistics**						
	TRIX	Salinity	Mussel size	Mussels density	df	transformation	AICc
Prevalence *P. timondavidi*	+	+	+	+	6	None	238.8
	+	+	+	-	5	None	236.8
	+	**+**	**-**	**-**	**4**	**None**	**234.8**
	-	-	-	-	2	None	236.1
Prevalence *Myticola* sp.	+	+	+	+	6	None	150.1
	-	+	+	+	5	None	148.1
	-	-	+	+	4	None	146.7
	-	-	-	+	3	None	146.4
	-	-	+	-	3	None	146.5
	-	**-**	**-**	**-**	**2**	**None**	**145.4**
Prevalence *E. inquilina*	+	+	+	+	6	None	122.6
	+	-	+	+	5	None	120.6
	+	-	-	+	4	None	119.2
	+	**-**	**-**	**-**	**3**	**None**	**117.6**
	-	-	-	-	2	None	128.8
Prevalence *U. cyprinae*	+	+	+	+	6	None	79.9
	+	-	+	+	5	None	77.8
	+	**-**	**+**	**-**		**None**	**75.8**
	+	-	-	-		None	77.0
	-	-	-	-		None	83.3

Table S4. Model selection for the generalised linear models (GLMM, negative binomial family) applied in the analysis with *Parvatrema timondavidi* and, *Eugymnanthea inquilina* abundance. Predictors present in the model are indicated with "+" and predictors absent with "-". Model performance statistics shows the degrees of freedom (df), the transformation applied to the dependent variable and the corrected Akaike Information Criterion (AICc). The model with the lowest AICc is presented in bold.

Dependent Variable	**Model performance statistics**						
	TRIX	Salinity	Mussel size	Mussels density	df	transformation	AICc
Abundance *P. timondavidi*	+	+	+	+	8	None	539.2
	+	+	-	+	7	None	537.354
	-	+	-	+	6	None	537.351

	-	-	-	-	3	None	
Abundance *E. inquilina*	**+**	**+**	**+**	**+**	**8**	**Log**	**235.0**
	+	+	+	-	7	Log	237.6
	+	-	+	-	6	Log	241.0
	-	-	-	-	3	Log	258.3

CHAPTER 2

Correlation among parasites infection, upstream host density and salinity in mussels of the South-Western Baltic Sea

ABSTRACT

Parasite prevalence and abundance are affected by abiotic and biotic drivers, and the unique features of the Baltic Sea make this basin a natural laboratory to better understand parasite dynamics under a rapidly changing climate. This field study 1) investigated patterns in parasite prevalence and abundance along the steep salinity gradient of the south-western Baltic Sea and 2) verified correlations between digenean trematode prevalence and abundance in the second intermediate host (the blue mussel *Mytilus edulis*) and salinity, mussel size and local density of infected periwinkles (*Littorina littorea*). A field sampling was run from the Danish Baltic Sea coast (salinity of 22) to the eastern German coast (salinity of 13). Individuals of periwinkles and mussels were collected at each site, and parasite presence, abundance and host condition index were assessed. Salinity and mussel size were the principal factors determining digeneans prevalence and abundance in mussels, while the local density of infected periwinkles affected digeneans abundance only. Two species dominated the parasite community in mussels, *Renicola roscovita* and *Himasthla elongata.* While salinity and mussel size explained 41% of the variance of *R.roscovita* prevalence, only salinity explained 37% of the variance of *H. elongata* prevalence. Salinity, mussel size and density of infected periwinkles explained the 57% of the variance of *R. roscovita* abundance, but only salinity and infected periwinkles density explained the 53% variance *H. elongata* abundance. This study emphasizes the interactive impact of salinity and first host density parasitism in Baltic mussels.

INTRODUCTION

Spatial heterogeneity in parasite distribution is driven by a wide variety of environmental (both abiotic and biotic) conditions (Anderson and Sukhdeo 2010). Among biotic conditions, a leading role is played by the distribution of the various hosts of the parasites' life-cycle (Poulin 1998; Marcogliese 2001; Morley and Lewis 2004). This is especially important for digenean trematodes presenting a particularly complex life cycle (Poulin and Mouritsen 2003; Fredensborg et al. 2005). In vertebrates, which often represent the final host of many parasite species, the adult digenean commonly develops and spawns eggs through sexual reproduction. Eggs are then released into the environment and develop to the miracidium stage, which will infect a first intermediate host, often a mollusc (e.g. snail). In the first intermediate host, mature sporocysts or rediae will asexually reproduce and develop cercariae, the digeneans' free-swimming infectious stage. Cercariae released into the environment will penetrate an invertebrate (e.g. bivalve) or vertebrate species as second intermediate host, and encyst as metacercariae, waiting to be ingested by the final host. Spatial heterogeneity of digeneans can, therefore, be mediated by abundance and infection status of the various host species at the population (i.e. density of host population) or at the individual level (trait-mediated effects).

At the population level, a general trend shown in previous studies is that upstream host (i.e. the preceding host species in a parasite's cycle) density and infection levels largely determine trematode prevalence and load in the next target host (Hechinger and Lafferty 2005; Thieltges 2007; Thieltges and Reise 2007; Galaktionov et al. 2015). Hence, a higher density of infected upstream host should, in turn, results in higher supply of trematodes' infectious stage in the next downstream host (Grosholz 1994; Thieltges and Reise 2007). However, up to date, most of the studies focused on the density of the final host neglecting the influence of the population densities of preceding hosts. At the individual level, host conspecifics may differ in their susceptibility to parasite infection. This intraspecific heterogeneity may result from differences in habitat, diet and defence or other traits (Carney and Dick 1999). Previous studies found size and age correlated with infection levels in various marine host-parasite systems (de Montaudouin et al. 2000; Mouritsen and Poulin 2002; Mouritsen et al. 2003; Thieltges and Reise 2007; Goedknegt et al. 2019). Heterogeneity in upstream host density at the population level and target host traits at the individual level may be enhanced by other abiotic fluctuations or gradients.

The importance of abiotic factors on parasite transmission is generally recognized. Temperature, salinity, pH or light affect free-living stages – and, thus, transmission efficacy - of many trematode species (Pietrock and Marcogliese 2003). In previous experimental studies, sub- or supra-optimal salinity proved to mediate survival and infectivity of cercariae (Koprivnikar 2010; Studer et al. 2012; Bommarito et al. 2020). The majority of field studies, except for one in Ria de Aveiro (Magalhaes et al. 2018), suggested a significant correlation between salinity and parasites community distribution and diversity (Schmidt et al. 2003; Baltic and North Sea: Thieltges et al. 2010; Miramichi and Bouctouche rivers: Blanar et al. 2011). In their study, Schmidt et al. (2003) found reduced salinity positively influence parasite community richness (parasite richness in Elbe estuary < Helgoland) and Thieltges et al. (2010) found a decline in similarity of the parasite community with distance along the salinity gradient of the Baltic Sea (salinity range:8 to around 20). Contrarily, Blanar et al. (2011) detected a negative effect of salinity in parasite richness (salinity range: 11-24). However, the effect of environmental salinity gradients in parasite distribution is still overlooked in many marginal habitats.

The semi-enclosed Baltic basin is characterized by different gradients of relevant environmental drivers, such as salinity and temperature decreasing from the south-west to the north-east (Snoeijs-Leijonmalm et al. 2017). Salinity drops from over 30 in the Skagerrak and Kattegat region to about 2 in the Gulf of Bothnia, representing one of the main drivers for benthic communities (Zettler et al. 2014). This persistent gradient is combined with dynamic climate change drivers such as warming, desalination and eutrophication (Reusch et al. 2018). Given these unique features, the Baltic Sea became a suitable area for field studies on the environmental effects on parasitism. Further particularities are that, 1) parasite diversity in the Baltic Sea is relatively low compared to other oceans (Zander and Reimer 2002), 2) parasite communities are composed of marine, brackish and freshwater species (Zander and Reimer 2002), and 3) the common hosts are established in numerous regions around the world (i.e. *Littorina* sp., *Hydrobia* sp., *Mytilus* sp.). Consequently, studies on the distribution of parasites in the Baltic Sea are relevant not only for this basin itself but also for other estuarine ecosystems or rock pool habitats (Buschek et al. 2012; Hall et al. 2013).

The study-system chosen in this study is a gastropod-bivalve-marine bird parasite system, involving the periwinkle *Littorina littorea* (first intermediate host) and the blue mussel *Mytilus edulis* (as second intermediate host). Periwinkles are known for harbouring many trematode species as well as other parasites/symbionts. Moreover, periwinkles are common grazers of the Baltic Sea benthic community, modulating the ecology of macroalgae populations and consequently the habitats of many other marine species. Common digenean trematode species detected in periwinkles in the Baltic Sea are *Renicola roscovita*, *Himasthla elongata*, *Cryptocotyle lingua* and *Microphallus pygmaeus* (Werding 1969). As periwinkles, mussels are spread all over Europe, playing a crucial role in creating habitat with different niches suitable for other organisms (Krauvfelin and Diaz et al. 2015) and enhancing the benthopelagic coupling (Kautsky and Evans 1987). The mussel *M. edulis* represents a frequent second intermediate host of *Renicola roscovita* and *Himasthla elongata*, as well as other commensals such as turbellaria or copepods.

Aims of the study

Based on this model system, our field study 1) investigated the geographical patterns of parasites prevalence and load in periwinkles and mussels along a regional salinity gradient and 2) assess whether prevalence and abundance of *R. roscovita* and *H. elongata* in the second intermediate host (mussel) are correlated with the density of infected periwinkles, mussel size and salinity. With these aims, a field sampling along the western Baltic coast was run.

METHODOLOGY

Sampling area and sample collection

A large-scale sampling was run during the summer months (June and July) of 2017 along the south-western coastline of the Baltic Sea (Fig. 1) which features the steepest salinity slope of the entire Baltic. Parasitic infection is known to be highest in warmer seasons (Thieltges and Rick 2006).

Six stations were sampled from Hou, Denmark to Niendorf, Germany (Fig. 1). The stations were selected based on geographical distribution and logistical feasibility. The sampling did not reach locations with salinity below 13, since the presence of periwinkles drastically decreases below this salinity (author pers. observ.;

Lauckner 1984). Periwinkle density was registered and categorised in three classes 50 cm^2 plot: low = 0–30 ; medium = 30–50 ; high 50–100 individuals per 50x50 cm plot.

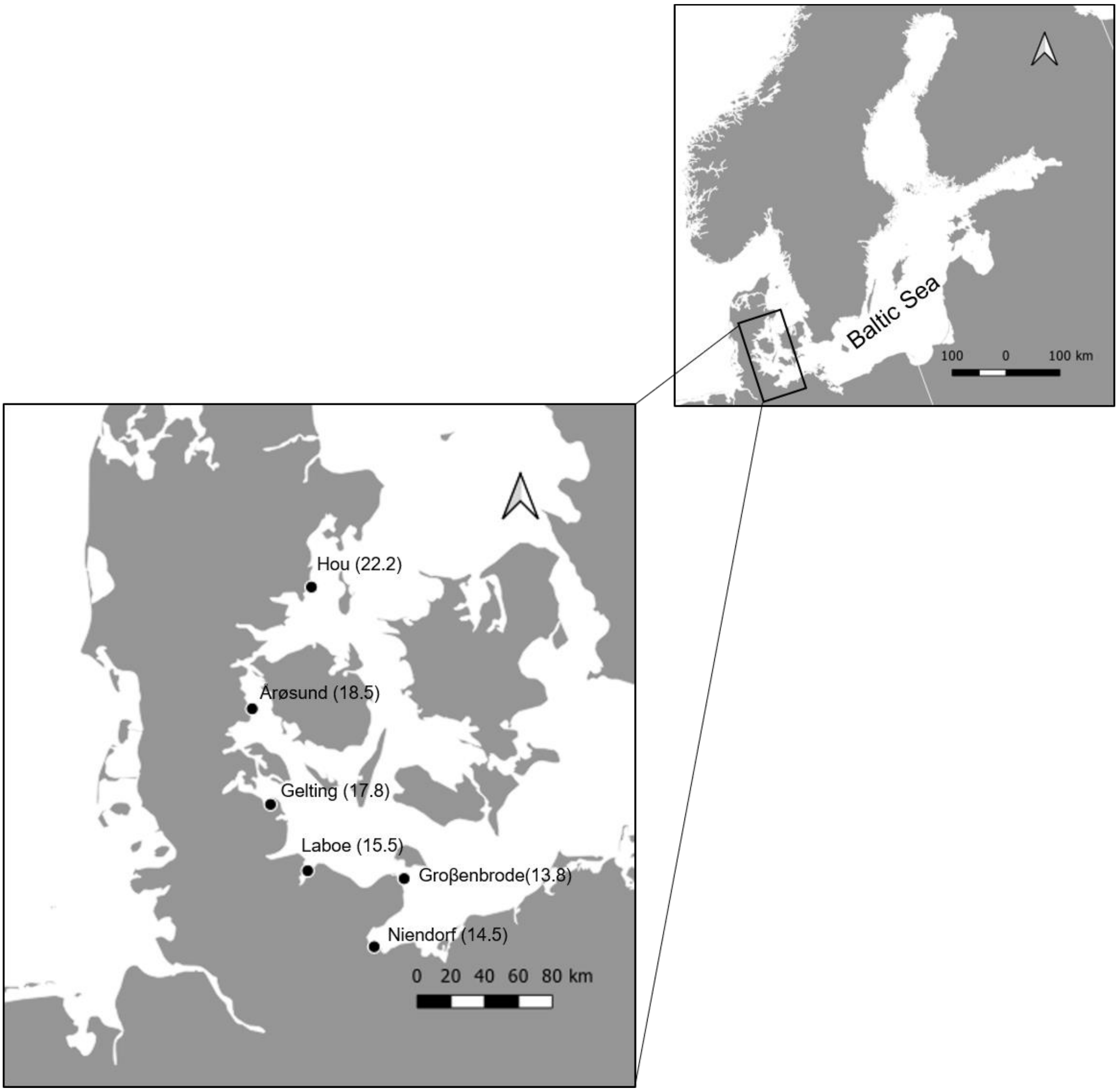

Figure 1. Map of the sampling stations following a steep Baltic Sea salinity gradient (from 13 to 22). Yellow dots represent sampling stations where periwinkles (*Littorina littorea*) and mussels (*Mytilus edulis*) were sampled.

Table 1. Environmental and biological features of the sampled stations. Salinity is reported based on annual means of the year 2017, temperature based on the summer months mean (June, July, August) (Copernicus dataset, see methodology in the main text).

Station	**Salinity (psu)**	**Temperature (°C)**	**Periwinkle density (# per 50x50 cm)**	**# of periwinkles sampled**	**# of mussels sampled**	**Mean mussel length (mm)**
Niendorf	14.5	17.8	0-30	25	30	41
Groβenbrode	13.8	17.7	0-30	30	25	40
Laboe	16.5	17.7	30-50	35	30	43
Gelting	17.8	17.0	50-100	35	25	49
Årøsund	18.5	16.7	50-100	30	30	47
Hou	22.2	16.5	50-100	30	30	47
Total				220	195	

At each site, samples were collected in three replicate 50x50 cm plots, ~100 m apart from each other. A total of 30 to 35 periwinkles and 25–30 blue mussels were collected at each station (Table 1). Niendorf deviated from these samplings as only 25 periwinkles were collected due to their very low densities. Only specimens larger than 17.0 mm for periwinkles and 30 mm for mussels were collected, as these sizes are well-suitable to be infected by common trematodes (Mouritsen et al. 1999; Goedknegt et al. 2017). Samples were transferred to the GEOMAR laboratory, shell length and total weight were measured, and the tissue was smashed between two glass slides (compressors). Parasite determination was applied referring to previous descriptions (Werding 1969; Zander 1998; de Montaudouin et al. 2009). For mussels, parasite prevalence (the percentage of infected host), mean individual richness (mean parasite species per host), intensity (mean number of individuals of a parasite species within infected host) and abundance (mean number of individuals of a parasite species in infected and not infected host) were registered. For periwinkles, parasite prevalence and richness were registered only. Indeed, when periwinkles are infected, almost all tissue area was infested with cercariae and rediae, and accurate estimation is not possible.

Following parasite determination, the tissue of mussels was transferred to pre-weighed aluminium pots (one for each individual) and dried at 80°C for 24 hours. The condition index (CI) was assessed as the ratio of the dry weight of the tissue and shell size. For mussels, the following formula was used: $CI=DW/L^3$, where DW is dry tissue weight (mg) and L is shell length (mm) following Riisgård (2001) and Stier et al. (2015).

Salinity and temperature data for all the stations were retrieved from the Baltic Sea physics reanalysis product provided by Copernicus Marine Environment Monitoring Service (http://marine.copernicus.eu). The reanalysis product is based on the coupled physical-biogeochemical model system NEMO-SCOBI (Nucleus for European Modelling of the Ocean - Swedish Coastal and Ocean Biogeochemical model). The data were extracted for a depth of 1.5 m. Annual average salinity and summer (June, July, August) average temperature were calculated for each station.

Data analysis

Prevalence, as the percentage of hosts infected with one or more individuals of a particular parasite species, was calculated for each parasite species found: *Renicola roscovita*, *Himasthla elongata*, *Cryptocotyle lingua*, *Microphallus pygmaeus*, *Podocotyle atomon*, ciliates and polychetes for periwinkles; and *R. roscovita*, *H. elongata*, *Paravortex cardii*, nematodes and copepods for mussels. Parasite intensity (the average intensity of a particular species of parasite among the infected members of a particular host species), abundance (the average number of parasites among all members of a particular host species), and species richness were calculated for *R. roscovita*, *H. elongata* (the two most common digenean trematodes) and *P. cardii* in mussels. Intensities and abundance of other parasites/symbionts were not considered since numbers were too low. Spatial differences between sampling locations were tested using a chi-square test, correcting the significance level for multiple testing using the Bonferroni approach.

For plots and analyses, the densities of periwinkles were calculated as the central value between the two extreme numbers of each density class (i.e. 0 and 30, 30 and 50, 50 and 100). Then, the density of infected periwinkles was obtained by multiplying the prevalence of the considered digeneans species by the periwinkle population density.

The effects of salinity and density of the periwinkle population on the prevalence of *R. roscovita* and *H. elongata* in *L. littorea* were tested using generalised linear models (GLM) with binomial family.

The effects of salinity, mussel size (defined as shell length, measured with a calliper) and density of infected periwinkles (predictors) on the prevalence of *R. roscovita* and *H. elongata* in mussels (response variables) were tested using GLMs with binomial family. In all models, prevalence was considered as parasite species presence/absence per individual mussel. The effects of the same predictors were tested on the log-transformed abundance of *R. roscovita* and *H. elongata* by using GLMs with gaussian family. The log-transformation of the abundances and the gaussian distribution were chosen over negative binomial distribution after inspection of the residuals plots. Like prevalence, abundance was considered at the individual level, as the number of metacercariae of *R. roscovita* or *H. elongata* in infected and not infected individual hosts. Temperature was not included as the range of average summer temperature of 2017 among stations was too small to detect potential effects (between 15.7 and 17.5°C). GLM models were run separately for *R. roscovita* and *H. elongata*.

First, a GLM including all predictors was run, followed by a series of GLMs excluding one or two predictors. A GLM including only an intercept was run representing the null model. After running the models, collinearity among predictors was tested with the “vif” function (packages “car” and “mctest”). Among all predictors, only prevalence of *R. roscovita* and *H. elongata* in periwinkles was excluded since highly collinear with density of infected periwinkles (vif > 200). For all other predictors, vif values resulted in values < 4. After testing for collinearity, all models were compared through AICc (Akaike Information Criterion corrected for small sample size). The model with the lowest AICc was chosen as driver model. All statistical analyses were performed using the software R 3.5.0 (R Development Core Team 2018).

RESULTS

Spatial patterns of parasite prevalence and abundance

Both, periwinkles and mussels were found along the salinity gradient between 13 and 22, from Niendorf to Hou (Table 1). The two most common digeneans infesting periwinkles and mussels as intermediate host, *R. roscovita* and *H. elongata,* had a mean prevalence of 6.1% and 9.7% in periwinkles, and 41.7% and 52.7% in mussels, respectively (Table 2). Other parasites found in periwinkles were *C. lingua, M. pygmaeus, P. atomon* (Table 2, Fig. 1Sa Supplementary Material). Ciliates were also found, with lower prevalence (Table 2, Fig.1Sa Supplementary Material). Other parasite species found in mussels were turbellaria (*P. cardii*), nematodes, copepods and ciliates (Table 2, Fig.1Sc Supplementary Material). Species richness of parasites in *L. littorea* varied from 1.0 to 1.4 between locations (Supplementary material, Fig 1Sb). In mussels, parasite species richness varied from 1.0 to 2.2 (Fig.1Sd, Supplementary Material). For periwinkles, the highest species richness was found in Årøsund (salinity 18.5; mean species richness: 1.4). For mussels, highest species richness was found in Laboe (salinity 16.5; mean species richness: 2.2).

Table 2. List of parasite and symbiont/commensal species identified in *Littorina littorea* and *Mytilus edulis* as first and second intermediate hosts, their taxon, their mean prevalence among sampling stations (pooled over stations) ± Standard Error (SE), as well as all other hosts known to be part of the parasites' life cycle.

Host species	**Parasite species**	**Taxon**	**Mean prevalence ± SE**	**1st intermediate host**	**Mean intensity**	**Mean abundance**	**2nd intermediate host**	**Final host**
Littorina littorea	*Renicola roscovita*	Platyhelminthes	6.1±4.2	*L. littorea*	/	/	molluscs	Sea birds
	Himasthla elongata	Platyhelminthes	9.7±6.0	*L. littorea*	/	/	molluscs	Sea birds
	Crytpocotyle lingua	Platyhelminthes	6.2±2.6	*L. littorea*	/	/	fishes	Sea birds
	Mycrophallus pygmaeus	Platyhelminthes	1.8±1.2	*L. littorea*	/	/	*L. littorea*	Sea birds
	Podocotyle atomon	Platyhelminthes	1.6±0.7	*L. littorea*	/	/	amphipods	Fishes
	Tricodina sp.	Ciliophora	0.6±0.6	/	/	/	/	/
Mytilus edulis	*Renicola roscovita*	Platyhelminthes	41.7±17.0	*L. littorea*	123	90.0	molluscs	Sea birds
	Himasthla elongata	Platyhelminthes	52.7±15.2	*L. littorea*	38	22.5	molluscs	Sea birds
	Paravortex cardii	Platyhelminthes	19±9.5		3	0.6		
	unknown trematode		1.3±1.3	/	154	1.5	molluscs	/
	Modiolicola sp.	Arthropoda	0.5±0.5	/	/	/	/	/
	Trichodina sp.	Ciliophora	4.2±2.2	/	/	/	/	/
	Nematodes	Nematoda	1.1±1.1	/	/	/	/	/

Spatially, in both *L. littorea* and *M. edulis*, *R. roscovita* and *H. elongata* prevalences were highest in Laboe, Årøsund and Hou (salinity ranging from 16.5 to 22.2; Fig.2a and b). Specifically, *R. roscovita* prevalence was significantly higher in Årøsund than in other stations ($X^{2,}$ p=0.001). *H. elongata* prevalence was slightly higher in Hou (X^2, p=0.07), but was significantly higher in Årøsund (X^2, p=0.001) than in other stations. In *M. edulis*, a high prevalence of *P. cardii* was found in Niendorf (X^2, p=0.05) and Laboe (X^2, p=0.001; Fig. 1Sc, Supplementary Material), here often co-occurring with other parasite species (especially *R. roscovita*). The highest intensity of *R. roscovita* and *H. elongata* in *M. edulis* was detected in Årøsund and in Hou (Fig. 2c).

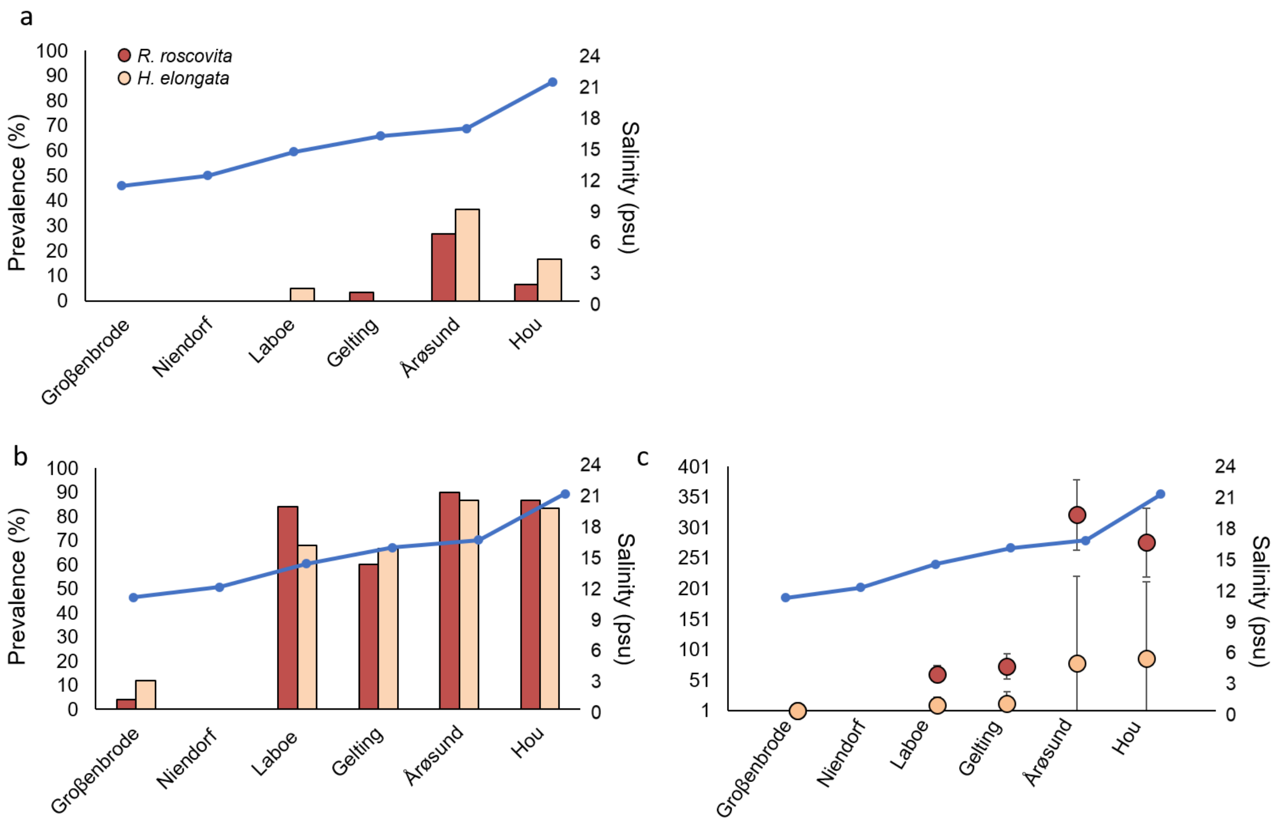

Figure 2. *Renicola roscovita* and *Himasthla elongata* prevalence (a) in *Littorina littorea*, and *Renicola roscovita and Himasthla elongata* prevalence (b) and intensity (c) in *Mytilus edulis* and mean annual salinity (line: psu). Error bars represent standard deviations (SD).

Abiotic and biotic correlations with parasite prevalence and abundance

Effects of salinity, mussel size and density of infected periwinkles on parasite prevalence in mussels

Prevalence (modelled as parasite presence/absence per individual mussel) of *R. roscovita* in mussels increased significantly with salinity (GLM, p<0.001; Table 4) and mussel size (GLM, p=0.005; Table 4) (Fig. 2S, Supplementary Material). Density of infected periwinkles and *R. roscovita* prevalence in mussels were unrelated (p=0.35). The model including only salinity and mussel length was identified as the best explanatory model for *R. roscovita* prevalence in mussels, with ΔAICc of 1.2 with respect to the model also including density of infected periwinkles (Table S1, Supplementary Material). The two predictors included in the model explained 41% of the variance in *R. roscovita* prevalence. *H. elongata* prevalence in mussels increased with salinity (GLM, p<0.001; Table 4; Fig. 2S, Supplementary Material). AICc selection identified the model including just salinity as the best explanatory model. The ΔAICc with the model including salinity and density or mussel length was of 1.1 and 1.4, respectively (Table S1, Supplementary Material). In the model, salinity explained 34% of the variance in *H. elongata* prevalence.

Table 3. GLM following binomial distribution on the effects of density of infected periwinkles and mussel size on the prevalence of *Renicola roscovita* and *Himasthla. elongata* in blue mussels (*Mytilus edulis*). The symbols "*", "**" and "***" indicate p-values <0.05, <0.01, <0.001, respectively.

Host species	**Trematode species**	**Predictors**	**Estimate**	**Std. Error**	**Z value**	**Pr(>|z|)**
Mytilus edulis	*R. roscovita*	Intercept	-15.227	2.277	-6.686	<0.001***
		Salinity	0.696	0.117	5.949	<0.001***
		Size	0.084	0.030	2.756	<0.005**
	H. elongata	Intercept	-12.055	1.876	-6.423	<0.001***
		Salinity	0.723	0.111	6.482	<0.001***

Effects of salinity, mussels size and infected periwinkles density on parasite abundance in mussels

R. roscovita abundance increased with salinity, mussel size and density of infected periwinkles (p<0.001; Table 5, Fig.3S, Supplementary Material). AICc selection identified the best model being the one that included all predictors (ΔAICc=12.0; Table S1, Supplementary Material). The three predictors in the model explained 57% of the variance in *R. roscovita* abundance.

H. elongata abundance increased with salinity and density of infected periwinkles (p<0.001; Table 4; Fig. 4S, Supplementary Material). AICc selection identified the best model being the one that included both predictors, i.e. salinity and density (ΔAICc=1.6 with respect to the model also including mussel size; Table S1, Supplementary Material). Salinity and density together explained 53% of the variance in *H. elongata* abundance.

Table 4. GLM following Gaussian distribution on the effects of salinity, mussel size (length) and density of infected periwinkles on the abundance of *Renicola roscovita* and *Himasthla elongata* in mussels (*Mytilus edulis*). The symbols "*", "**" and "***" indicate p-values <0.05, <0.01, <0.001, respectively.

Parasite species	**Predictors**	**Estimate**	**Standard Error**	**Z value**	**Pr (>|z|)**
R. roscovita	Intercept	-3.530	0.418	-8.445	<0.001***
	Salinity	0.187	0.022	8.283	<0.001***
	Size	0.026	0.006	3.788	<0.001***
	Density of infected periwinkles	0.042	0.008	5.074	<0.001***
H. elongata	Intercept	-2.769	0.381	-7.265	<0.001***
	Salinity	0.210	0.022	9.258	<0.001***
	Density of infected periwinkles	0.044	0.008	5.203	<0.001***

DISCUSSION

General patterns of parasite prevalence and intensity

The parasite community in periwinkles (*Littorina littorea*) and mussels (*Mytilus edulis*) appeared dominated by digenean trematodes, among which the species *R. roscovita* and *H. elongata* showed the highest prevalence in both host species. In the periwinkle, *C. lingua* was the third species with high prevalence. These patterns of prevalence were also observed by Lauckner (1984), who, however, reported *C. lingua* as the dominant species in periwinkles over *R. roscovita* and *H. elongata*. We found *C. lingua* and *M. pygmaeus* generally more prevalent at salinities below 17.8, while *R. roscovita* and *H. elongata* were dominant at salinities above 17.8. Our findings are coherent with other previous studies (Reimer 1970; Möller 1978; Lauckner 1984), revealing high success of *C. lingua* and *M. pygmaeus* in brackish waters. In the case of *M. pygmaeus*, this success could derive from a shorter life cycle, involving a single intermediate host where cercariae directly develop into metacercariae, and are likely less subjected to environmental stress, as instead the free-living cercariae (Marcogliese 2001). In the case of *C. lingua*, better tolerance to a broader range of salinities may derive by the mobile intermediate hosts (i.e. fish).

Role of biotic factors

Previous studies observed trematodes prevalence and abundance to be positively correlated with the prevalence and abundance of the final host (Hechinger and Lafferty 2005; Fredensborg et al. 2006; Song and Proctor 2020). A higher abundance of competent final host is more likely to introduce a higher prevalence of infective stages into the environment, where then the intermediate host will get infected (Song and Proctor 2020). Our results showed that first intermediate host density can be equally important, as observed by other few studies (Thieltges and Reise 2007; de Montaudouin and Lanceleur 2011; Correia et al. 2020).

Density of infected periwinkles related positively to the abundance of *R. roscovita* and *H. elongata* in the mussel host. Our results are generally coherent with the findings of Thieltges and Reise (2007), where they investigated the effects of density of the upstream host and host size on prevalence and abundance of common trematode species in cockles of the North Sea (Wadden Sea). Increased abundance with infected host density could be considered as a direct correlation since higher densities of infected upstream host individuals should provide higher numbers of free-living cercariae, which then infect the second intermediate host (Thieltges and Reise 2007).

Both, prevalence and abundance of *R. roscovita* in mussels increased with host size (mussel length), while this was not true for *H. elongata*. A positive correlation between parasite prevalence, abundance and host size was already shown in previous studies (Thieltges et al. 2010; Goedknegt 2019) and could be attributed to higher filtration rates occurring in larger mussels (Nikolaev et al. 2006), which enhance the chance of more cercariae to enter the mussel through the inhalant current (Wegeberg et al. 1999; Thieltges and Reise 2007). In terms of time of exposure, the age of mussels could be another explanation. Hence, the high number of metacercariae detected in larger and older mussels could be a result of parasite accumulation through time (Nikolaev et al. 2006). About the reason why this did not occur in *H. elongata* we can only speculate. One possibility is that contrarily to *H. elongata*, *R. roscovita* metacercariae are known to grow inside the second intermediate host (Lauckner 1980, 1983). This characteristic may affect *R. roscovita* cercariae preference for larger-size host individuals. Alternatively, *R. roscovita* cercariae are mainly transmitted during filtration of the host through the inhalant current. A higher filtration rate could, therefore, be more relevant for *R. roscovita* than *H. elongata* cercariae, which also transmit through active penetration of the hosts' foot. Experimental studies on different mechanisms of transmission of the two species will be needed to fully understand this difference in abundance found in the field.

Role of salinity

R. roscovita and *H. elongata* prevalence and abundance in mussel hosts, significantly increased with increasing salinity in the range investigated here. Besides a general positive correlation among parasite prevalence and salinity, we found a slight difference between the two main parasite species, *R. roscovita* and *H. elongata*. Indeed, the prevalence of *H. elongata* more strongly correlated with salinity than that of *R. roscovita*. As reported by Lauckner (1984), *H. elongata* infects periwinkles through swimming and salinity-sensible miracidia. In *R. roscovita*, eggs are directly ingested by *L. littorea*, and consequently possibly less impacted by salinity changes.

There is no field study available that correlates the steep salinity gradient within the western Baltic Sea to parasite prevalence and abundances. In their sampling along the Ria de Aveiro coast, Magãlhaes et al. (2018) did not observe salinity effects on parasite abundance within a salinity gradient from 4 to 30. However, the high spatial homogeneity detected was probably caused by the low abundance of parasites in general (Magãlhaes et al. 2018). This is not the case for the western Baltic Sea coast, where high abundances of trematodes were previously reported (Werding 1969; Lauckner 1984; Zander 1998). Also, Goedknegt et al. (2019) during their sampling in the Wadden Sea, did not detect any significant correlation between prevalence and abundance of *H. elongata* and *R. roscovita* and salinity. However, their study was run in a different system, where the salinity gradient is not that steep as it is in the western Baltic. Other field studies focusing on parasites diversity, but not on prevalence and abundance, assessed salinity as one of the most critical factors influencing parasite distribution, superimposing even on local effects such as eutrophication (Schmidt et al. 2003; Blanar et al. 2011; Poulin et al. 2011).

As previously observed by Zander (1998), trematode distribution could be an indirect consequence of the intermediate host (periwinkles and mussels) distribution, which themselves have limits of adaptive osmoregulation to changes in salinity. A critical threshold for periwinkle activity is represented by salinities lower than 13 (Taylor and Andrews 1988). Mussels of the genus *Mytilus* spp. instead are still found in the Northern Baltic Sea at salinities below 6.5, where they reach their margin of tolerance (Riisgård et al. 2013). However, *Mytilus* populations of the Baltic Sea show a clear pattern of interspecific gene flow among *M. edulis* and *Mytilus trossulus*. In the central Baltic Sea the genotype is *M. trossulus*-like, instead in the western Baltic Sea, at salinity around 15 (Kiel Fjord, including Laboe) the genotype is *M. edulis*-like (Stuckas et al. 2017). Salinity effects can also strongly impact the free-living stages of trematodes. Generally, if we assume the marine nature of the parasite species analysed, their distribution and reproductive capacity are directly affected to the hydrology dynamics (i.e. shifts in salinity) of the habitat, as mentioned by Schmidt et al. (2003). This tight association among marine parasites and habitat hydrology is confirmed by experimental studies, which detected cercarial emergence, survival and transmission negatively affected by lower salinities (Lei and Poulin 2011; Studer et al. 2012; Bommarito et al. 2020). Negative effects of reduced salinity in cercarial emergence might be reconducted to a stress response by the first intermediate host (Lei and Poulin 2011; Bommarito et al. 2020), while negative effects on survival and infectivity could be attributed to the vulnerability of the cercaria itself (Pietrock and Marcogliese 2003). In their experimental approach, Bommarito et al. (2020) simulated a salinity range of 13 to 19. The study found *H. elongata* emergence, activity and infectivity to be significantly reduced at low salinity (i.e. 13), probably due to osmotic stress of cercariae. In the same study, not only infectivity but also mussel susceptibility to infection significantly diminished with decreasing salinity, highlighting the mediator role of the host during the infection.

Comparison experimental and field study

The cumulative experimental results detected in previous studies (Studer et al. 2012; Bommarito et al. 2020) are matching with our field data, generally suggesting that at reduced salinity trematode transmission to the downstream host drastically decrease, due to the combined osmotic stress of the cercariae and their hosts. However, in the field the effect of salinity on transmission might be further enhanced or buffered by biotic components. Our field results detected a positive effect of upstream host density in *H. elongata* abundance. Upstream host density clearly increase at the highest salinities (Table 1), further promoting the chance of trematode transmission. In the future, salinity in the Baltic Sea is expected to decrease (by 2-3 psu, see Meier et al. 2012). With salinity around 13 representing the margin of tolerance of *L. littorea* (Hylleberg and Christensen 1978; Taylor and Andrews 1988), locations which currently present an average salinity of 16 will might be subjected to a decrease in periwinkle population density. This might lead to a decrease in abundance of *H. elongata*.

The other biotic factor which positively affected trematode prevalence and abundance (in *R. roscovita*), was mussel size. The range of size used by Bommarito et al. (2020) was similar to the one observed in this field study (mussels of 40-50 mm). Average mussel size in our field study appeared to increase with salinity, from 40 (salinity 13.8) to 47-49 (salinities 17.8-22). This lead to argue that at higher salinities cercarial infectivity and host susceptibility might further raise due to cercariae preference for larger mussels and to an increase of mussel filtration rate. With Baltic freshening, a reduction in mussel size might also occur. Hence, this phenomenon has already been observed in the dwarfed mussels (hybrids between *Mytilus trossulus* and *M. edulis*) of the proper Baltic Sea (Riisgård et al. 2013). At salinities below 16 the negative effect of freshening will might be also stressed by smaller mussel size. However, to confirm this hypothesis, the additive effects of salinity and downstream host size should be experimentally tested to different marine trematode species.

CONCLUSIONS

In conclusion, our study emphasised the relevance of salinity, upstream host density and size as drivers of trematodes infection levels in mussels with respect to trematode species very common in the Baltic Sea. In our study clearly emerged the importance of biotic drivers, both at the individual (host size) and at the population (upstream host density) level. Under a global change perspective, the distribution of *L. littorea* might be shifted, by increasing osmotic stress in a future Baltic Sea of less saline waters, while *M. edulis* size might also be affected. Furthermore, the correlation of trematode prevalence and abundance with salinity emerged in our field observations and aligns well with the experimental data achieved on the same host-parasite system. Here, salinity affected many essential steps of the cercaria stage, from the first to the second intermediate host. As our experimental data stressed also, a potential freshening of the Baltic Sea coasts under global change, could lead to reduced trematode transmission success. This could happen especially with *H. elongata*, which distribution appeared more associated with salinity than the distribution of *R. roscovita.* Further field studies should include the presence and abundance of the final host, as well as experimental studies that involve density of the first intermediate host and different size classes of the target host as a proxy of metacercarial abundance.

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SUPPLEMENTARY MATERIAL

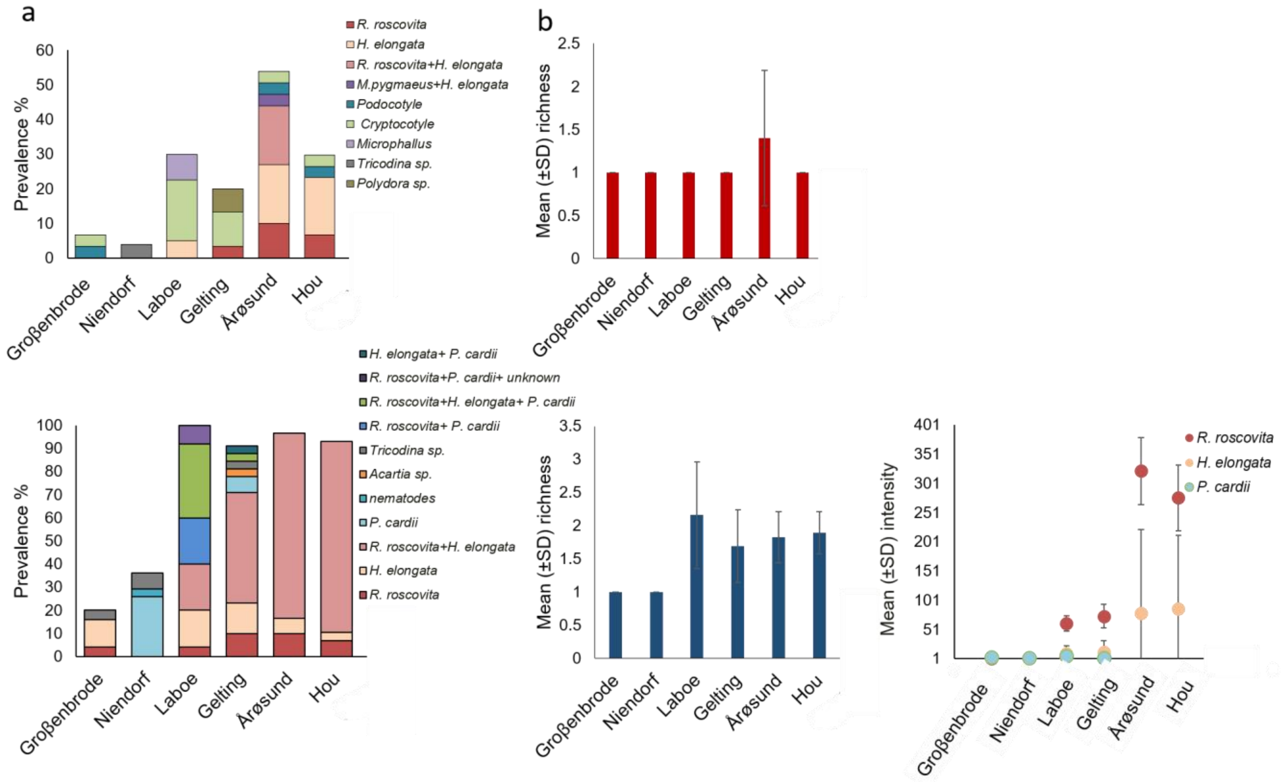

Figure 1S. Parasite prevalence (a) and mean species richness of parasites (b) in *Littorina littorea*, and prevalence (c), richness (d) and intensity (e) in *Mytilus edulis*; the latter ones only for *Renicola roscovita*, *Himasthla elongata* and *Paravortex cardii*. All sampling locations are presented based on annual mean salinities observed at the sites (x-axes). Prevalence of co-infections are shown separately. Error bars represent standard deviations (SD).

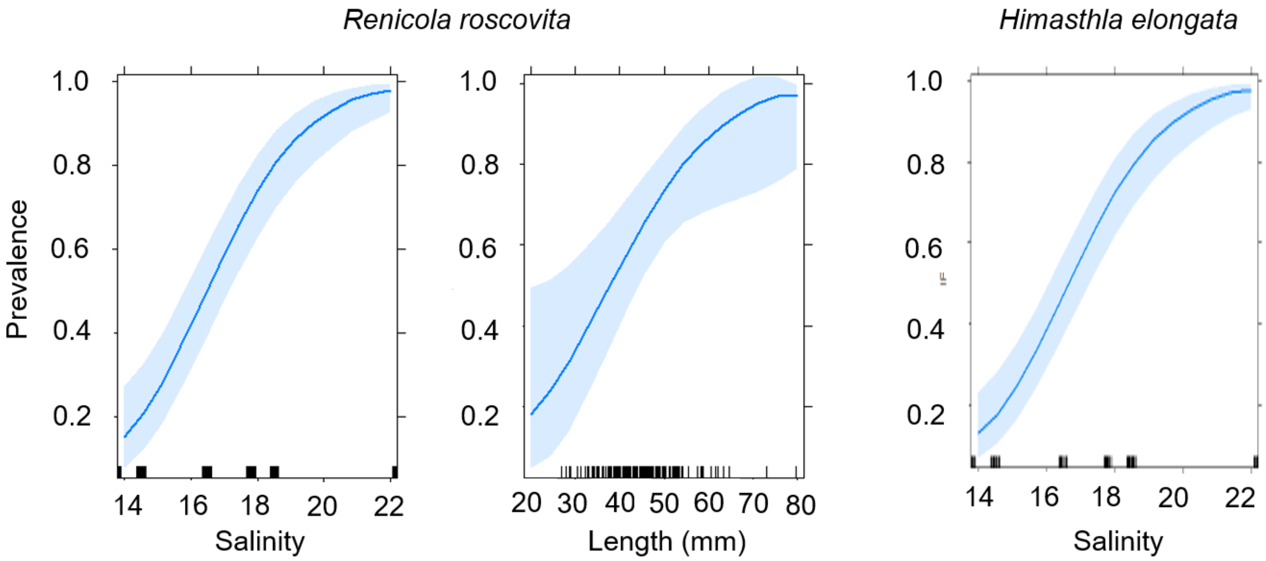

Figure 2S Generalised linear models showing the effects of salinity and mussel size (length) on *Renicola roscovita* prevalence in mussels (a and b) and on the effect of salinity of *Himasthla elongata* prevalence in mussels (c). The y-axes indicate the effects of salinity and length (for *Renicola roscovita*) and salinity only (for *Himasthla elongata*) and are shown as solid lines. The 95% confidence intervals are presented as blue shaded areas.

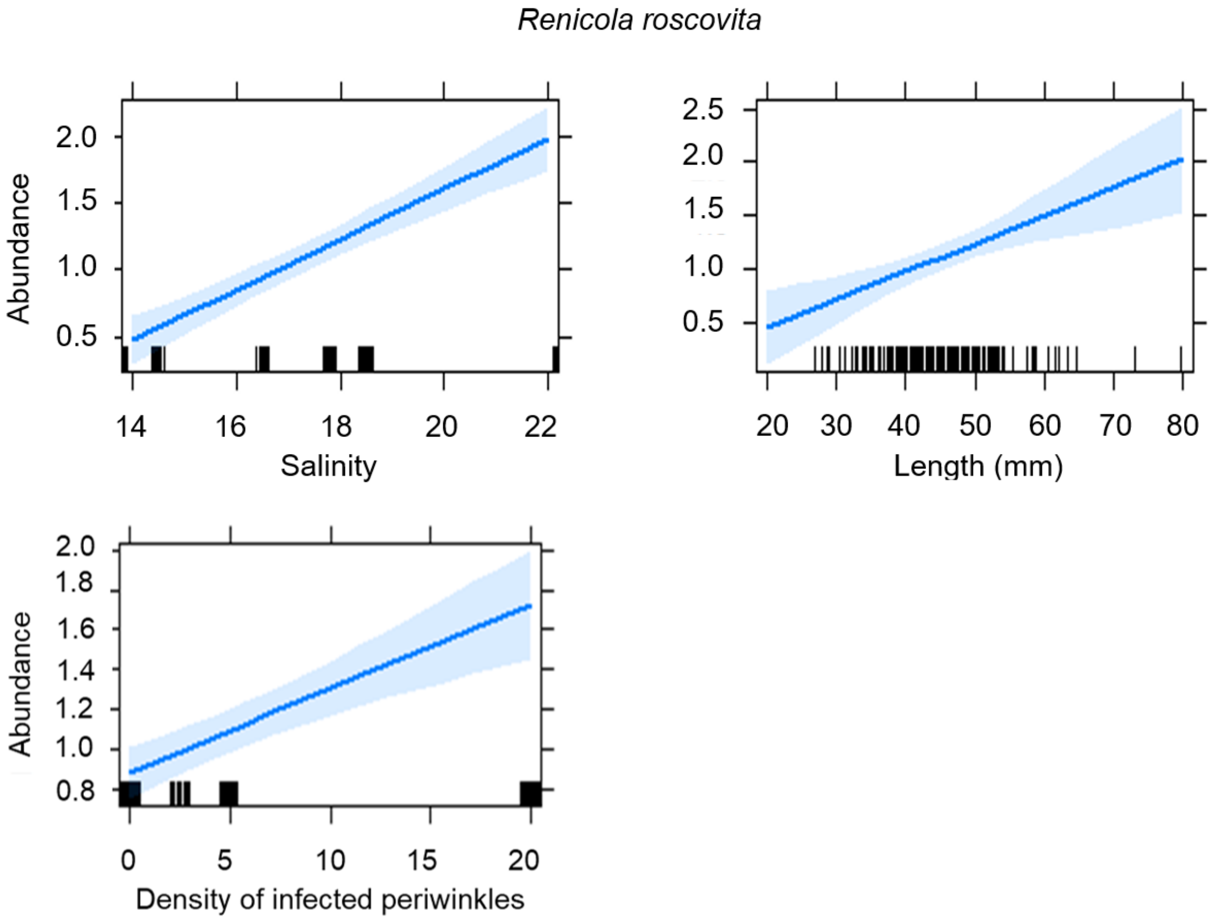

Figure 3S Generalised linear model (GLM) on the effects of salinity (a), mussel size (length) (b), and density of infected periwinkles (c) on *Renicola roscovita* abundance in mussels. The y-axes indicate the effects of salinity, length and density of infected periwinkles, and are shown as solid lines. The 95% confidence intervals are presented as blue shaded areas.

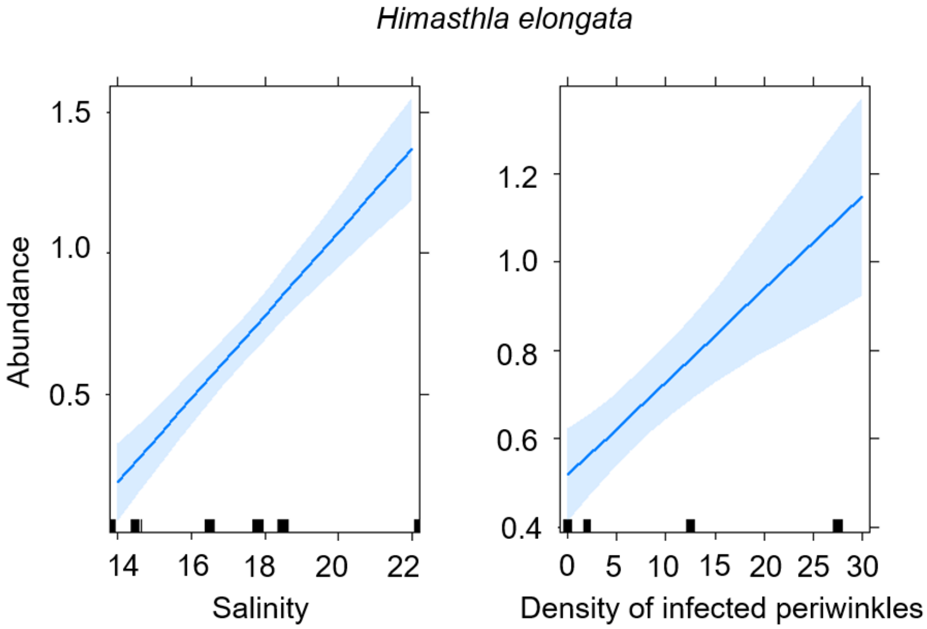

Figure 4S Generalised linear model (GLM) on the effects of salinity (a) and density of infected periwinkles (b) on *Himasthla elongata* abundance in mussels. The y-axes indicate the effects of salinity and density of infected periwinkles, and are shown as solid lines. The 95% confidence intervals are presented as blue shaded areas.

Table 1S. Model selection for the generalised linear models (GLM) applied in the analysis with *Renicola roscovita* and *Himasthla elongata* prevalence and abundance. Predictors present in the model are indicated with "+" and predictors absent with "-". Model performance statistics shows the degrees of freedom (df), the transformation applied to the dependent variable and the corrected Akaike Information Criterion (AICc). The model with the lowest AICc is presented in bold.

Dependent Variable	Predictors			Model performance statistics		
	Salinity	Mussel size	Density of infected periwinkles	df	transformation	AICc
Prevalence *R. roscovita*	+	+	+	4	None	143.1
	+	**+**	**-**	**3**	**None**	**141.9**
	+	-	+	3	None	149.6
	-	+	+	3	None	177.1
	+	-	-	2	None	148.4
	-	+	-	2	None	201.4
	-	-	+	2	None	195.3
Prevalence *H. elongata*	+	+	+	4	None	157.7
	+	+	-	3	None	156.7
	+	-	+	3	None	156.4
	-	+	+	3	None	187.9
	+	-	-	2	None	**155.3**
	-	+	-	2	None	219.8
	-	-	+	2	None	193.5
Abundance *R. roscovita*	+	+	+	5	Log	**364.0**
	+	+	-	4	Log	386.4
	+	-	+	4	Log	376.0
	-	+	+	4	Log	420.6
	+	-	-	3	Log	2226.3
	-	+	-	3	Log	2252.6
	-	-	+	3	Log	2222.0
Abundance *H. elongata*	+	+	+	5	Log	264.7
	+	+	-	4	Log	281.9
	+	-	+	4	Log	**263.1**
	-	+	+	4	Log	316.7
	+	-	-	3	Log	1931.1
	-	+	-	3	Log	1950.2
	-	-	+	3	Log	1930.7

CHAPTER 3

Freshening rather than warming drives trematode transmission from periwinkles to mussels

ABSTRACT

In the Western Baltic Sea, climate change is happening at much faster rate than in most other seas and organisms are additionally exposed to a steep and variable salinity gradient. Climate change has previously been shown to affect parasite transmission in other marine ecosystems, yet little is known about potential effects of warming and desalination on parasite-host interactions. In laboratory experiments, we determined the combined effects of projected seawater warming and freshening on the emergence, activity, survival and infectivity of cercariae (free-swimming infectious stage) of the trematode *Himasthla elongata* (Mehlis 1831), shed from its first intermediate host, the periwinkle *Littorina littorea* (Linnaeus 1758), in the Baltic Sea. We also assessed the susceptibility of the second intermediate host, the mussel *Mytilus edulis* Linnaeus, 1758, to cercarial infections. Generally, salinity was the main driver, particularly of cercarial activity, infectivity and mussel susceptibility to infection. At the lowest salinity (13) cercariae were 50% less active compared to the highest salinity (19). Infection success and host susceptibility followed a similar pattern, with 47% and 43% less metacercariae (encysted stage) present at salinity 13 than at salinity 19, respectively. In contrast, effects of simulated warming were found only for cercarial survival, with cercarial longevity being higher at 19 than at 23°C. No significant interactions between temperature and salinity were found. In contrast to the literature, the results suggest that a climate change driven freshening (partly also warming) may lead to a general decline of marine trematodes, with possible beneficial effects for the involved hosts.

INTRODUCTION

Climate change is expected to affect a wide range of interactions among marine organisms, including interactions between parasites and their hosts (Poulin and Mouritsen 2006; Marcogliese 2008). In general, parasites are widespread in aquatic environments, and often interact with various ecosystem stressors, both at the population and the community level of the hosts they infect (Sures 2008; Nachev and Sures 2015; Vidal-Martínez and Wunderlich 2017). At the same time, parasites have been shown to have profound impacts on aquatic ecosystems by numerous direct and indirect effects on host populations and communities (Mouritsen and Poulin 2009; Sures et al. 2017). For instance, as reported by Wood et al. (2007), grazing activity of infected *Littorina* sp. on macroalgae decreased by 40% compared to non-infected individuals, indirectly causing changes in community structure of ephemeral algae. Given the important ecological roles of parasitism, an understanding of the links between parasite infection levels in hosts and changing environmental conditions is crucial to identify the effects of climate change on parasites and their hosts in marine ecosystems.

Many parasites have complex life cycles, often involving several sequential hosts. The free-living transmission stages in between hosts are known to be particularly sensitive to abiotic factors (Pietrock and Marcogliese 2003; Koprivnikar et al. 2010). Of those factors, temperature has been studied most intensively (Marcogliese 2016), in particular in cercarial life cycle stages of trematodes. Cercarial stages emerge from a first intermediate gastropod host and penetrate the second intermediate host tissue, in which they transform into the following life cycle stage (metacercariae), awaiting ingestion by the final host (marine bird). Diverse experimental studies have demonstrated positive correlations between increasing seawater temperature and cercarial emergence and infectivity (Thieltges and Rick 2006; Koprivnikar and Poulin 2009; Studer and Poulin 2013). Both emergence and infectivity occasionally decrease after a certain threshold depending on the trematode species studied (McCarthy 1999; Thieltges and Rick 2006; Koprivnikar and Poulin 2009). Temperature can also affect transmission to the next host, by direct effects on the infectivity of free-living cercariae (Pechenik and Fried 1995; Studer et al. 2010) or changes in the susceptibility of the second intermediate host to parasite infection (Laverty et al. 2017).

Considering that global change is a complex of different interacting changes, abiotic factors such as salinity or pH changes, other than temperature may further modify the emergence, survival and infectivity of free-living stages of trematodes, as well as the resistance of their hosts (Zander 1998; Mouritsen 2002; Pietrock and Marcogliese 2003; Leiva et al. 2019). Moreover, previous comparative analyses (Morley 2011; Morley and Lewis 2013; Marcogliese 2016) confirmed a large range of thermal tolerance beyond optimum temperatures for cercarial emergence and survival. These findings suggest that temperature may not represent the most relevant variable for parasitism as thought previously. In coastal marine environments, salinity can play a prominent role in parasite transmission and previous studies found decreases in cercarial emergence and infectivity at reduced salinities (Lei and Poulin 2011). Salinity may interact with temperature as indicated by multifactorial experiments. For example, Koprivnikar et al. (2010) found mean survival of cercarial stages of *Acanthoparyphium spinulosum* compromised at 35 and 40 at 25°C but not at 20°C. However, many of these multi-factorial experiments focused on single phases of cercarial life cycles only, not including other possibly relevant transmission and developmental steps.

In the Baltic Sea, salinity gradients represent one of the major drivers of benthic community composition (Ojaveer et al. 2010; Jasper et al. 2011; Vuorinen et al. 2015). The inflow of high-saline bottom water from the North Sea and the outflow of shallow low-saline water, mostly from river-runoff and precipitation (Müller et al. 2016), create a strong Baltic-wide salinity gradient from about 30 (Skagerrak and Kattegat region in the west, southwest) down to 3 in the northeast area of the Gulf of Bothnia. The steepest salinity gradient is found in the South-Western Baltic Sea (i.e. eastern coast of Denmark and northeastern German coast), where salinity drops from 20 to 8 over a four hundred kilometres (Bonsdorff 2006). Due to this strong gradient of osmotic stress its land-locked nature, intense anthropogenic pressures and its strong environmental fluctuations, the Baltic Sea represents a hot-spot to study climate change effects. Regional impacts are accompanied by global changes such as warming and ocean acidification. The Baltic Sea was indeed identified as the fastest-warming sea worldwide, and one of the fastest under increased nutrient load and oxygen depletion in bottom waters (with the Black Sea and the East China Sea; Reusch et al. 2018).

In this study, we investigated the single and combined effects of seawater warming and freshening on different life stages of the trematode *Himasthla elongata*, one of the dominant trematode species in the Western Baltic Sea (Lauckner 1984). This parasite uses marine snails (*Littorina littorea*) as first intermediate host and blue mussels (*Mytilus edulis*) and other bivalve species, as second intermediate host. Coastal birds feeding on mussels represent the final host (Lauckner 1984). *Himasthla* sp. cercariae normally survive up to a maximum of two days, with 100% survival rate within 24 hours (de Montaudouin et al. 2016). Cercariae of *H. elongata* at 20°C were found to maintain infectivity for around 20 hours (Greve 1997). We assessed the different transmission steps from the first to the second intermediate host (Fig. 1): i) cercarial emergence from periwinkles, ii) cercarial activity and survival after emergence, iii) cercarial infectivity in mussels, and iv) susceptibility of mussels to cercarial infection. For iii) cercariae were treated but not the mussels, whereas for iv) mussels were treated but not the cercariae. With this study, we contribute to a better understanding of the effects of expected warming and freshening on parasite transmission success in a Baltic Sea host-parasite system.

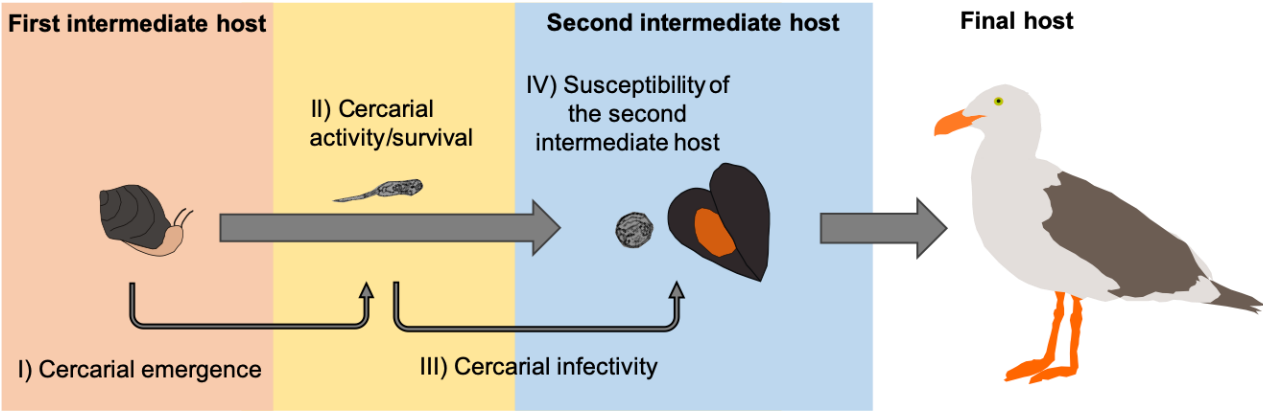

Fig. 1 Life cycle of the trematode *Himasthla elongata* and transmission steps investigated in this study: **Exp. I**: cercarial emergence from the first intermediate host, the periwinkle *Littorina littorea*; **Exp. II**: cercarial activity/survival; **Exp. III**: cercarial infectivity; **Exp. IV**: susceptibility of the second intermediate host, the blue mussel *Mytilus edulis*, to cercariae infection. Different colours represent the different phases of the life cycle on which we focused: the first host/cercarial phase (red), the cercarial free-living phase (yellow) and the phase within the metacercarial stage in the second intermediate host (blue).

METHODOLOGY

Parasite and host collection

Approximately 500 periwinkles (*L. littorea*) were haphazardly collected by hand at Årøsund, Denmark (55.25°N, 9.70°E) in August 2017, and transported to the GEOMAR laboratory in Kiel, Germany. Here, periwinkles were kept together in plastic containers of 25 L filled with seawater at 19 and 16°C (i.e. the conditions at the site and time of sampling), aerated and fed *ad libitum* with *Ulva lactuca* and *Fucus vesiculosus*. On the day after collection (measurement 0), each periwinkle was individually placed in a petri dish containing 10 mL of seawater (19) and incubated under constant illumination underneath heating lamps at 25 to 27°C for 4 hours to promote cercarial emergence (e.g. Mouritsen 2002). Subsequently, petri dishes were checked under a stereomicroscope for the presence of cercariae. Periwinkles shown to be infected with *H. elongata* by this method were then kept in 10 L plastic containers in aerated seawater at 19and 16°C (fed *ad libitum* with *U. lactuca* and *F. vesiculosus*).

Mussels (*M. edulis*) of 40–50 mm of shell length (i.e. 1–1.5 years of age) were collected from the 'Kieler Meeresfarm', a marine aquaculture facility in the inner Kiel Fjord (54.36°N, 10.16°E), as these are known to be free from trematode parasite infection. To confirm the uninfected status of mussels, 50 individuals were dissected immediately after collection and tissues (squeezed between two glass slides) were checked for parasites under a stereomicroscope. No infected mussels were found.

General experimental design and set-up

All experiments were conducted using temperature and salinity (fully crossed) as well as time (only for cercarial output) as fixed factors, and periwinkle/mussel identity nested within water bath as random factor (See Electronic Supplementary Material, ESM Fig.S1). Temperature levels applied were 19 and 23°C. These two temperature levels were chosen based on representative summer temperatures in shallow Western Baltic Sea habitats (19°C as shown for the past 15 years in Kiel Fjord; Pansch et al. 2018), and the expected increment by 2100 (+4°C; IPCC Report 2019). Salinity levels applied were 13, 16 and 19. Following predictions for the Baltic Sea, salinity is expected to drop due to increasing precipitation rates and freshwater runoff (Vuorinen et al. 2015; Johnson et al. 2018). We, thus, chose two severity levels of desalination, i.e. reduction by 3 and 6, resp., from 19 which is the average salinity of Årøsund, the site where *L. littorea* was collected. Furthermore, the activity of *L. littorea* was shown to considerably decrease at salinities below 13.5 (Rosenberg and Rosenberg 1972).

The experiments were conducted in six temperature-controlled water baths (50x50x50cm) located in a single climate chamber (16°C). Cercarial motility and survival was assessed in a different room, using six smaller temperature-controlled water baths (8 L). Three of the six water baths were set at 23 and three at 19°C, and each water bath included all salinity levels. There were i) five independent containers (one periwinkle per container) per salinity level per water bath for the cercarial emergence phase, ii) one 96 well-plate (15 cercariae, one cercaria per well) per salinity level per water bath for the functional activity/survival phase and iii) four independent containers (one mussel per container) per salinity level per water bath for the infection success and susceptibility phases. For each salinity level, filtered Kiel Fjord seawater was used and either marine salt (Seequasal) or deionized water was added, until the desired salinity was reached. In each experiment, temperature and salinity were measured with a digital salinity meter (ProfiLine, Cond 3110, WTW), within the experimental jars every other day.

Exp. I Cercarial emergence from periwinkles

Cercarial emergence was assessed by counting all cercariae that emerged from single periwinkles exposed to the different treatments. Infected periwinkles (20 to 25 mm length) were haphazardly assigned to the different treatment combinations. Periwinkles were placed individually into 250 mL glass jars filled with seawater at the different salinities. Water at the respective salinity conditions was prepared by adding salt (Seequasal) or DI water as necessary. After individually adding the snails, each jar was tightly closed with a lid preventing evaporation and provided with a small hole to allow oxygenation through a tube (from air pumps). Salinity and temperature were checked every other day before the water change, and only minor salinity increments were registered (+0.2–0.3). Five jars per salinity treatment were placed into each waterbath (three baths for each temperature level x five periwinkles/jars for each salinity level, i.e. 15 periwinkles in total per treatment combination), to acclimate periwinkles to the respective temperature and salinity levels. Periwinkles were fed daily *ad libitum* with *U. lactuca* and the water was exchanged every second day. Acclimation to the different temperatures and salinities lasted for one week, increasing/decreasing temperature and salinity by 1°C and 1 psu per day, respectively.

In order to measure cercarial emergence, periwinkles were individually transferred from the jars into 50 mL Plexiglas beakers (Omnilab, ESM Fig. S2a), one day after the 7-days acclimation (measurement 1), one week after the start of exposure to the treatments (measurement 2), and two weeks after the start of exposure to the treatments (measurement 3), representing three sequential measurements for each individual periwinkle. Each incubation lasted eight hours. Each beaker was filled with 40 mL of seawater of the respective salinity and small amounts of *U. lactuca* were added. The beakers were covered with a net and incubated for eight hours in the same water baths from which the periwinkles originated (ESM Fig.S2b) At the end of the incubation period, the periwinkles were individually placed back into their 250 mL glass jars at treatment conditions, while the water from each beaker was transferred into 50 mL Falcon tubes. In addition, the beakers were immediately washed with 5 mL ethanol (99%), and the solution was also added to the Falcon tube to ensure the collection of all cercariae and their preservation for later counting. Samples were centrifuged for five minutes at 800 g, excess water was discarded and cercariae at the bottom were poured into a petri dish for counting under a stereomicroscope (Nikon, SMZ1000). Periwinkles that did not shed any cercariae over all the three incubations were excluded from the analysis.

Exp. II Cercarial activity and survival

Infected periwinkles were acclimated for three weeks under the different treatments (*n*=10 individuals per treatment) and incubated as described above for four hours to allow for cercarial release. At the start of the experiment, approximately 45 cercariae per treatment, obtained from the pooled cercariae released within four hours from the ten snails per treatment, were added individually to wells of three 96-well plates (*n*=15 wells into three replicate 96-well plates per salinity; 45 wells in total). The amount of water (of the respective salinity levels of 13, 16 and 19) in each well was around 0.25 mL. The 96 well-plates were tightly closed with the respective manufacturer's lids throughout the 45 hours period preventing evaporation, except when cercariae were examined for activity and survival. The 96-well plates were then exposed to the different temperature treatments placing them in the six thermobaths (three thermobaths for each temperature level x one 96-well plate for each salinity level = three 96-well plates per treatment combination). Activity and survival were then assessed by visual assessment under a stereomicroscope after 4, 6, 9, 18, 27, and 45 hours. Cercariae were considered fully active when they were constantly swirling around (category: *fully active*). When cercariae were laying at the bottom of a well and not reacting after physical stimuli (through pipette's tip), these were considered dead (category: *dead*), when still reacting, even if slowly, these were still considered alive (category: *alive*). This last category included "*fully active*". Wells that accidentally received more than just one cercaria during the inoculation, were excluded from the analysis.

Exp. III Cercarial infectivity in mussels

The infectivity of *H. elongata* cercariae was investigated by counting metacercariae in *M. edulis*, after a standardized exposure to cercariae acclimated at different temperatures and salinities. Infected periwinkles were acclimated to the treatments for four weeks prior to the infection success assays. Mussels were collected two days prior to the start of the assays, kept in ambient salinity and temperature conditions and fed with *Rhodomonas salina*. A total of 72 mussels (*n*=12 for each treatment combination) of 40–50 mm shell length, kept at 19 psu and 16°C, were individually placed in 50 mL beakers at the respective temperatures and salinities, and exposed to a standardized number of cercariae released from the all periwinkles from the different treatment combinations. In each waterbath, four beakers per salinity combination were placed, i.e. a total of twelve beakers per bath. To obtain cercarial stages for the infection assays, periwinkles were individually placed in petri dishes at the different salinities and incubated for four hours under constant light (see above). After release, all cercariae (4–5 hours old) of a given treatment combination were collected mixing the water from the different petri dishes into one single container, counted under the stereomicroscope and immediately added to the mussels. This accounted for genotypic and phenotypic variation among cercariae. Each of the 12 mussels of a given treatment combination was inoculated with 22 (13–19°C, 16–19°C and 19–23°C), or 23 (19–19°C) cercariae, except for the 16–23°C treatment, in which only five mussels where infected with 20 cercariae each due to the low number of cercariae obtained from such pre-treated periwinkles. The 13–23°C treatment was excluded from the analyses due to extremely low numbers of cercariae released under this particular treatment combination. The beakers with mussels were then returned to the water baths at the treatment temperature and incubated for 24 hours. This incubation period was chosen in order to ensure cercarial encystation (de Montaudouin et al. 2016), but to also limit possible metabolic stress for the host, that could have affected the experiment. After the incubation, each mussel was collected, dissected, and *H. elongata* metacercariae were identified and counted under a stereomicroscope.

Exp. IV Susceptibility of acclimated mussels to cercarial infection

The effects of temperature and salinity on the susceptibility of *M. edulis* to infection by *H. elongata* cercariae were assessed by acclimating mussels to the above-mentioned treatment combinations (salinity x temperature) for one week, before these were exposed to non-acclimated cercariae. In this experiment cercariae did not receive any temperature/salinity treatment and only the mussels did (after acclimation), in order to specifically investigate a putative shift in susceptibility under the treatments by the second intermediate host. We assumed acclimation effects to be stronger in the (acclimated) hosts and less likely on the very short-term basis in the added (non-acclimated) cercariae. However, some potential confounding factors by cercariae very quickly acclimatised cannot be fully excluded. Cercariae were obtained as described above from periwinkles collected two days earlier from the sampling site at Årøsund, Denmark. The exposure to cercariae of each mussel (n=12 mussels per treatment) was realized as described for Exp. II, but this time, the number of cercariae added to individual mussels was 54 (16–19°C, 16–23°C and 19–23°C), 53 (13–19°C) and 56 (19–19°C). Beakers with mussels were incubated at the different treatments for 24 hours. Mussels were then dissected and the number of metacercariae in each mussel (squeezed between two glass plates) was counted under a stereomicroscope.

Statistical analysis

Prior to analysis, the normality of data distribution was tested through Shapiro Wilks tests (Shapiro and Wilk, 1965) and residual plots were checked visually. Non-parametric Generalised Linear Mixed Models (GLMMs), using the packages lm4 and MASS, were then applied to cercarial emergence, infectivity and mussel susceptibility. Residual extraction, the marginal R^2 and conditional R^2 were used to reveal individual identity and thermobath effect, using the function r.squared GLMM (package MuMIn; Nakagawa & Schielzeth 2013). Individual identity nested within thermobath was then used as random factor in all the steps analysed with GLMM. The Akaike Information Criterion (AIC) was used to select the best GLMM model, from models including all fixed factors and reduced models (without temperature or salinity as fixed factors). Two-way ANOVA was instead used for activity and survival. All statistical analyses were performed using the software R 3.5.0 (R Development Core Team 2018).

For cercarial emergence, a negative binomial GLMM, using the lme4 package, was used to test for the effects of temperature, salinity and sampling time (fixed factors) on the number of *H. elongata* cercarie (Exp. I). Negative binomial structure was chosen due to high over-dispersion and presence of zeros in the data.

For cercarial activity/survival (Exp. II), the analysis applied was selected in order to link Exp II with Exp. III. For this purpose, we accounted of the differential slope of decreasing activity/survival during these 24 hours by considering the integral over time (integrated area), which should correspond to the (changing) infection pressure that the mussels underwent in the infection experiments. The average proportion of "*fully active*" and "*alive*" cercariae present in one 96-well plate was considered as one replicate. Proportions were calculated through the 'survfit' function (package survminer). The integral areas of both 0–24h and 0–45 hours intervals were calculated using the mean proportions of the three 96 well-plates to fit the curves. Curves of activity were fitted using geometric model (Power Low family), curves of survival using logistic power model (Sigmoidal models) (software: CurveExpert Professional 2.6.5). To test the effects, two separated two-factorial ANOVA were applied for each interval. Integrated motility and survival at 24 hours and 45 hours were the dependent variables, temperature and salinity the independent variables. Post-hoc tests (Tukey honestly significant difference (HSD)) were performed following ANOVA.

Cercarial infectivity in *M. edulis* (Exp. III), was tested by a GLMM fitted with a Poisson structure with the number of metacercariae found in mussel tissue as dependent variable, and temperature and salinity were set as fixed factors. The same methodology as for infectivity was applied for susceptibility, however, here applying a GLMM fitted with a negative binomial structure.

Using the raw data, the effects of i) strong future freshening (salinity 13) in relation to present conditions (salinity 19), and ii) strong warming (23°C) in relation to present condition (19°C), were expressed as logarithmic response ratios (Lajeunesse 2015). For cercarial activity, only the 0–24h interval was re-considered, linking this period to the infectivity experiment, in which mussels were incubated for 24 hours. Effects were considered significant when the confidence interval (CI) bar was not overlapping zero. Effect sizes (in percentages) of the different life cycle steps were calculated applying the inverse of the log mean ratio for i), all temperature levels were pooled, for ii), all salinity levels were pooled.

RESULTS

Exp. I Cercarial emergence

For cercarial emergence, model selection through AIC revealed four top models having ΔAICs of ≤ 2.1, with models including temperature or salinity and time, or an interaction between both (ESM Table S1). However, the model including only time as fixed factor described the data best (ESM Table S2). Cercariae output significantly decreased by 31% from the first to the second incubation in all treatments (GLMM, estimate value (*ev*)= -0.78, z= -2.65, *P*= 0.007, ESM Table S2), and by 49% from the first to the third incubation (GLMM, *ev*= -1.26, *z*= -3.92, *P*<0.0001, ESM Table S2). However, this happened with clearly variable patterns among treatments. The strongest decrease of cercarial release over time was observed at salinity 13, while at salinity 19 it remained fairly constant throughout the two weeks (Fig. 2). At 13–19°C only the 50% of the periwinkles shed cercariae, compared to 70-80% as observed in the other treatments (ESM Fig. S3).

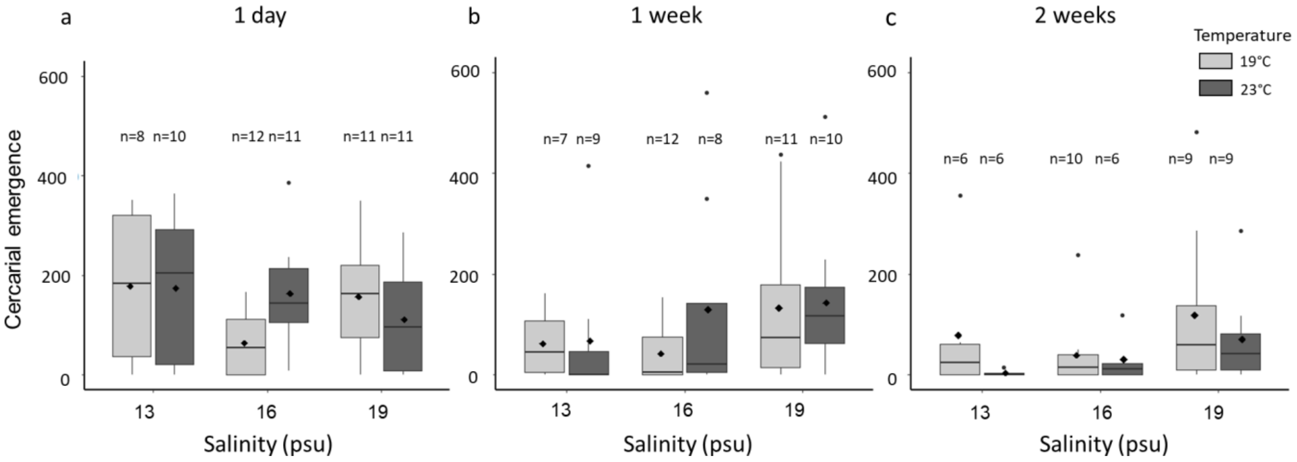

Fig. 2 Effects of temperature, salinity and time on *Himasthla elongata* cercarial emergence during 8 hours from *Littorina littorea* at three measurement points: a) one day after acclimation, b) one week after acclimation and c) two weeks after acclimation week in 19 and 23°C seawater conditions at salinities of 13, 16 and 19. Each *n* indicates the number of periwinkles shedding cercariae in each treatment. The boxes represent the 75 (upper) and 25 (lower) percentile, the whiskers denote the lowest and the highest value, the black lines within the boxes the medians, the black diamond-shaped dots identify the means, and the black dots the outliers. Periwinkles that did not shed any cercariae in all the three incubations were excluded from the analysis and the plots.

Exp. II Cercarial activity and survival

In the integrated 0–24 hours and 0–45 hours, cercarial activity was significantly decreasing with salinity (ANOVA, 0–24h: $F_{2,12}$ = 0.10, *P*= 0.001; 0–45h: $F_{2,12}$ = 12.45, *P*= 0.001, Table 2). Cercarial activity over 24 hours was 31% lower at salinity 16 than salinity 19 and 37% lower at salinity 13 than 19 (Tukey test:16–19: *P*= 0.004; salinities 13–19: *P*= 0.002). Cercarial activity over 45 hours was 36% lower at salinity 16 than salinity 19 and 42% lower at salinity 13 than salinity 19 (Fig.3; Tukey test: 16–19: *P*= 0.003; 13–19: *P*= 0.002). Cercarial survival showed no significant effects in the integrated 0–24 hours interval. Contrarily, temperature had a negative effect in the integrated 0–45 hours, with survival at 23°C 19% lower than at 19°C (ANOVA, $F_{1,12}$= 32.12, *P*= 0.001, Table 2).

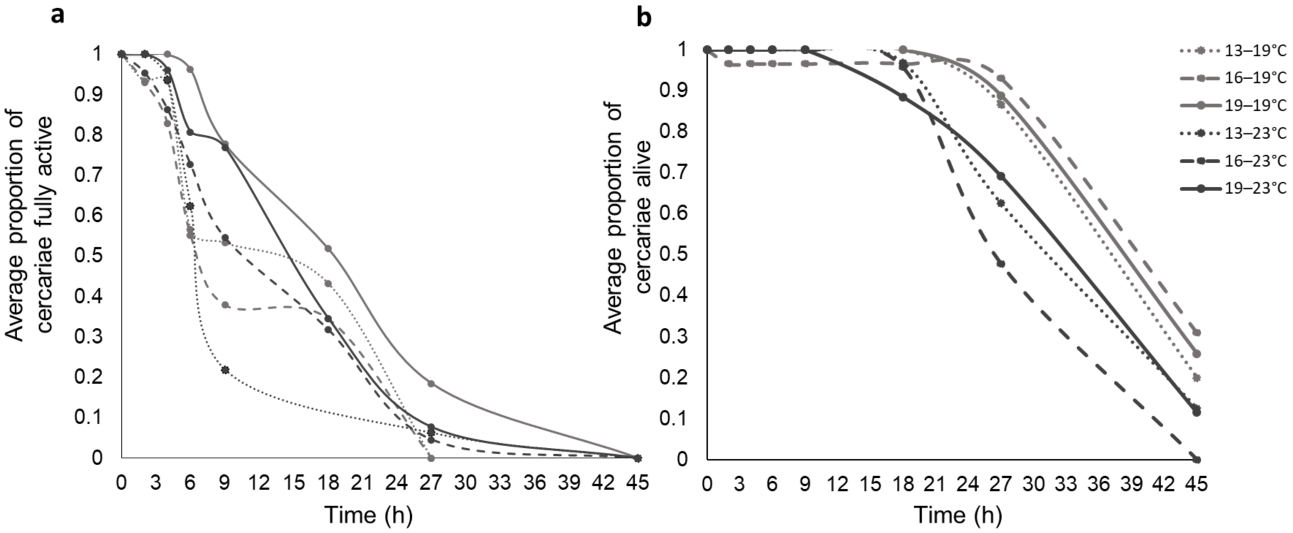

Fig. 3 Proportion of *Himasthla elongata* cercariae considered as a) fully active (i.e. constantly swirling around; and b) alive over 2, 4, 6, 9, 18, 27 and 45 hours post release in 19 and 23°C seawater conditions at salinities of 13, 16 and 19.

Table 2 Results of a two-way ANOVA testing for the single and interactive effects of temperature (19 and 23°C) and salinity (13, 16, 19) on the integrated 24 hours and 45 hours activity and survival of *Himasthla elongata* cercariae. The "**" symbol indicate *P* values <0.01 and "***" indicate *P* values <0.001. Bold text indicates significant *P* values.

Experiment		**Treatments**	**Df**	**MS**	**F value**	**P value**
Motility	*Integrated 24 hours*	Temperature	1	2.03	0.429	0.525
		Salinity	2	57.05	12.035	**0.0013****
		Temperature × Salinity	2	10.95	2.309	0.142
	Integrated 45 hours	Temperature	1	7.51	0.775	0.396
		Salinity	2	120.77	12.459	**0.0011****
		Temperature × Salinity	2	20.77	2.143	0.160
Survival	*Integrated 24 hours*	Temperature	1	1.43	1.812	0.203
		Salinity	2	0.08	0.101	0.905
		Temperature × Salinity	2	1.00	1.268	0.316
	Integrated 45 hours	Temperature	1	227.06	32.128	**0.0001*****
		Salinity	2	1.54	0.218	0.807
		Temperature × Salinity	2	11.63	1.645	0.234

Exp. III Cercarial infectivity

Model selection indicated the model which only included salinity to be the best model (ESM Table S3). Infectivity significantly decreased with decreasing salinity. At salinity 16 the proportion of cercariae successfully infecting mussels was 33% lower than at salinity 19 (GLMM, *ev*= 0.46, *z*= 2.03, *P*= 0.041, ESM

Table S4) and at salinity 13 this trait was 47% lower than at salinity 19 (GLMM, *ev*= 0.65, *z*= 2.97, *P*= 0.002, Fig.4, ESM Table S4). Models including temperature as an additional additive or interactive factor, even if not significant, also received substantial support from the data (ΔAIC≤2; ESM Table S3). While at salinity 19 infectivity was 21% higher at 23°C compared to 19°C, it was the opposite at salinity 16 (at 23°C infectivity 44% lower than 19°C) (Fig. 4). Only very few cercariae were shed from periwinkles at the 13–23°C treatment. Therefore, infection success could not be tested for 13–23°C.

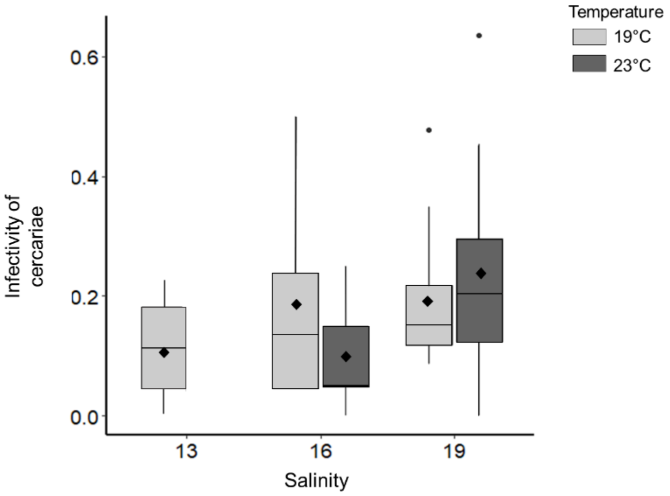

Fig. 4 Proportion of acclimated cercariae successfully infecting non-acclimated *Mytilus edulis* (n=12 for each treatment, apart from 16–23°C, where n=5) following a 24 hours incubation in 19 and 23°C seawater conditions at salinities of 13, 16 and 19. The 13–23°C treatment was excluded from the experimental analyses due to the very low number of cercariae shed from the snails acclimatised to this respective treatment. The boxes represent the 75 (upper) and 25 (lower) percentile, the whiskers denote the lowest and the highest value, the black lines in the boxes are the medians, the black diamond-shaped dots identify the means, and the black dots the outliers.

Exp. IV Susceptibility of mussels

Model selection identified the model including only salinity as the best model (ESM Table S5). The proportion of cercariae infecting acclimatised mussels decreased significantly with salinity. At salinity 16 the proportion was 19% lower than salinity 19 (GLMM, *ev*= 0.32, *z*= 1.59, *P*= 0.110, ESM Table S6) and at salinity 13 it was 43% significantly lower than salinity 19 (GLMM, *ev*= 0.61, *z*= 3.03, *P*= 0.002, ESM Table S6). The model including temperature and salinity also explained the data reasonably well (ΔAIC 1.85, ESM Table S5). The patterns in the plot (Fig. 5) show a similar susceptibility between hosts at salinity 16 and at salinity 19 at 19 and 23°C, but lower susceptibility at 23°C than at 19°C at salinity 13.

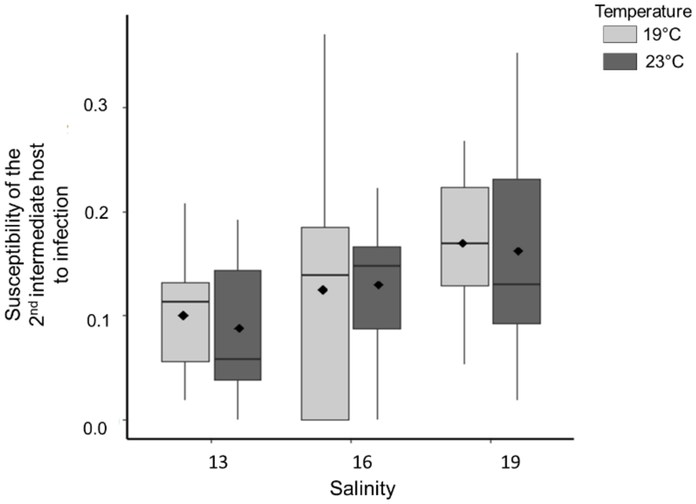

Fig. 5 Proportion of non-acclimated cercariae infecting acclimated *Mytilus edulis* (*n*=12 for each treatment) following a 24 hours incubation in 19 and 23°C seawater conditions at salinities of 13, 16 and 19. The boxes represent the 75 (upper) and 25 (lower) percentile, the whiskers denote the lowest and the highest value, the black lines within the boxes the medians and black diamond-shaped dots identify instead the means.

Future versus present conditions in our study system

After pooling the temperature levels, log response ratios showed a significant decrease of cercarial emergence (to 54%), cercarial activity (to 80%), cercarial infectivity (to 70%), and second intermediate host susceptibility (75%) in an extreme future freshening scenario from salinity 19 (ambient condition) to 13 (future condition; Fig. 6a). Contrarily, after pooling the salinity levels, and comparing future global warming (23°C) with today's temperatures (19°C), only cercarial emergence and long-term survival (45h) decreased significantly, to 70% and 90% respectively (i.e. confidence intervals not overlapping zero; Fig. 6b).

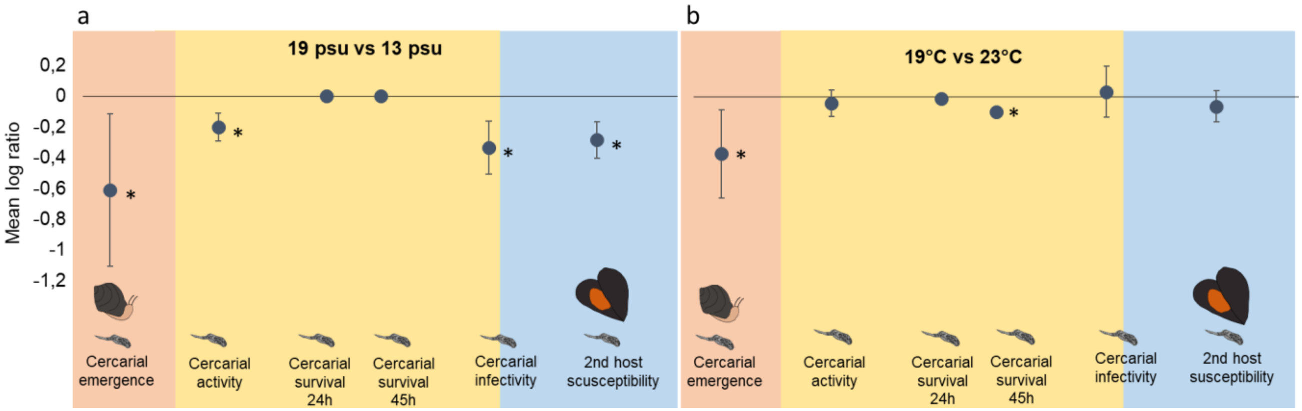

Fig. 6 Logarithmic response ratios for: cercarial emergence, cercarial activity after 24 hours, cercarial survival after 24 and 45 hours, cercarial infectivity and mussel (second intermediate host) susceptibility. a) Effects of desalination (log [(trait at 13)/ (trait at 19)]) with data from the two temperature treatments pooled, and b) Effects of warming (log [(trait at 19°C)/ (trait at 23°C)]) with data from the three salinity levels pooled. Error bars represent 95% confidence intervals. Significant effects are indicated by '*'. Different colours represent the different effects on: i) cercarial shedding from periwinkles (red), ii) cercarial survival and infectivity (yellow), and iii) susceptibility of mussel to infections (blue).

DISCUSSION

To date, studies of environmental effects on the free-living stages of parasites in the Baltic Sea are rare, apart from experiments by Möller (1978) about the combined influence of temperature and salinity on the survival of fish parasites. In this study, we assessed the possible impact of warming and freshening on specific transmission stages from the first to the second intermediate host of a common marine trematode of the western Baltic Sea. Salinity had the strongest effects on most transmission steps: cercarial emergence, activity, infectivity and 2nd intermediate host susceptibility all decreased with decreasing salinity. In contrast, only cercarial emergence and survival were affected by temperature, both decreasing with the warming scenario. Interaction effects between salinity and temperature were weak or absent.

Cercarial emergence

Cercarial emergence decreased over the experimental duration, accordingly time was the factor contributing to the model that fitted the data best. However, over the entire experimental period, cercarial emergence significantly decreased to 54% with sea water freshening (salinity 13) and to 70% with warming compared to the ambient condition (salinity 19; Fig. 6). Both observations are in line with previous studies which showed a positive relationship between cercarial emergence and increase in salinity (Sindermann and Farrin 1962; Lei and Poulin 2011; Studer and Poulin 2012) as well as increase in temperature (Mouritsen 2002; Studer et al. 2010). Cercarial emergence typically increases with temperatures until an optimum, usually determined by the host tolerance and by acclimation to changed environmental conditions (Erasmus 1972; Ataev 1991; Morley and Lewis 2013). The number of cercariae released during our measurement 1 (after 7 days of acclimation) was relatively high compared to the next two incubations, especially at the lowest salinity (13). Here, the number of cercariae released was 35% higher than at ambient condition (salinity 19). This could hint at an initial response of periwinkles to the abrupt changes in salinity/temperature, which then possibly decreased with time. Hence, high fluctuation of environmental factors characterising the Western Baltic shallow coast (Franz et al. 2019), might favour a higher tolerance to stressors by benthic organisms. This is especially valid for *L. littorea*, which has been shown coping well with temperature peaks in a study by Pansch et al. (2018). The substantial variance of the responses within the treatment combinations could be also attributable to within-host and -parasite population variance of sensitivities (Al Janabi et. 2016, Leiva et al. 2019). Lastly, since *Littorina* sp. inhabits shallow coasts, other factors could influence cercarial emergence, such as water level changes, as reported by Leiva et al. (2019). Further experiments applying frequent peaks of temperature and salinity changes over a short period, and accompanied by sea level changes, would be valuable to investigate whether cercarial emergence is reacting stronger to short-term triggers than to longer-term acclimations.

Cercarial activity and survival

Cercarial activity decreased significantly with freshening of seawater to 80% of the activity at ambient conditions (salinity 19; Fig. 6). Again, these salinity effects are consistent with previous studies from other marine trematode species of the genus *Acanthoparyphium* (Echinostomatidae) and *Maritrema* (Microphallidae) and (Koprivnikar et al. 2010; Studer and Poulin 2012), generally showing cercariae to fare better with increasing salinity. The reason for the decreased cercarial activity at lower salinities is probably attributable to osmotic stress. Body fluids of periwinkles are known to be isosmotic down to 15, below which the fluids become hyperosmotic (Todd 1964; Rumsey 1973). Cercariae released from hosts under such low-salinity conditions might been subjected to an osmotic shock after emergence (Lauckner 1984). Supporting this, in coastal waters with salinity below 15 *H. elongata* infections were registered only in periwinkles at the bottom of shallow waters (Lauckner 1984). Stratification of the water column in the Baltic Sea often leads to the deeper water layer being higher in salinity and lower in temperature (Liblik and Lips 2019). In contrast to activity, cercarial survival was negatively affected by temperature only. Over 45 hours, survival decreased significantly by 10% at the higher temperature (23°C) (Fig. 6) compared to ambient (19°C). Similar results have been reported previously (Mouritsen 2002; Koprivnikar et al. 2010), possibly resulting from higher temperatures accelerating the metabolism of cercariae leading to a faster depletion of their finite energy reserves (Pechenik and Fried 1995). This in turn might have direct consequences for their transmission success in a future warmed ocean.

Cercarial infectivity and susceptibility of mussels

Freshening had a negative effect on cercarial infectivity which decreased significantly with desalination to 70% compared to ambient condition (salinity 19; Fig. 6). Freshening has been shown previously to reduce cercarial infectivity, possibly because of the vulnerability of the free-living stages of marine trematodes to low salinity (Pietrock and Marcogliese 2003). Furthermore, as shown by Stunkard and Shaw (1931), osmotic stress could provoke slower cyst formation during infection. A previous study by Riisgård et al. (2013) found no differences in filtration rate of blue mussels collected at salinity 17.5 and exposed for two weeks to a range of salinities (10–30). Hence, the reduced infectivity with freshening could be merely attributed to the acclimatised cercariae. The susceptibility of the mussel host was also negatively affected by seawater freshening to 75% of the ambient condition (Fig.6). Accounting that filtration rate at lower salinity is not significantly reduced during one-week of acclimation, other reasons narrowed to susceptibility should be attributed, which remain unknown. However, the difference of 5% between decreased infectivity (70%) and susceptibility (75%) with desalination leads to argue that acclimation mattered. To exclude any effect of changes in filtration on susceptibility, a longer acclimation period to the mussels would be required. Moreover, further experiments investigating physiological responses of mussels while infected by cercariae could also be useful for a better understanding of mussel susceptibility. Interestingly, no significant effects of temperature or interaction between temperature and salinity were observed for both cercarial infectivity and mussel susceptibility. This suggests a limited importance of temperature for cercarial infectivity and mussel susceptibility in this parasite-host system. However, it may be that stronger warming scenario (>4°C) or extreme future conditions during heatwaves could have resulted in stronger temperature responses. For example, Pansch et al. (2018) found *M. edulis* to tolerate temperature peaks better than other bivalve species. Simingly, only at very high temperatures the mussel immune system may become compromised leading to an increase of cercarial infection.

Expected shifts in parasitism in response to desalination and warming

In general, our experiments suggest that future desalination scenarios for the Baltic Sea may lead to lower cercarial transmission rates, and therefore lower infection levels in mussel hosts, with salinity having more profound effects than temperature. Based on the combined effects of decreasing cercarial emergence (reduced to 54% with freshening) cercarial activity (reduced to 80% with freshening), cercarial survival (reduced to

90% with warming over 45 hours) and mussel susceptibility (reduced to 75% with freshening), future climate scenarios could lead to a decrease in infection levels of *H. elongata* in mussels to around 30% (0.54 x 0.8 x 0.9 x 0.75). Such lower infection levels in mussels with decreasing salinity are also supported from field data along the Western Baltic coast, in which prevalence of trematodes in *M. edulis* at salinities below 15 was reduced by 80% relatively to higher salinities (Bommarito et al. unpublished.). These predictions suggest that global change may not always lead to elevated levels of parasitism as predicted by some studies (Poulin 2006; Studer et al. 2010), which could be beneficial for the host populations. It must be emphasized that the direction of global change effects is probably not universal, but conditional on the respective parasite-host systems, and the particular environmental context. Steep salinity gradients can be found in many coastal seas worldwide, for example areas in which glacier meltwaters release freshwater into the sea, or the many estuaries resulting from larger river runoffs. Therefore, the data collected on the host-parasite system in the present study are not relevant to the Baltic Sea only but might be applied to other host-parasite systems. Furthermore, *H. elongata* is distributed worldwide, and testing whether the response of this species to changes in temperature or salinity differs with latitude would be beneficial. Our experiments only investigated the transmission from the first to the second intermediate host. Further experiments on other life cycle stages of *H. elongata*, such as on eggs or miracidial stages, are needed to fully understand the climate change effects on this entire host-parasite system, and whether our findings can be extended to other marine trematode species. Acidification could be also included, especially in experiments involving molluscs as intermediate hosts. In addition, parasite transmission and host infection levels under climate change will also be affected by other factors such as the density of the intermediate hosts, the presence of the final hosts, as well as other environmental factors. All of these are important to consider in developing a full picture of the climate change effects on host-parasite interactions.

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SUPPLEMENTARY MATERIAL

Experimental set-up

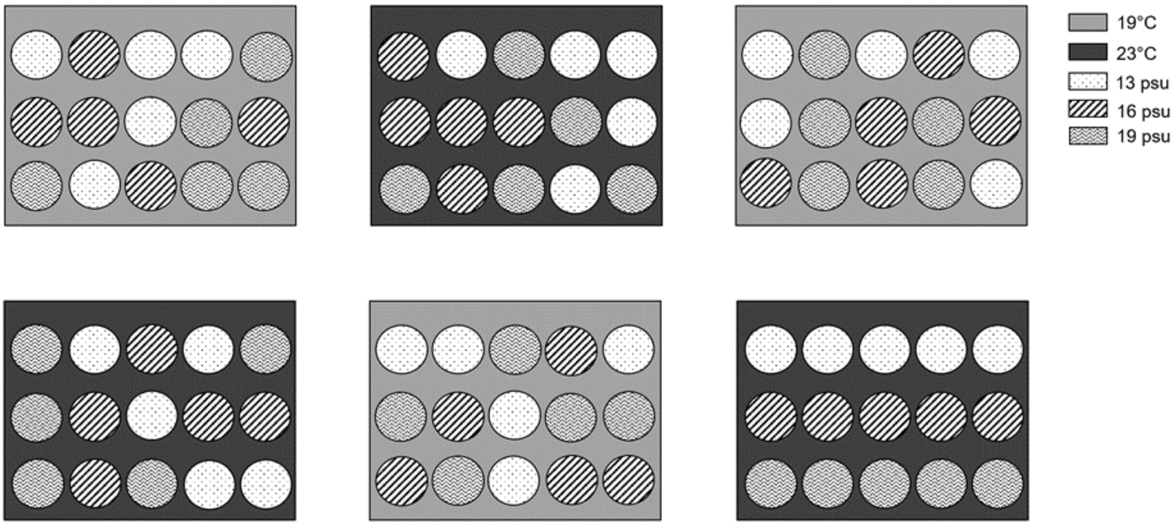

Fig. S1 Experimental set-up of Exp. I, III and IV. Six water baths were used, three with temperature set at 19°C and three with temperature set at 23°C. In each water bath five (Exp. I) or four (Exp III and IV) individual replicates of snail/mussel per each salinity level were haphazardly located. Thus, each water bath included: five replicates at salinity 13, five at salinity 16 and five at salinity 19. For activity/survival (Exp. II) six water baths were also used, three at 19°C and three at 23°C, but instead of individual beakers, one 96-well plate containing 15 cercariae per single well for each salinity level was located in each water bath. Thus, each water bath included three 96-well plates: one at salinity 13, one at salinity 16 and one at salinity 19.

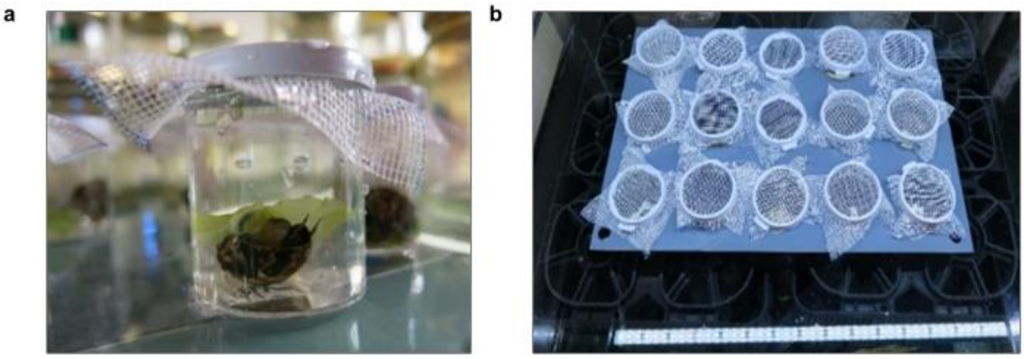

Fig. S2 Set-up used during all the three incubations for Exp.I (cercarial emergence) with **a)** the 50 ml plexiglass beakers in which each snail was contained, and b) the placement of the 15 beakers in each water bath.

Cercarial emergence

With regard to the percentage of infected hosts shedding cercariae in the pooled sampling events, only 50% of the snails released cercariae at 13psu/19°C, compared to 70-80% in the other treatments (Fig. S1). However, the difference between the treatments was not statistically significant (GLMM binomial; p>0.1).

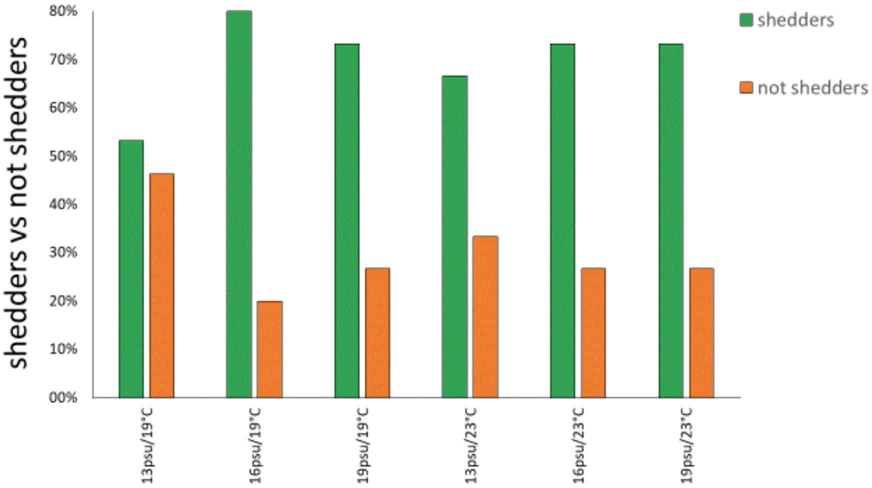

Fig. S3 Percentage of periwinkles shedding and not shedding cercariae at different treatments during the three incubations.

Table S1 Results of the AIC selection for the best model explaining cercarial emergence. All interactive and additive and single effects of temperature (19 and 23°C) and salinity (13, 16, 19 psu) were tested. The best model is indicated in bold.

Model	**Df**	**AIC**	**ΔAIC**
GLMM negative binomial time only	**6**	**1760.7**	**0.0**
GLMM negative binomial Temperature + Time	7	1762.2	1.5
GLMM negative binomial Salinity + Time	8	1762.5	1.8
GLMM negative binomial Salinity × Temperature	9	1762.8	2.1
GLMM negative binomial Temperature + Salinity + Time	9	1764.0	3.3
GLMM negative binomial Salinity × Time	9	1765.5	4.8
GLMM negative binomial Temperature × Time	12	1765.2	4.5
GLMM negative binomial Temperature × Salinity × Time	21	1775.8	15.1

Table S2 Time effect (sampling 1, 2 and 3) on cercariae released by infected hosts. Time is considered to be a fixed factor and snail identity was nested within waterbath as random factor. Reference category is sampling 1. Results of the General Linear Mixed Model (GLMM) negative binomial. Significant effects are indicated in bold

Factor and level	**Estimate**	**Std. Error**	**Z value**	**Lower-95 CI**	**Upper-95 CI**	**Pr (>|z|)**
Sampling 2	-0.7879	0.2964	-2.658	-1.368827	-0.2069049	**0.0078**
Sampling 3	-1.2684	0.3234	-3.922	-1.902207	-0.6346046	**0.0001**

Table S3 Results of AIC selection for the best model explaining cercarial infectivity. Both interactive, additive and single effects of temperature (19 and 23°C) and salinity (13, 16 and 19 psu) were tested based on the number of *Himasthla elongata* metacercariae found in the mussel host tissue. The best model is indicated in bold

Model	**Df**	**AIC**	**ΔAIC**
GLMM Poisson Salinity	**5**	**255.58**	**0.00**
GLMM Poisson Temperature × Salinity	7	256.33	0.75
GLMM Poisson Temperature + Salinity	6	257.58	2.00
GLMM Poisson Temperature	4	263.79	8.15
GLMM Poisson null	3	263.05	7.47

Table S4 Effect of salinity (13, 16 and 19 psu) on cercarial infectivity in *Mytilus edulis.* Salinity was considered to be a fixed factor and mussel identity was nested within tank as random factor. Salinity reference category: 13 psu. Results of a GLMM Poisson. Significant effects are indicated in bold

Factor and level	Estimate	Std. Error	z value	Lower-95 CI	Upper-95 CI	Pr (>|z|)
Salinity 16	0.4650	0.2284	2.035	0.0172531	0.9126801	**0.0418**
Salinity 19	0.6528	0.2197	2.971	0.2221494	1.0835121	**0.0029**

Table S5 Results of AIC selection for the best model explaining susceptibility of mussel hosts. Both interactive, additive and single effects of temperature and salinity were tested based on the number of *Himasthla elongata* metacercariae found in the mussel host tissue. The best model is indicated in bold

Model	Df	AIC	ΔAIC
GLMM negative binomial Salinity	**6**	**423.50**	**0.00**
GLMM negative binomial Temperature + Salinity	7	425.35	1.85
GLMM negative binomial Temperature × Salinity	9	429.13	5.63
GLMM negative binomial Temperature	5	429.94	6.44
GLMM negative binomial null	4	428.06	4.56

Table S6 Effect of salinity (13, 16 and 19 psu) on *Mytilus edulis* susceptibility to infection. Salinity was considered to be a fixed factor and mussel identity was nested within tank as random. Salinity reference category: 13 psu. Results from GLMM negative binomial. Significant effects are indicated in bold

Factor and level	Estimate	Std. Error	z value	Lower-95 CI	Upper-95 CI	Pr (>|z|)
Salinity 16	0.3268	0.2046	1.598	-0.07414943	0.7277901	0.1101
Salinity 19	0.6099	0.2009	3.036	0.21620480	1.0036977	**0.0024**

GENERAL CONCLUSIONS

Understanding the role of parasites: tiny but fundamental players too long neglected

The ecological impact of global change is expected to rise as fast as human exploitation of the environment increases (Sala et al. 2000). At the age of fast global change, networks of biotic interactions are fundamental to maintain diversity, ecosystem function and to modulate global change effects (Bascompte et al. 2006; Dobson et al. 2006; Suttle et al. 2007). Hence, as experimentally emphasised by Davis et al. (1998), ecological interactions can themselves influence the responses by the single species to global change. In the theatre of ecological interactions, parasites have a protagonist role, by direct effects on their host population or trait-mediated indirect effects on the entire community (Hatcher et al. 2014). As illustrates by Sures et al. (2017), the crucial contribution of parasites to the ecosystem should require more attention and an increase in links between ecologists and parasitologists.

The present study mainly aimed to raise the attention on the host-parasites interaction and how this can be modulated by different environmental conditions, under a global change perspective. Through the three main chapters, it has been highlighted the role that abiotic and biotic factors can have on parasites distribution and transmission as well as their potential future modifications under global change scenarios.

Different basins, different parasites…

Regardless of the choice of the *Mytilus* genus as typical host, the parasite communities reported in our field studies on the Adriatic and the Baltic basins highly differed. In the Western Baltic Sea, the most common parasites species found were the marine trematodes *Renicola roscovita* (family: Renicolidae) and *Himasthla elongata* (family Echinostomatidae) both in *Mytilus edulis* and *Littorina littorea*. In *L. littorea* also other trematodes, such as *Cryptocotyle lingua* (family: Hetereophydae) and *Microphallus pygmaeus* (family: Microphallidae) were detected. Species composition was similar to the ones previously described in the Baltic coast (Lauckner 1984; Zander and Reimer 2002; Thieltges et al. 2009). In the North Adriatic Sea, the most common species found was the trematode *Parvatrema timondavidi*. Other trematodes were not detected, and other abundant species were parasites/symbionts such as *Eugymnanthea inquilina* and *Urastoma cyprinae*, as also previously described by Ryyan et al. (2004). The variation in the species composition is not surprising, given the marked differences between the Adriatic and the Baltic Sea in terms of evolutionary histories and environmental condition, the former old and species-rich (Hsü et al. 1977), the second young and species-poor (Lass and Matthäus 2008; Schiewer 2008). Despite these differences, both semi-enclosed basins are subjected to high anthropogenic pressure and are experiencing global change effects faster than other oceans. The semi-enclosed nature lead to these two basins to exibit own characteristics (Araldi et al. 1999; Zettler et al. 2007) which in turn may lead to segregation of the host populations. Hence, a study conducted in the Adriatic by Štambuk et al. (2013) detected really low genetic variation among subpopulations of mussel over a large geographical scale, compared to the population of the Atlantic coast.

…but common outcomes

Abiotic factors, temperature is not everything!

As highlighted by previous studies, in the environment parasites are exposed to many variables other than temperature (Marcogliese 2016). This thesis included the effect of abiotic environmental factors, namely salinity, eutrophication and temperature, on the parasite distribution and transmission. Salinity was recognised as a primary driver in both the study areas, but especially in the Baltic Sea. Increasing salinity appears to promote parasites prevalence and abundance, as previously reported by other few studies (Schmidt et al. 2003; Poulin et al. 2011; Marcogliese 2016). Prevalence and abundance of the analysed trematode species in the Baltic, *R. roscovita* and *H. elongata* increased towards the Kattegat strait (salinity above 16 psu). Influence of salinity appears especially valid in trematodes, supporting the assumption of strong links between the distribution of parasites and the one of their hosts (Zander 1998). Hence, although known as highly tolerant organisms, snails and mussels show limits of adaptive osmoregulation (Taylor and Andrews 1988; Riisgård 2012), which could be reflected also on their parasites. Our field sampling was followed by the experimental study, in which second intermediate susceptibility was negatively affected by decreasing salinity. As parasites and hosts display this close association, the osmotic tolerance of a parasite species could serve as an essential indicator of host stress and could help to predict shifts in host distribution. In our experimental study it was shown that reduced salinity negatively affects trematode free-living cercariae, especially in their activity and infectivity phases, while temperature affected only cercariae survival. In a future freshening scenario of the Baltic Sea reduced salinity could have negative effects on trematodes, while the second intermediate host may benefit from a decreasing infection pressure.

As opposed to the Baltic, in the Adriatic Sea salinity may increase in the future (Solidoro et al. 2009; Lionello et al. 2012), enhancing an increase of parasites and symbionts abundance. Beside salinity, results of the field study in the Adriatic suggested a dominant role of eutrophication in parasites prevalence and abundance. Mussels inhabiting the high-nutrients load environment of the North-Western Adriatic coasts (Libralato et al. 2004; Rampazzo et al. 2013) may display higher condition index and higher metabolic rates (i.e. filtration) (Peterson et al. 2015), which could enhance trematode infection. Furthermore, a healthier status can increase the host tolerance towards stressful condition, which may benefits parasitism (Aalto et al. 2015). A potential decrease in eutrophication in the future (Giani et al. 2012) could be detrimental for the hosts and, consequently, for parasites strictly associated with them, such as trematodes. As opposite, an increase in eutrophication could benefit host-parasites association, but until a critical limit. Hence, nutrients level beyond a certain threshold could provoke hypoxia or anoxia events and have negative effects on parasites free-living stages as well as on their hosts (Pietrock and Marcogliese 2003). Our findings about eutrophication and parasites distribution could contribute to emphasise the role of parasites as indicators of the ecosystem health and, therefore, as useful tools for the management of shellfish activity. In general, insights from our field and experimental studies suggest a leading role of salinity and eutrophication, stressing the necessity to combine field monitoring multi-factorial, near-natural, experiments.

Intermediate hosts do matter

As mentioned above, parasites can have a broad variety of effects in their host, influencing their population dynamics. The flip side of the coin is that host population- and individual-traits can also deeply affect parasites distribution (Poulin 1997; Hechinger and Lafferty 2005; Thieltges and Reise 2007; Galaktionov et al. 2015). Gastropods usually serve as first intermediate host for parasites, since here miraciudium develops in sporocyst/redia, proliferates and eventually produces the cercariae stage. Our field study in the Baltic was one of the first one including both the first (*L. littorea*) and the second intermediate host (*M. edulis*). Obtained results suggest a positive correlation between snail density (upstream host) and abundance of trematodes metacercariae in mussels (downstream host). A higher density of first intermediate host possibly facilitates higher abundance by producing more cercariae (Thieltges 2007; Thieltges and Reise 2007). Under the global change scenarios, snails density in the Baltic Sea is expected to decline due to freshening or warming (Vuorinen et al. 2015) and this could in turn affect parasites abundance.

The results of the Adriatic sampling showed as relevant the density of the second intermediate host, as previously reported also by other studies (Galaktionov 2015; Goedknegt 2019). At the individual level, mussel size correlated positively with trematodes prevalence and abundance. This is the first study of parasite community in bivalves all along the North Adriatic coast, including both a spatial and a temporal scale. As a further novelty, we attempted to correlate richness in individual mussel with local richness, detecting a significant positive correlation. These findings highlighted the importance of considering parasite richness at different hierarchical levels of organisation (Poulin 1997), which should be considered in further studies. Moreover, these findings support experimental studies on parasites transmission (i.e. infectivity, host susceptibility to infection and survival) involving biotic factors, such as intermediate host traits (i.e. different levels of density and different individual sizes).

FUTURE PERSPECTIVES

Host-parasites interaction is often neglected in the field of ecology. This thesis provided a broad frame on parasites diversity, distribution and transmission on two apparently similar but really different basins, giving an insight of the variability in parasites spatial patterns occurring at different latitudes. Moreover, this thesis represents a trade-off between a more profound knowledge of a host-parasite system in a specific habitat and a broader knowledge of different host-parasites systems in a larger spatial range, with differing environments. The main aim of this thesis was to emphasise the importance of considering different abiotic factors and stress the fundamental role of the intermediate hosts. However, this study included a limited group of host-parasite systems the and only a part of the leading environmental drivers of parasites transmission, consisting of a narrowed example of the magnitude of factors involved in the host-parasite interaction. As stressed by Poulin (2016) in his review, the research effort in ecoparasitology is still mainly addressed to the definitive hosts, which are more relevant for humans, and parasites species are often described only in their adult stages, without clues of larval stages harboured by intermediate hosts. Invertebrates as mussels and snails are among the most relevant benthic organisms, spread worldwide in coastal environments, and deepening their role in parasites transmission become crucial under global change. With this thesis, I would like to increase the awareness of the crucial role of parasites in the ecological interactions, as well as the synergy among the fields of parasitology and ecology. Driving the conclusions, future possible questions to answer to in the near future could be:

1) **Not just cercariae, neither adults: how global change can affect parasites eggs and miracidia transmission to the first intermediate host?**

   So far, really few was investigated infectivity of these early free-living stages of trematodes life cycle, and most of them focused on *Schistosoma* sp. (Anderson et al. 1982; Morley 2011). Further experiments investigating on abiotic effects in miracidial infectivity and susceptibility of the first intermediate host to infection would be needed. Experiments could be preceded by a review on environmental effects in trematodes eggs and miracidia, in order to identify better the gaps that need to be filled.

2) **In the natural environment, where fluctuations of abiotic factors are occurring, what will be the outcome of different effects of environmental variables in parasites emergence, infectivity and host susceptibility and survival?**

   A near-natural approach for further experiments on emergence, infectivity and second host susceptibility and survival, involving the combination of different factors (i.e. salinity, eutrophication, acidification) could help for a deeper understanding of parasites transmission under global change. A possible way could be represented by mesocosm experiments, which systems which simulate the natural environmental fluctuations.

3) **Which are the main drivers among intermediate host population and/or individual traits affecting parasites distribution?**

Many studies assessed the important of population and individual traits of intermediate host in parasites prevalence, abundance and distribution (i.e. density for the former, size, length), but still a broad frame is missing. A more comprehensive knowledge could be acquired by pooling historical traits-based datasets from different latitudes and different intermediate host-parasites system. In habitats where the incoming of invasive species is increasing at fast rates, new organisms may represent alternative host. Hence, traits of potential alternative host should be also considered, in order to predict potential effects in parasites distribution.

4) **How other intermediate hosts-parasites system rather than *Littorina* sp., *Mytilus* sp. and their trematodes respond to global change?**

As illustrated by Poulin et al. (2016) all that is known about intermediate host-parasites system turns around really few host species, i.e. *Hydrobia* and *Littorina* sp. Thus, field and experimental studies focusing on different host-parasites system, including overlooked parasites groups such as turbellarians, would be fundamental to understand better the effect of climate change on the entire food-web network.

5) **Organisms exhibit different threshold to environmental stressors (i.e. warming) which are habitat- and species-specific. How host susceptibility to parasitism differ at different latitudes?**

In their review, Lafferty and Mordecai (2016) described the multitude of factors influencing parasites transmission under global warming, including host susceptibility. This is known to be affected by the host critical temperature threshold, which can be estimated by examining the performance curves of the different organisms. Performance curves based on other abiotic factors than temperature are often missing and new experiments could fill these gaps.

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