



Parasite Assemblages in a Bivalve Host Associated with Changes in Hydrodynamics

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Abstract

Studies on marine community structure and their modulation by horizontal and vertical abiotic gradients have focused on free-living species, neglecting parasites despite their important role in ecosystems. Field observations, a laboratory flume experiment and modelling were used to examine parasite infection patterns related to host tidal and spatial position along a Portuguese coastal lagoon (Ria de Aveiro). Cockles (*Cerastoderma edule*) and trematodes were used as a host-parasite model to test the hypotheses: (1) higher infection levels in subtidal cockles, positioned downstream and (2) higher current velocity in downstream areas with consequent higher cockle infection. Cockles were collected from two spatial and tidal positions in two seasons. The relationship between current velocity and cockle infection was examined experimentally by exposing cockles to cercariae of *Himasthla elongata* at two current velocities. Intertidal cockles displayed higher infection, highlighting vertical position as an important driver of infection possibly due to first host proximity and/or trematode life cycle facilitation (higher cockle exposure to final host predation). The hydrodynamic model showed that the Ria de Aveiro was abiotically heterogenous with cockles displaying higher infection levels in the downstream area. Multiple regression analysis demonstrated a positive correlation between salinity, dissolved oxygen, current velocity and trematode abundance. The influence of current velocity on infection success was corroborated experimentally. The present study demonstrated the importance of host vertical and horizontal position on trematode infection, highlighting the positive influence of current velocity by supporting settlement and promoting favourable abiotic conditions.

Keywords Cockles · Trematode parasites · Tidal position · Abiotic drivers · *Cerastoderma edule*

Introduction

The ecological functioning of Atlantic semi-sheltered ecosystems, such as coastal lagoons and estuaries, is commonly driven by abiotic factors that follow gradients with horizontal

and vertical dimensions. The horizontal dimension, related to ocean connection and to freshwater inputs (Dyer 1997; Prandle 2009), results in downstream areas (closer to the ocean) with greater current velocity, higher salinity and less pronounced temperature shifts than upstream areas (closer to freshwater inflows) (e.g. Vaz et al. 2005). On the other hand, the vertical dimension, related to water movement associated to tides (Stephenson and Stephenson 1949), causes strong gradients (following an intertidal gradient) in terms of desiccation, water temperature and irradiance, with the most extreme values being displayed in the upper limit of the tidal zone (e.g. Rawlings 1999). However, the relative strength of these gradients and drivers depends on several features inherent to each ecosystem, namely geomorphology, hydrology and oceanography. For instance, the width of a coastal system connection with the ocean has an effect on flood/ebb ratios, altering water exchanges with the ocean and consequently conditioning horizontal saline and water temperature shifts (Chanson 2004; Liu et al. 2007). In the same sense, geometry

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and morphology of coastal systems influence tidal amplitude, resulting in different emersion periods, water temperature and light exposure (Liu et al. 2007; Wolanski and Elliot 2015).

As a result of these strong environmental gradients, marine species in semi-sheltered ecosystems occur in horizontal and vertical zones according to their ability to cope with the different abiotic conditions. Abiotic gradients are therefore responsible for the structure of faunal communities, as has been described by multiple studies (Bianchi 1991; Kallianiotis et al. 2000; Massutí and Reñones 2005). For instance, the benthic diversity of more dynamic areas is usually greater and composed mainly of suspension feeding fauna (Rosenberg 1995; Thistle 2003), whereas lower current velocities promote deposit feeding organisms (Thistle 2003). Similarly, salinity and water temperature have an influence on benthic marine species composition (Sousa et al. 2006; Cochrane et al. 2009; Palmer et al. 2016), increasing its diversity within a certain range of values considered as optimal (Montagna et al. 2002; Villnäs et al. 2012). However, most of these studies were focused on free-living species (e.g. bivalves, gastropods or polychaetes) and neglected parasite species, despite their omnipresence and reputed impact on populations, communities and ecosystems (Mouritsen and Poulin 2002; Kuris et al. 2008; Torchin and Hoeg 2008; Dairain et al. 2019).

Trematodes are the most prevalent macroparasites in coastal waters (Lauckner 1983), representing a high percentage of the marine biomass (Kuris et al. 2008) with the potential to modulate host population dynamics (Thieltges 2006; Gam et al. 2009; Magalhães et al. 2015). They display a complex and heteroxenous life cycle, generally comprising three parasitic and two free-living stages (Bartoli and Gibson 2007). After hatching from an egg, the miracidium (a free-living larval stage) infects the first host (an invertebrate) by penetrating their tissues, where it metamorphoses into a sporocyst or redia. In this host, trematodes multiply asexually and emerge in the form of cercariae (a free-living stage) that actively swims in pursuit of a second host to infect, which may be an invertebrate or a vertebrate. This free-living stage has a short lifespan, in which it must ensure a successful transmission between hosts. However, cercariae are highly affected by abiotic factors (Pietroock and Marcogliese 2003). For example, cercariae survival and infection success benefit from increasing temperatures (Mouritsen 2002; de Montaudouin et al. 2016) and salinities (Koprivnikar et al. 2010; Studer and Poulin 2013), within an optimal range. Nevertheless, studies concerning the influence of hydrodynamic conditions are still very scarce (e.g. de Montaudouin et al. 1998; Waser et al. 2016), despite the planktonic host-to-host dispersal mechanism handled by cercariae. The life cycle is completed when the second host is predated by the final host, a vertebrate (fish or birds).

In the present study, cockles (host), trematodes (parasite) and the Ria de Aveiro were used as an ecosystem model to address the following main objectives: (1) To determine,

through field monitoring, whether parasite infection is related to the host tidal and/or spatial position (i.e. intertidal/subtidal and upstream/downstream, respectively) in the lagoon. Since cockles are infected by trematode parasites through their feeding activity (while submersed), the postulated hypothesis is that infection is higher in subtidal areas (more filtration and exposure to be infected) and lower in the upstream position (more unstable and adverse abiotic conditions); (2) To examine if there is an actual heterogeneity of the water body within the Ria de Aveiro, by implementing a hydrodynamic numerical model and field data (water temperature, pH, salinity, dissolved oxygen, redox potential, sediment median grain-size and organic matter content), and to what extent the variation of small-scale abiotic factors overlaps with the effects of tidal and stream position on cockle infection levels. The postulated hypothesis is that the current velocity is lower in the inner position of the lagoon (near to riverine influence), leading to more pronounced abiotic variations and consequent lower trematode infection success on cockles (correlating with the formerly enunciated hypothesis); (3) To examine the influence of water velocity on trematode infection success, following a laboratory experimental approach. The postulated hypothesis is that cockles exposed to higher water velocity (by promoting contact between cercariae and the second intermediate host) are more infected than cockles exposed to lower water velocity.

Among bivalves, cockles (*Cerastoderma edule*) were selected as the host model because of their great contribution to the benthic fauna biomass (Vaughn and Hoellein 2018), broad distribution in coastal water systems in the Northeast Atlantic coast (Tebble 1966; Honkoop et al. 2008) and high diversity of pathogens, especially trematodes (de Montaudouin et al. 2009; Longshaw and Malham 2013). The Ria de Aveiro coastal lagoon was used as a field model for its ecological importance (Sousa et al. 2013). This ecosystem is part of Natura 2000, a European network to protect and conserve areas of high biodiversity, and a LTER (Long-Term Ecological Research) site—therefore representing a valuable natural laboratory where it is possible to develop research in different scientific fields from which results can be extrapolated and applied to other coastal systems.

Material and Methods

Field Monitoring

Study Area and Sampling Design

The Ria de Aveiro is a shallow coastal lagoon located on the Northwest Atlantic coast of Portugal (40° 38' N, 8° 44' W). This lagoon is approximately 45 km long and 8.5 km wide covering a total surface of 83 km², with 17 km² of intertidal

flats (Dias et al. 2000). The Ria de Aveiro is subdivided into four main channels (S. Jacinto, Espinheiro, Ílhavo and Mira) and is supplied with freshwater by Antuã, Boco, Cáster and Vouga rivers (Lillebø et al. 2015). The average depth of the lagoon is about 1 m (Dias et al. 2000).

The present study was conducted in the S. Jacinto channel of the Ria de Aveiro. This channel is the longest and widest of the lagoon, running parallel to the coast from the mouth of the lagoon up to Ovar (northern boundary of the Ria de Aveiro lagoon). Sampling was performed in two seasons (summer July 2018, and winter February 2019), at low tide in the intertidal and subtidal (upper infralittoral) zones, and at two spatial positions/areas (downstream and upstream), in a total of eight sampling sites (Fig. 1).

Collection and Treatment of Biological Samples

Intertidal cockles (from four sampling sites) were collected by sieving the sediment of six quadrats of 0.25 m² through a 1-mm mesh. Cockle density (per m²) was determined. At the four subtidal sites (close to each of the four intertidal sites), cockles were collected with a hand dredge and cockle density (d) was calculated following the equation:

$$d(\text{ind. m}^{-2}) = \frac{n}{a \times t \times l}$$

where n corresponds to the number of cockles collected; a is the area of the hand dredge (m²); t is the number of trawls; and l is the total number of times the dredge was launched.

Shell length (SL) of cockles was measured to the nearest millimeter with a digital calliper. Then, twenty cockles of the dominant cohort were selected according to the SL distribution, dissected, squeezed between two glass slides and observed with a stereomicroscope for trematode identification. All trematodes were identified to the species level following de Montaudouin et al. (2009) identification key. Abundance (per cockle) and prevalence (percentage of infected cockles) of each parasite species were determined according to Bush et al. (1997).

Collection and Treatment of Abiotic Field Data

From each site, two sediment samples were collected from the sediment surface to perform median grain-size analysis (MGS) and to determine total organic matter content (TOM). Sediment from the subtidal samples was collected using a 0.05 m² Van Veen grab. Water temperature (°C), total pH, salinity, dissolved oxygen (DO) and redox potential (ORP) were measured in the nearest water column (intertidal sites) or in the sediment-water interface of the sites where cockles were collected (subtidal sites), using a handheld multiparameter probe (YSI Pro Plus Multiparameter Instrument). Only one measure was taken in each sampling site, at each

period, which may underestimate temporal variations. However, in these areas, the variations are presumed low in between replicates (e.g. near the downstream sampled area, salinity: 29.6 ± 0.2; temperature: 15.5 °C ± 0.06 °C; unpublished data). Sediment MGS was conducted by wet sieving the silt and clay fraction (fine particles, diameter < 0.063 mm) and dry sieving the remaining sediment fractions (sand and gravel) following Quintino et al. (1989). For TOM analysis, sediment was dried at 60 °C for 48 h and ground to powder with a mortar and a pestle. Results from TOM analyses were obtained from the difference between the dried samples (approximately 1 g) and the combusted samples (4 h at 450 °C (Kristensen and Andersen 1987)).

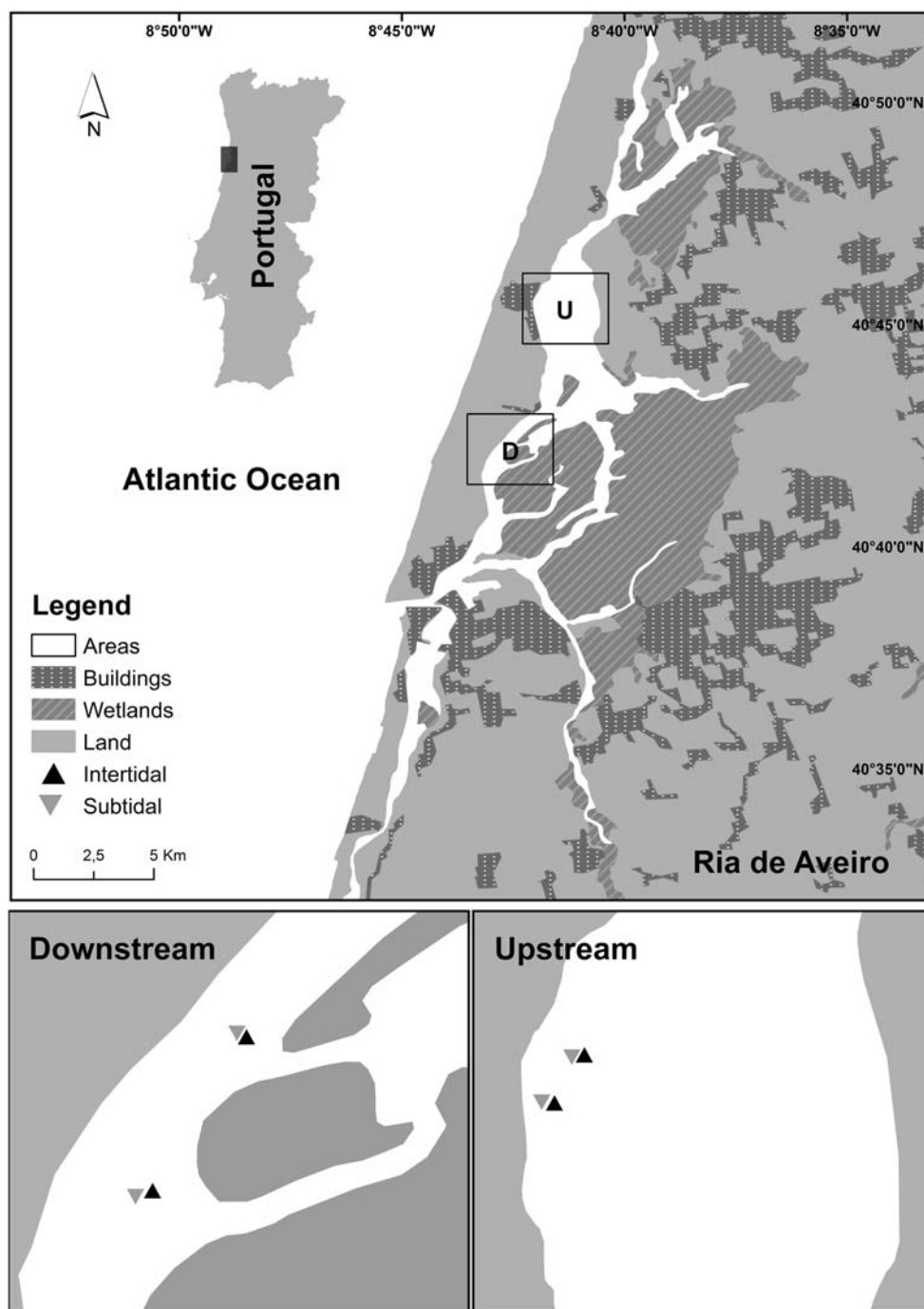
Hydrodynamic Characterization and Particle Dispersion

The MOHID water modelling system, previously implemented and calibrated for the Ria de Aveiro (Vaz et al. 2007, 2009), was used to characterize the lagoon hydrodynamics for the sampled periods (July 2018 and February 2019). MOHID is a three-dimensional model that uses a finite volume approach to perform the spatial discretization and solves the incompressible equations, assuming hydrostatic equilibrium, the Boussinesq and Reynolds approximations (for details see Martins et al. (2001)). The Ria de Aveiro was discretized with a rectangular grid with a variable spatial resolution: 40 × 40 m in the central area and 40 × 100 m in the upper lagoon (Vaz et al. 2007; Picado et al. 2013). The bathymetric dataset available for the present study was collected in a general survey carried out in 1987/1988 by the Hydrographic Institute of Portuguese Navy (IH). The bathymetry was updated for the majority of the lagoon with recent data provided by “Polis Litoral Ria de Aveiro” for the main channels of the lagoon in 2011 and by the Aveiro Harbour Administration, SA in 2012, for the inlet. One simulation per sampled period was performed in order to characterize the lagoon hydrodynamics at the time of the field surveys (July 2018 and February 2019). Maximum velocity (MV), root mean square velocity (V_{RMS}) and residual velocities (RV) were computed for the entire lagoon. Maximum velocity (MV) corresponds to the maximum absolute value determined for the current velocity during the sampled period and is used as an indicator of the local maximum transport of properties. V_{RMS} is used instead of the average since for a tidal dominated flow the net velocity is close to zero and therefore, the ebb and flood velocities have almost symmetric opposite directions. It was computed through the equation:

$$V_{\text{RMS}} = \left(\frac{1}{N} \sum_{i=0}^N V^2 \right)^{1/2}$$

where $V = \sqrt{u^2 + v^2}$ is the velocity modulus.

Fig. 1 Study area: Ria de Aveiro, Portugal. Geographical distribution of the studied sites. Sampling areas: U, upstream area; D, downstream area



Residual velocity (RV) corresponds to the mean velocity over periods of fundamental tidal frequencies, therefore representing the long-term transport of properties, and was determined over a period of 14 days, 18 h, 51 min and 24 s (multiple of $\frac{T_{M_2} \times T_{S_2}}{T_{M_2} - T_{S_2}}$ where T_{M_2} and T_{S_2} are the period of M_2 and S_2 tidal constituents, respectively). MOHID handles the covering and uncovering of boundary cells by a wetting/drying scheme, which is described in Martins et al. (2001). Therefore, the emersion time was estimated through the

analysis of the time series of sea surface height at each sampling station.

The Lagrangian model, which is a subset of the MOHID modelling system based on the hydrodynamic information, was also implemented. It uses the concept of passive particles advection, characterized by their spatial coordinate (x, y, z) . The major factors responsible by particle movement (u_p) are generally the mean velocity (u_M) provided by the model and a small-scale random fluctuation (u_F), so that $u_p = u_M + u_F$.

Taking advantage of the Lagrangian model, two particles were released, one in the upstream and other in the downstream areas at the instant of the local high tide to guarantee the submersion of the first intermediate host and the possibility of cercariae emission. Particles trajectories were tracked for 48 h, the maximum described cercariae lifetime (Sukhdeo and Sukhdeo 2004).

Laboratory Experiment

Hosts and Parasites

Parvatrema minutum are dominant trematode parasites in cockles from Aveiro (see Results) but are difficult to handle for experiments, mainly due to their very low prevalence in the first intermediate host (*Scrobicularia plana*) which are themselves fragile in microcosm conditions (Fermer 2009). Hence, *Himasthla elongata* was the trematode parasite species used in this study—also a common trematode infecting cockles in the Ria de Aveiro (e.g. this study; Magalhães et al. 2018a). This parasite uses the common periwinkle, *Littorina littorea*, as the first intermediate host and the cockle, *C. edule*, as the second intermediate host.

Cockles ranging from 17 to 20 mm SL (i.e. small cockles to minimize natural infection (Wegeberg et al. 1999, Mouritsen et al. 2003)) were collected in the Mira channel of the Ria de Aveiro coastal lagoon, Portugal (40° 38' 31.7" N, 8° 44' 10.9" W), on March 2019. A cockles' subsample ($n = 10$) was dissected and observed under a stereomicroscope for assessment of natural infection. The remaining cockles were acclimated in laboratory for a week at 30 salinity and 18 °C temperature, corresponding to the sampling area conditions (Magalhães et al. 2018a), and daily fed with Algamac Protein Plus ® at a concentration of 730 cells $\mu\text{L}^{-1} \text{day}^{-1}$ adapted from Pronker et al. (2015).

Infected periwinkles were collected in Texel, The Netherlands, and kept in laboratory in an aquarium filled with water at 35 salinity and 14 ± 1 °C and fed with *Ulva* sp. *ad libitum*, according to Magalhães et al. (2018b). These periwinkles hosted *H. elongata* sporocysts and were already successfully used in previous infection experiments (Magalhães et al. 2018b). Cercariae of *H. elongata* were collected by stimulating emission from the first intermediate host by transferring the infected snails to individual containers with water at 35 salinity and approximately 24 °C. Released cercariae were immediately (< 4 h) collected with a pipette and counted under a stereomicroscope.

Experimental Design

A current velocity laboratory experiment was carried out in two pre-designed recirculating water mini-flumes (Online Resource, Fig. 1S) at two different current surface velocities

(0.1 m s^{-1} and 0.3 m s^{-1}). Each mini-flume was composed by a main tank (1.7 m \times 0.2 m \times 0.2 m) and a reservoir (0.2 m \times 0.2 m \times 0.5 m). The water column height was approximately 0.10 m. The recirculation of the water was provided by an EHEIM 3400 universal pump located in the reservoir and entered the flume through a PVC tube of 25 mm diameter. To homogenise the current velocity inside the flume, the water entrance in the main tank was structured by two watercourses. Inside the flume, the water was kept at 30 salinity and at a temperature of 18 ± 1 °C. This temperature was controlled through a water bath at temperature 14 ± 1 °C where the reservoir was inserted. The water current was measured in the middle of the working section (approximately 0.85 m distance to the water entrance) with a JCD Flowwatch meter.

For the experiment, ten cockles of similar size per water flow/velocity treatment were transferred to the working section of the flume, a delimited area (0.15 m \times 0.18 m) by a 1-mm mesh size net, located 0.85 m from the water entrance (Online Resource, Figure 1SA). Subsequently, 500 cercariae of *H. elongata* (< 4 h lifespan) were released per flume between the bifurcation of the water entrance (Online Resource, Fig. 1S). After 48 h, the period of time needed for cercariae to encyst in the second intermediate host (de Montaudouin et al. 2016), cockles were dissected, squeezed between two glass slides and the metacercariae of *H. elongata* were identified and counted under a stereomicroscope.

Data Analysis

In order to compare the trematode metacercariae mean abundance per cockle, a three-way nested ANOVA was performed using 'season' as fixed factor (two levels: summer 2018 and winter 2019), 'sampled area' as fixed factor (two levels: downstream and upstream) nested in 'season' factor and the 'tidal position' as fixed factor (two levels: intertidal and subtidal) nested in 'sampled area' by its turn, nested in 'season'. The abundance of trematodes per cockle was $\log_{10}(y + 1)$, transformed and used as dependent variable.

To test the heterogeneity of the S. Jacinto channel of the Ria de Aveiro, comparisons among the abiotic variables (water temperature, pH, salinity, dissolved oxygen (DO), redox potential (ORP), MGS, TOM, emersion time and hydrodynamics characteristics (MV, RV and V_{RMS})) of the downstream and upstream areas of the lagoon in each season and between seasons were performed through independent samples Student's *t* test. Subsequently, a multiple linear regression was performed using the same abiotic factors (water temperature, pH, salinity, DO, ORP, MGS, TOM, emersion time and hydrodynamics characteristics) and biotic variables (cockle density and shell length) as independent variables and the $\log_{10}(y + 1)$ transformed mean trematode abundance of parasites per tidal position of each sampled area in each season as dependent variable. Multiple linear regression was used to

obtain the explanatory model of trematodes abundance variability among all environmental conditions. The data was tested for normality, but it failed this assumption. Nonetheless, multiple linear regressions are robust tests even when the normality of samples is not reached (Marôco 2011).

The infection success of the laboratory experiment was tested through a Kruskal-Wallis H test followed by post-hoc pairwise tests to compare metacercariae.cockle⁻¹ in each water flow/ velocity treatment (0.1 m s⁻¹ vs. 0.3 m s⁻¹) and naturally infected cockles.

All statistical analyses were performed using the IBM SPSS Statistics software v.25, by first testing Levene's test for equality of variances and by assuming normality.

Results

Field Monitoring

Trematode Community

The results obtained from this study showed that out of 320 dissected cockles (20 per sampling site × 2 seasons), 115 were infected by eight trematode species of four different families (Table 1). Three species infecting cockles as first intermediate host, *Bucephalus minimus* (prevalence (P) = 1.9%), *Gymnophallus choledochus* (P = 0.6%), and *Monorchis parvus* (P = 1.3%), and five species infecting cockles as second intermediate host, *Himasthla continua* (P = 8.1%), *H. elongata* (P = 20.9%), *H. interrupta* (P = 12.2%), *H. quissetensis* (P = 1.3%) and *Parvatrema minutum* (P = 19.7%). Overall, trematodes were absent at four sites (subtidal site 1 and 4 in the summer and subtidal sites 3 and 4 in the winter) while intertidal sites 1 and 2 in winter presented the highest trematode species richness—both with 6 different species.

Trematode mean abundance ranged between 0 and 477 metacercariae.cockle⁻¹, the maximum abundance observed

at intertidal site 1 in the winter. *Parvatrema minutum* was the most abundant species representing approximately 97% of the total abundance, while *H. elongata* and *H. interrupta* each represented 1.2%. *Himasthla quissetensis* was the rarest species infecting only 4 cockles and represented < 0.1% of the total abundance. Trematode mean abundance was not significantly different between seasons (F (1, 312) = 0.27; *p* = 0.66). Conversely, 'sampled area', the nested factor within each season, showed a significant difference in terms of trematode mean abundance (F (1, 312) = 24.78; *p* < 0.001), with higher values displayed in the downstream area. Trematode mean abundance of cockles living in intertidal sites was significantly higher than those in the subtidal (F (1, 312) = 18.74; *p* < 0.001) within each sampled area nested in each season, except in the upstream area in summer (F (1, 312); *p* = 0.601) (Fig. 2; Table 2).

Abiotic Characterization of the S. Jacinto Channel

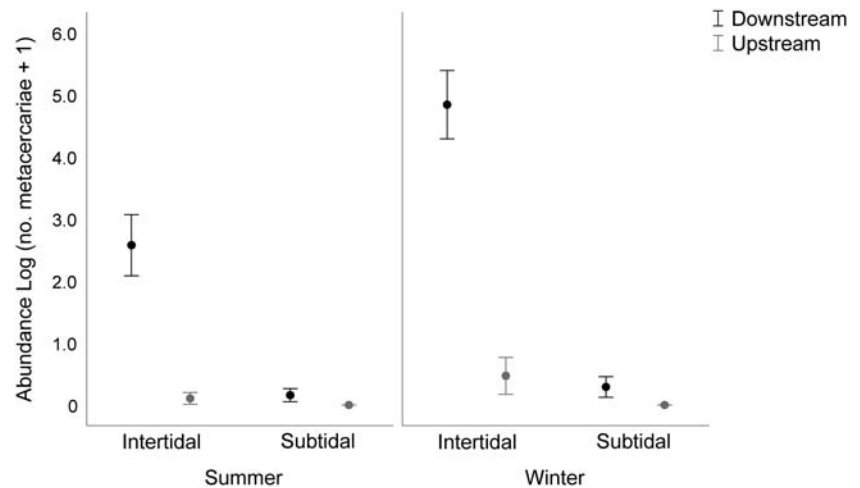
Regarding hydrodynamics, the model results demonstrated a heterogeneity along the S. Jacinto channel with increasing current velocity towards the mouth of the lagoon (Fig. 3). The downstream areas of the lagoon, in both summer 2018 and winter 2019, presented higher MV (Fig. 3a), RV (Fig. 3b) and *V*_{RMS} (Fig. 3c) compared to values obtained for the upstream areas (MV: *t*₃₀ = 26.424; *p* < 0.001; RV: *t*₃₀ = 5.935; *p* < 0.001; *V*_{RMS}: *t*₃₀ = 33.425; *p* < 0.001) of the S. Jacinto channel (Table 3). When comparing hydrodynamic characteristics between seasons, independent samples Student's *t* test showed that MV, RV and *V*_{RMS} displayed significant higher values in winter (MV: *t*_{29.9} = -2.323; *p* < 0.05; RV: *t*₃₀ = -6.383; *p* < 0.001; *V*_{RMS}: *t*_{29.1} = -5.215; *p* < 0.001) compared to summer (Table 3).

In the downstream area, the Lagrangian model (Fig. 4) showed that the particle followed an initial dispersion trajectory towards the mouth of the lagoon in the first hours; however, after 24 h, the particle was positioned around the release site. Between 27 and 31 h after release, the particle followed a

Table 1 List of the digenean trematode species found in the eight sampling sites of the present study with the indication of the hosts involved in their life cycle. Adapted from de Montaudouin et al. (2009)

Trematode species	Family	1st intermediate host	2nd intermediate host	Final host
<i>Bucephalus minimus</i>	Bucephalidae	<i>Cerastoderma edule</i>	<i>Pomatoschistus</i> spp.	<i>Dicentrarchus labrax</i>
<i>Gymnophallus choledochus</i>	Gymnophallidae	<i>Cerastoderma edule</i>	<i>Cerastoderma edule</i>	Water birds
<i>Monorchis parvus</i>	Monorchidae	<i>Cerastoderma edule</i>	<i>Cerastoderma edule</i>	<i>Diplodus</i> spp.
<i>Himasthla continua</i>	Himasthidae	<i>Peringia</i> spp.	<i>Cerastoderma edule</i>	Water birds
<i>Himasthla elongata</i>	Himasthidae	<i>Littorina littorea</i>	<i>Cerastoderma edule</i>	Water birds
<i>Himasthla interrupta</i>	Himasthidae	<i>Peringia</i> spp.	<i>Cerastoderma edule</i>	Water birds
<i>Himasthla quissetensis</i>	Himasthidae	<i>Tritia reticulata</i>	<i>Cerastoderma edule</i>	Water birds
<i>Parvatrema minutum</i>	Gymnophallidae	<i>Scrobicularia plana</i>	<i>Cerastoderma edule</i>	<i>Haemotopus ostralegus</i>

Fig. 2 Average abundance of trematodes per cockle (\pm SD) in intertidal and subtidal zones of the upstream and downstream areas sampled in summer (July) and winter (February)



rapid dispersion towards the south (mouth of the lagoon) where it remained (Fig. 4a). Concerning the upstream area, the particle also dispersed towards the south, persisting in the studied downstream area within the first 24 h. After 24 h, the particle dispersed into the inner parts of the lagoon (Fig. 4b).

Field abiotic data demonstrated that the two sampled areas (downstream and upstream) were significantly different in terms of water temperature during the winter season ($t_{158} = 5.764$; $p = 0.016$), with higher values displayed in the downstream area, whereas between seasons, water temperature was higher in summer ($t_{318} = 41.999$; $p < 0.001$) (Table 3). In the downstream area, TOM was higher during summer ($t_{158} = 11.992$; $p = 0.002$), while in the upstream area, ORP presented significant higher values during the same season ($t_{158} = -6.123$; $p < 0.001$).

Influence of Abiotic and Biotic Variables on Trematode Abundance

The multiple linear regression analysis identified salinity, dissolved oxygen (DO) and V_{RMS} as the significant main

Table 2 Three-way nested ANOVA results for $\log_{10}(y + 1)$ transformed metacercariae abundance per cockle (dependent variable). Season (summer 2018 vs. winter 2019) used as a fixed factor, sampled area (upstream vs. downstream) and tidal position (intertidal vs. subtidal) used as nested factors

	df	MS	F	p value
Season	1	7.19	0.27	0.657
Area	1	265.93	318.77	< 0.001*
Tidal position	1	284.83	341.44	< 0.001*
Season (area)	1	20.67	24.78	< 0.001*
Season (area (tidal position))	1	15.64	18.74	< 0.001*
Error	312	0.84		

*Statistically significant at the 0.05 level

predictors of trematode abundance per cockle. Salinity ($p = 0.003$), DO ($p = 0.035$) and V_{RMS} ($p = 0.002$) displayed a significant positive correlation and the following model explained 71% of the total variation of trematode abundance per cockles (Table 4):

$$\log_{10}(\text{abundance} + 1) = -6.319 + 0.090 \text{ Salinity} + 0.414 \text{ DO} + 2.310 V_{RMS}$$

The remaining variables under study (water temperature, pH, ORP, MGS, TOM, emersion time, cockle density and shell length) were excluded from the final model since these variables did not significantly influence trematode abundance.

Laboratory Experiment

Cercariae infection success was significantly different according to different water current conditions and to local natural infection ($H(30) = 16.05$; $p < 0.001$). In this experiment, cockles exposed to higher current values (0.3 m s^{-1}) presented higher infection levels (mean infection = $13.50 \pm 5.78 \text{ SD}$ metacercariae.cockle $^{-1}$, $n = 10$) compared to cockles exposed to lower water velocities (0.1 m s^{-1}) which presented $7.40 \pm 2.80 \text{ SD}$ metacercariae.cockle $^{-1}$ ($n = 10$). Naturally infected cockles demonstrated $2.6 \pm 1.75 \text{ SD}$ metacercariae.cockle $^{-1}$ ($n = 10$).

Discussion

In the present study, eight trematode species were found to infect cockles (*C. edule*) from the sampled sites and areas of the Ria de Aveiro. As commonly reported in other study areas (Arcachon bay, France (de Montaudouin et al. 2009), Exe estuary, England, (Goater 1993)), *Parvatrema minutum* was the most abundant species, representing more than 90% of total metacercariae abundance. Consequently, results

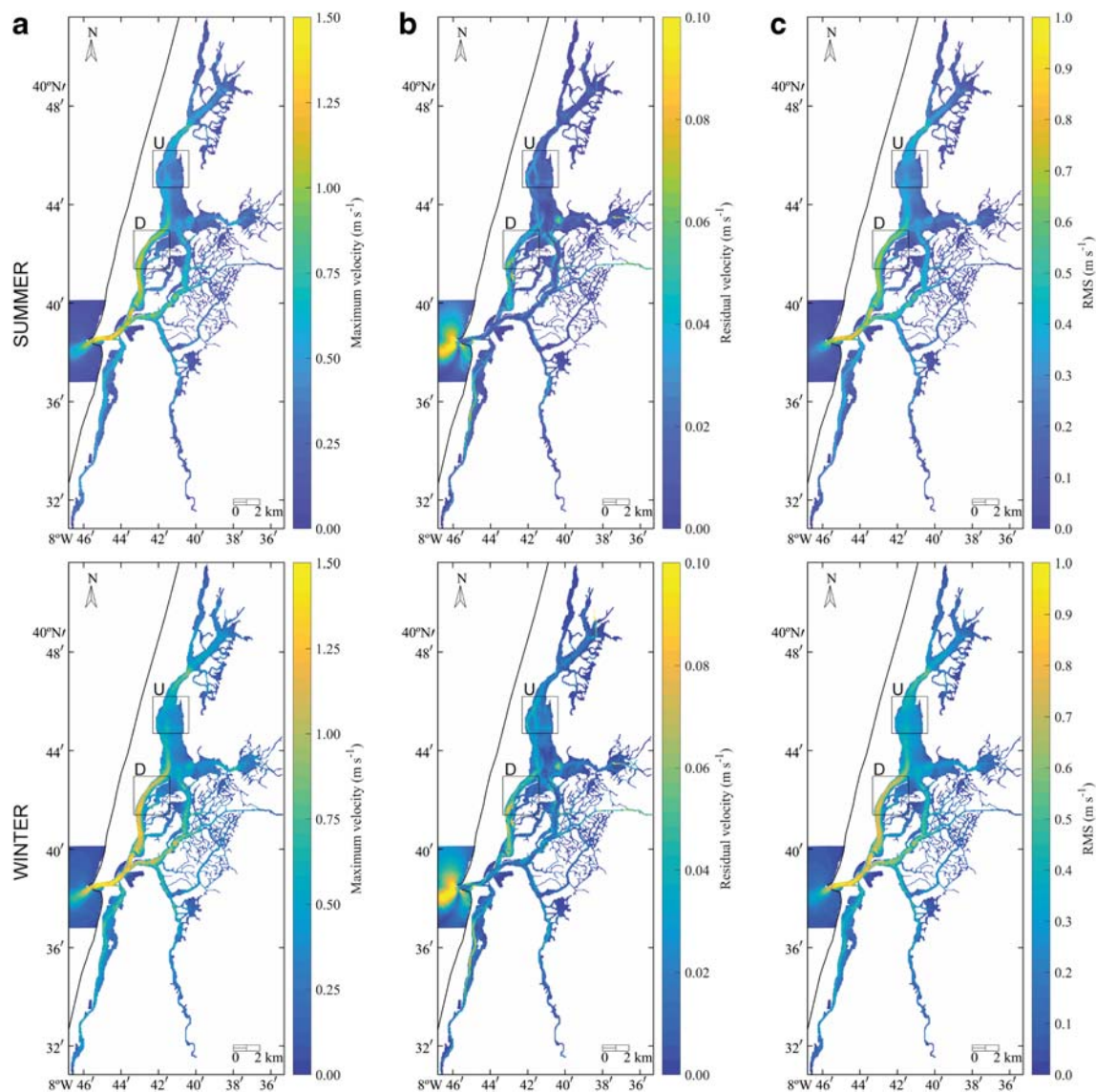


Fig. 3 Hydrodynamic characteristics of the Ria de Aveiro coastal lagoon sampling areas between 16 and 17 of July, 2018 (summer) and 21–22 of February, 2019 (winter). **a** Maximum velocity (MV); **b** Residual velocity

(RV); **c** Root mean square velocity (V_{RMS}). Sampling areas: U, upstream area; D, downstream area

concerning ‘total metacercariae abundance’ are mainly representative of *Parvatrema minutum*, but all trematode species were pooled with the assumption that cercariae behaviour was roughly similar between these species. This trematode uses the bivalve *Scrobicularia plana* as the first intermediate host which is a broadly distributed and abundant species in European and Mediterranean coastal systems (Green 1957). By inhabiting a wide range of environments, from muddy to sandy beaches (Verdelhos et al. 2005), *S. plana* promotes the abundance and presence of the trematode *P. minutum* in highly contrasting environments (Fermer et al. 2010).

There was no evidence of seasonality in species richness and metacercariae abundance per cockle. Trematode cercariae emission from the first host is usually linked to water temperature (Goater 1993; Desclaux et al. 2004). For

instance, in Arcachon bay, the emergence of parasites from the first intermediate host occurred during periods when water temperature was above 15 °C (de Montaudouin et al. 2016), leading to higher cockle infection levels (Thieltges and Rick 2006). The lack of seasonality in the Ria de Aveiro has been previously reported by Magalhães et al. (2018a) and interpreted as a result of an overall low trematode abundance in cockles, in addition to greater survival of trematodes in the metacercariae life stage, preventing the detection of temporal variations in the structure of trematode communities. The absence of seasonal infection could also be due to extreme weather events. Indeed, July 2018 (summer sampling period of the present study) was generally cold and followed by a month of unusually high precipitation (IPMA 2018), which may

Table 3 Characterization of each sampled area in terms of the salinity, dissolved oxygen (DO, mg L⁻¹), redox potential (ORP), pH, temperature (°C), organic matter content (TOM, %), sediment median grain-size (MGS, Φ), emersion time (s) and hydrodynamic factors: maximum velocity (MV), residual velocity (RV) and root mean square velocity (V_{RMS})

	Summer 2018		Winter 2019	
	Downstream	Upstream	Downstream	Upstream
Salinity	32.88	33.85	33.74	31.26
DO	6.47	6.56	7.16	6.97
ORP	184.50	246.60	152.78	158.30
pH	7.90	7.95	7.97	7.94
Temperature	22.93	20.63	13.63	12.90
TOM	1.42	1.25	0.70	0.88
MGS	2.04	1.95	1.96	1.91
Emersion time	123.75	87.50	130.00	82.50
MV	0.97	0.47	1.06	0.50
RV	0.04	0.03	0.05	0.04
V _{RMS}	0.63	0.29	0.72	0.32

have decreased salinity and consequently led to a decrease in cercariae emission (Koprivnikar et al. 2010). Conversely, February 2019 (winter sampling period of the present study) presented mild weather conditions with low precipitation (IPMA 2019) which could have promoted cercariae emission, levelling trematode abundance in cockles compared to the summer. Another plausible explanation is the relatively cold water temperatures in all

Table 4 Results of the multiple linear regression model used to find the predictors of trematodes metacercariae abundance per cockle (dependent variable, log₁₀(y + 1) transformed). DO dissolved oxygen; V_{RMS} root mean square velocity; SE standard error

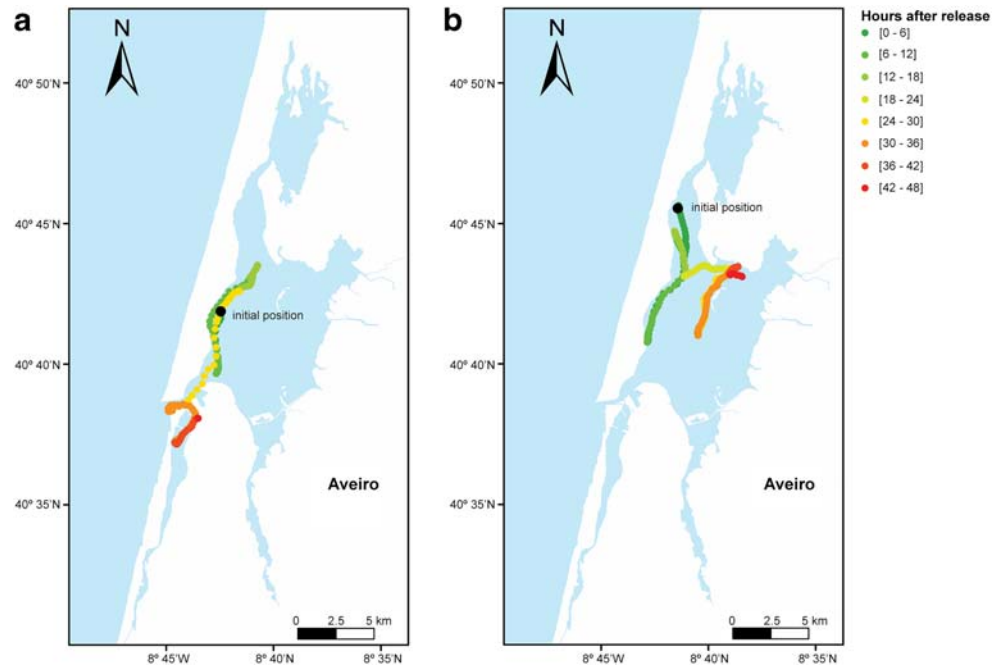
	Constant	Salinity	DO	V _{RMS}	Model
Estimate	-6.319	0.090	0.414	2.310	
SE	1.347	0.024	0.174	0.603	0.456
p value	0.001*	0.003*	0.035*	0.002*	< 0.001*
R ²					0.705

*Statistically significant at the 0.05 level

seasons, maybe related to the upwelling system in the Ria de Aveiro, that lowers trematode infection levels (Correia et al. 2020).

Surprisingly, and contrary to what was hypothesized, the prevalence and abundance of trematodes were higher in cockles from intertidal areas. The prevalence of some digenean trematode species has been previously recorded to differ with the tidal position of the host (e.g. Lim and Green 1991; Gam et al. 2008). One of the factors that can explain this variation is light exposure. In intertidal areas, light exposure and intensity are necessarily greater than in the subtidal. Kennedy (1979) experimentally demonstrated that light intensity had a positive effect on the activation and duration of the swimming capacity of cercariae of the digenean trematode *Bunodera mediovitellata*, which infects the pea cockle as first intermediate host and an insect larva as second intermediate host (*Limnephilus* sp.). Beyond light, trematodes rely on the presence of all their hosts to complete their life cycle (Bustnes

Fig. 4 Particle tracking released in (a) downstream area and (b) upstream area



and Galaktionov 1999). For example, the presence and density of the first intermediate host and the final host can explain differences between intertidal and subtidal sites in terms of diversity and abundance of trematode parasites in cockles (Hechinger and Lafferty 2005; Thieltges and Reise 2006). In the present study, the diversity and/or abundance of first hosts or water birds (final host of several trematode species) was not assessed. Nevertheless, the first hosts of the trematode species observed during this study are mainly intertidal gastropods which are found throughout the entire S. Jacinto channel, the sampled channel of the Ria de Aveiro of this study (Barroso et al. 2000). Furthermore, 173 different species of water birds (including final hosts of most of the identified trematodes in cockles) were reported in the Ria de Aveiro (Borrego 1996), considered as a hotspot of biodiversity and a habitat protected by the EU Birds Directive (79/409/CEE). Hence, in theory, subtidal cockles were more likely to be infected by free-living propagules of trematodes (due to their suspension feeding activity). Nonetheless, intertidal cockles are more attractive hosts, from the parasite perspective, due to their first host proximity. Moreover, intertidal cockles are more susceptible to be predated by water birds, the final host of several trematode species (de Montaudouin et al. 2009) (cf. Table 1), and complete the life cycle. However, no correlation between cockles emersion period and trematode abundance was found on the intertidal sites of the upstream area of the Ria de Aveiro, probably due to the general low trematode abundance in cockles.

The present study highlighted that the prevalence and abundance of a given parasite may not only be related to the final host presence and large-scale mobility (Feis et al. 2015) but is also dependent on the environment to which the parasite must quickly adapt, to ensure its transfer among hosts. In the Ria de Aveiro, the downstream area (near the lagoon's mouth) presented higher prevalence and abundance of trematodes. Outer areas (downstream) of transitional ecosystems, such as coastal lagoons, are usually characterized by higher current velocity and lower variation of salinity and water temperature due to higher oceanic proximity and influence, and display a higher abundance of trematodes (e.g. Arcachon bay, France (de Montaudouin and Lancelour 2011), Merja Zerga lagoon, Morocco (Gam et al. 2010), Northern Wadden Sea, Germany (Thieltges and Reise 2007)). Conversely, the more pronounced variations of water temperature and salinity characteristics of the inner areas in addition to the lower current velocity are likely to result into lower parasite abundance (Magalhães et al. 2018a). Indeed, the present results showed that the current velocity can be a predominant environmental factor in promoting trematode parasite abundance.

Fingerut et al. (2003) found that cercariae display effective vertical swimming in coastal systems with low current velocities, increasing the time needed to reach the bottom of the water column (where their second intermediate host inhabits)

when the current is higher. Moreover, the maximum speed of a trematode cercariae from the Echinostomatoidea superfamily (superfamily gathering 4 out of the 8 trematode species found in this study) is $\sim 3.6 \text{ mm s}^{-1}$ (Meyrowitsch et al. 1991), which is relatively low in comparison to the Ria de Aveiro current velocity determined by the MOHID model. Consequently, cercariae are likely not able to swim against the water current, tending to drift in the water column as passive particles, and carrying them away from the habitat of the first host. Indeed, simulations performed with the Lagrangian model showed that a released particle remains within the downstream area of the lagoon for at least 24 h, corresponding to the lifespan or the infective period of many trematode cercariae (Sukhdeo and Sukhdeo 2004). Conversely, in the upstream area, the particle is already far from the initial position, before reaching 12 h after release.

As expected, the water dynamics of the Ria de Aveiro were greater in the downstream position (closer to the mouth of the lagoon). These results are similar to those observed for other coastal systems (e.g. Seim et al. 2006; Iglesias et al. 2019). Hydrodynamics and coastal systems abiotic factors are interlinked (Nikora 2010). For instance, the greater current velocity of downstream areas promotes important gas exchanges in the air-water interface, increasing the concentration of important constituents in the water, such as oxygen (Broecker et al. 1980; Thomann and Mueller 1987; Wanninkhof et al. 1991). This study showed that sampled areas were heterogenous in terms of redox potential and organic matter content. Redox potential that is often associated with sediment grain-size, due to oxygen penetration rate and consequent microbial oxygen uptake (Florek and Rowe 1983), displayed higher values in the upstream position. On the other hand, organic matter correlated with sediment grain-size as well (Tyson 1995), and displayed significantly higher values in the downstream position.

The differences found among areas, especially regarding hydrodynamic features but also considering salinity and dissolved oxygen, were shown to be significant in influencing the abundance patterns of the trematode community infecting cockles. As stated before, the current velocity of the system has the ability to modulate the trematodes' life cycle, for example by affecting cercariae dispersion mode. Higher current velocities therefore promote cercariae infection success, resulting in higher infection levels in cockles. Furthermore, because there is a strong relationship between current velocity, namely ocean proximity (Lee et al. 2006), and salinity, the latter also showed a positive correlation to trematode abundance. Moreover, salinity can have a positive influence on trematode emergence from the first intermediate host (cercariae free-living stage), resulting in higher trematode abundance in the second intermediate host (Lei and Poulin 2011; Studer and Poulin 2012). Combined with current velocity and salinity, dissolved oxygen was shown to be related to higher

trematode abundance in cockles. Bivalves' filtering activity and respiration rate are enhanced by oxygen availability in the water column (e.g. Tang and Riisgard 2018), which might lead to higher trematode infection, as these parasites infect their second intermediate hosts through their feeding/respiration activity. Additionally, the emission of cercariae from the first intermediate host and their survival has been reported to severely decrease along with the non-availability of oxygen (Olivier et al. 1953; Vernberg 1963). In fact, Vernberg (1963) demonstrated that *H. quissetensis*, a trematode species that infects cockles (*C. edule*) as the second intermediate host, was partially inactive when exposed to an environment with reduced oxygen concentrations and died in the first hours after exposed to anaerobic conditions.

Previously discussed results concerning the influence of current velocity on trematode abundance were experimentally corroborated, i.e. cockles exposed to higher water velocity had a higher mean metacercariae infection than cockles exposed to the lower water velocity. Assuming that cercariae are unable to actively swim against the water velocity experienced in the flume, they would disperse, remaining in the water column for a longer period, increasing the parasite-host interactions, thus enhancing the chance to be filtrated by cockles. In the case of the flume with lower current velocity, cercariae would not have as much scattering capacity, eventually sinking and decreasing infection possibilities.

In conclusion, this study showed the influence of the host vertical and horizontal position in trematode prevalence and abundance. Cockles occurring in the intertidal position presented higher trematode abundance, probably due to life cycle facilitation (more favourable conditions for free-living stages transmission and proximity to the other hosts). Nevertheless, host-parasite interactions are complex and dependent on multifactorial processes occurring in the natural environment. More sheltered habitats with lower current velocity presented lower trematode abundance due to either a lower dispersion capacity by trematode cercariae, decreasing the chances of infection, or to lower air-water exchange that leads to a deterioration of the water quality. This study showed how current velocity could support trematode infection on second intermediate host and can be used as a baseline in understanding how current velocity alterations under climate change scenarios could influence trematode communities.

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