



Marine trematode parasites as indicators of environmental changes

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ABSTRACT

Coastal ecosystems are threatened by growing pressures related to global change. The evaluation of an ecosystem's biodiversity and status is by consequence of increasing importance to assess potentially undergone changes and predict their future trajectory for management and conservancy purposes. Biotic indicators sensitive to ecosystem changes are thus continuously sought for. Trematode parasite communities were examined as potential indicators of environmental changes, considering that the achievement of their complex life cycle is modulated by several factors. Indeed, the rule for trematode is that each species complete their cycle by a succession of three different host species and two free-living stages, thus depending on several biotic and abiotic factors. To this end, we examined the trematode community infecting the common cockle in Banc d'Arguin, Arcachon Bay, France. Monthly data extending over 16 years and sampled at a single station were compared to field book notes describing the changes of the closely surrounding landscape. In 2021, we also sampled cockles at 15 stations presenting different substrate features along the bank. Over time, seven out of nine changes of the trematode community structure presented concordances with changes in landscape. We hypothesize this was related to the environment heterogeneous substrate (*i.e.*, temporal succession of oyster parks, bare sands and seagrass) with cascading effects on host populations. However, some changes could not solely be explained by changes in landscape. Our spatial study showed that the trematode communities exhibited intricate infection patterns with a complex interaction between substrate heterogeneity and larval dispersal ability of parasites. Thus, trematode communities might be potential indicators of subtle changes in the environment. However, it remains unclear which scale of environmental changes trematodes are actually sensitive to.

1. Introduction

Oceans are facing unprecedented challenges leading vital habitats and species to high risk of extinction. Human-induced global change is presenting numerous threats to marine ecosystems such as climate change (Greene and Pershing, 2007), overexploitation of marine resources (Coll et al., 2008), habitat fragmentation and destruction (Gibson et al., 2007), pollution (Beman et al., 2005) and introduction of invasive species (Bax et al., 2003). These pressures affect marine ecosystems in ways we do not fully understand to this day. Indeed, combined with the complex dynamics which rule marine ecosystems, an accurate evaluation of an ecosystem's biodiversity and state is a particularly difficult task. However it is also a highly valuable task to assess the changes it might have undergone and predict the future trajectory it will follow, hereby representing a useful tool for management and conservation (Rice and Rochet, 2005; Jørgensen et al., 2016). To do so, indicator taxa can be used. Typically, these are groups of species

whose ecological traits or diversity are closely linked to those of other taxa, potentially more difficult to study (Olsgard et al., 2003; Hechinger and Lafferty, 2005).

More recently, European directives such as the Water Framework Directive (WFD) or the Marine Strategy Framework Directive (MSFD) required European states to maintain their marine waters in "good ecological status". Different indicators were considered in order to fuel this questioning, among them the structure of macrozoobenthic communities (Borja et al., 2010). Due to their intrinsic characteristics (relatively long lifespan, reduced motility), benthic organisms appear as good indicators of the environment (Dauvin, 1993), although they are often more influenced by the sediment characteristics than by the ecological quality of the surrounding water body, at least in (semi-) sheltered marine ecosystems (Lavesque et al., 2009). As a result and a complementary approach, parasites can be taken into consideration, more specifically those parasitic species which are linked to multiple hosts and with free-larval stages, like trematodes (Mouritsen and Poulin,

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2002; Hechinger and Lafferty, 2005; Hudson et al., 2006; Hechinger et al., 2007). Most dominant metazoan group of macroparasites in coastal waters (Lauckner, 1983; Schmidt et al., 2000), trematodes have a complex life cycle composed of two or three hosts. Marine adult trematodes sexually reproduce and spawn their eggs in the final host, often marine birds or fish. Eggs are released in the environment by the final hosts' feces and shed to develop into free-living miracidia larvae. Latter will infect a mollusc, most commonly a gastropod, as first intermediate host. As a sporocyst or redia at this stage, it will, once mature, release cercariae larvae in the environment. This second free-living form will rapidly infect the second intermediate host, an invertebrate or vertebrate species, and encyst specific tissues as a metacercaria. Finally, the second intermediate host will in turn be eaten by the final host, in which the parasite will develop into an adult and thus have completed its cycle. Throughout this cycle, various biotic and abiotic factors act as drivers on parasite success which will directly impact their distribution. To name a few, hydrodynamics (de Montaudouin et al., 1998), temperature (de Montaudouin et al., 2016), diversity of host species (Thieltges and Reise, 2007) and target host density (Magalhães et al., 2017) are important drivers underlying trematode dynamics such as infection temporality. Thus, we hypothesize that infection success will be particularly sensitive to environmental changes, and that subsequent parasite community structure in their hosts could alert on global change.

Bivalves can act as first but more often as second intermediate host or as both, as does the common cockle *Cerastoderma edule* (Lauckner, 1983; Schmidt et al., 2000). Europe's main wild-harvested bivalve species *C. edule* is recognized as a key species due to its ecosystem engineering abilities, creating, modifying and maintaining habitat for other species (Philippart et al., 2007; Sousa et al., 2009; Carss et al., 2020) and offers many ecosystem services including biodiversity support, carbon sequestration, nutrient removal and an important connection between trophic levels (Morgan et al., 2013; Rakotomalala et al., 2015; Carss et al., 2020). Cockles spawn from May to July/August when mean sea temperatures are around 13 °C (Boyden, 1970). The planktonic larvae resulting from the fertilized eggs live for two to five weeks. After settlement, a cohort can live up to 40 months and individuals may reach up to 50 mm in shell length (Magalhães et al., 2016). *Cerastoderma edule* is a dominant species in coastal waters which can be found along the north-eastern Atlantic coast, from the Barents Sea to Mauritania (Tebble, 1966; Honkoop et al., 2008; de Montaudouin et al., 2009). It can host a diverse parasite community with up to 16 trematode parasite species (de Montaudouin et al., 2009, 2021).

The present work aims to provide new insights to the potential of trematode communities as indicators of environmental changes. To do so, we examined the trematode community of *C. edule* according to temporal and spatial changes in landscape. In a first approach, "landscape" will be defined as the global environment, discriminating bare sand beach, oyster farming presence and seagrass beds. Data from a long-term monitoring campaign (1997–2013) allowed us to observe changes in the composition and structure of the trematode community, at one given bare sand station, in the National Reserve of Banc d'Arguin on the southwestern Atlantic coast of France, and to assess its relationship with the observed evolution of the surrounding environment ("landscape"). In complement to this temporal approach, the trematode community was assessed at multiple stations of this tidal flat, exhibiting different environmental features to offer supplementary elements for the understanding of trematode dynamics and how they reflect environmental changes. Our main hypothesis is that a change of the surrounding environment, at spatial or temporal scale, will beget a change of the structure of trematode communities in host subpopulations (*i.e.*, population at the scale of one station) of cockles.

2. Materials and methods

2.1. Study area

Sampling took place on Banc d'Arguin (44.60°N, 1.25°W), Arcachon Bay, France. Arcachon Bay is a 182-km² macrotidal lagoon situated on the southwestern Atlantic coast of France. It is connected to the Atlantic Ocean by a 24-km² wide channel where Banc d'Arguin is located (Fig. 1). This 4 km long and 2 km wide sand bank at low tide is a National Nature Reserve which includes sand dunes and semi-sheltered sandflats. The cockle population is distributed along the intertidal semi-sheltered part of the bank. The sediment is largely dominated by medium sands (median grain-size = 350 µm), salinity is high year-round (32–35, Robert et al., 1987) and surface sediment temperature in the intertidal varies seasonally between −0.2 °C and 30 °C (Dang et al., 2010). Banc d'Arguin is an area where various marine bird species winter, nest or rest during migrations and a diversity of fish species inhabits the surrounding waters. It hereby offers a variety of potential hosts for the trematode parasites.

2.2. Long-term monitoring of the trematode community

2.2.1. Sampling procedure

Cockles were collected monthly between October 1997 and October 2018 at a unique site located on an intertidal flat on the eastern side of Banc d'Arguin (Fig. 1). Six quadrats of 0.25-m² were sampled on bare sand and the sediment sieved through a 1-mm mesh. When cockles were present, their shell length was measured with a caliper at the least mm. Ten cockles per cohort (*i.e.*, age class) were dissected and squeezed between two glass slides for trematode observation under a stereomicroscope. Trematodes were identified to species level according to the identification key established by de Montaudouin et al. (2009). Metacercariae were counted to assess specific parasite abundances. For parasite species using the cockle as first intermediate host, it is impossible and irrelevant to count parasite entities (sporocysts and/or cercariae).

2.2.2. Data analyses

The original dataset was made up of 4,586 dissected cockles of shell lengths ranging from 1 to 39 mm. A positive relationship between cockle length, proxy of cockle age to a certain extent, and number of trematode metacercariae was shown (Thieltges, 2008). Cockles of small size are commonly poorly infested and exhibit low metacercariae abundances, whatever the year or the site is. To remove this size effect on the parasite diversity and abundance, we neglected cockles of shell lengths < 15 mm (Magalhães et al., 2015, 2020; Miura et al., 2019). Sampling dates at which less than ten dissected cockles remained were also removed from the dataset to homogenize it for upcoming calculations.

Even with cockles of shell length > 15 mm, there could be a size effect related to the fact that the older the cockles, the higher the probability is for them to be infected (interaction time, filtration rate, tissues space). In order to reduce this effect, we examined the relationship between cockle length and total abundance of metacercariae in our dataset (Fig. 2). A Spearman correlation test showed there was a significant nonlinear monotone relationship between the two variables. Least-squares analysis was used to fit a nonlinear model to our data. Abundances were thus weighted via the following established relationship between cockle shell length (*L*, in mm) and total abundances of metacercariae per cockle, (*Ab*): $Ab = 0.0025 \times L^{3.3647}$. Abundances of metacercariae per cockle were then brought back to a hypothetical standard sized cockle shell of 20 mm. From these weighted abundances, median metacercariae abundances were calculated per sampling date. The outgoing dataset hereby consisted in a [183 months × 7 species] time series ranging from October 1997 to May 2013.

Next, the structure of the parasite community was explored to identify potential changes in time. Non-metrical multidimensional

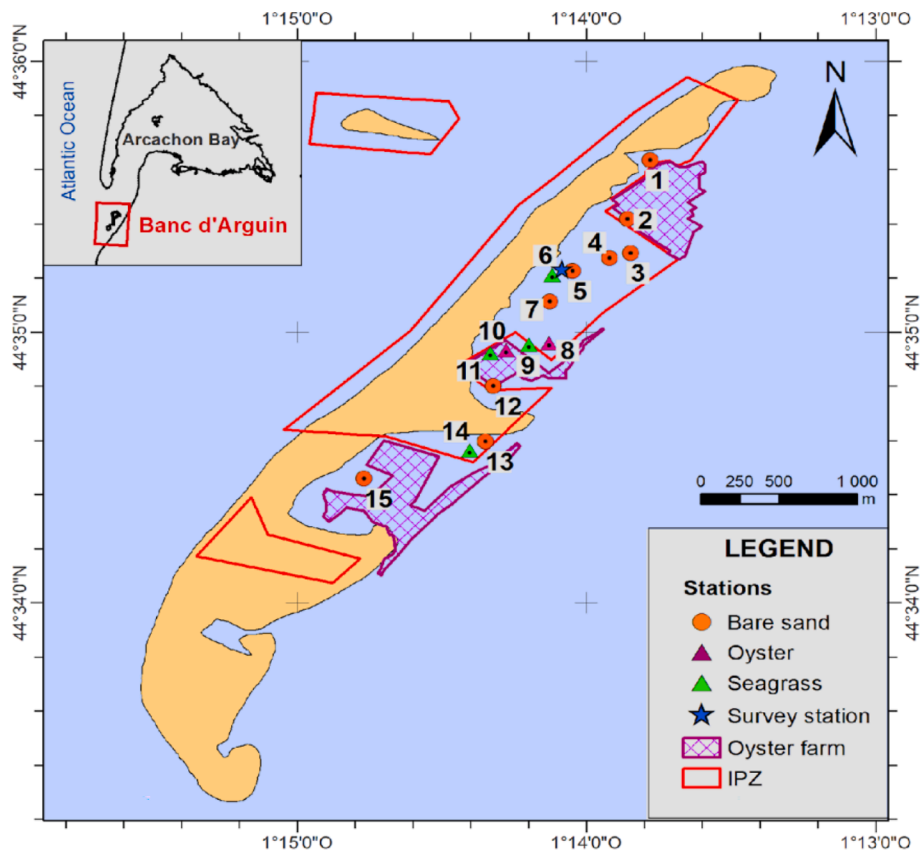


Fig. 1. Banc d'Arguin and location of the 16 sampling stations. Fifteen stations presenting either substrate homogeneity (circle), i.e., bare sand, or substrate heterogeneity (triangle), i.e., presence of oysters or seagrass (*Zostera noltei*), and the long-term survey station (blue star). IPZ: Integral Protection Zone.

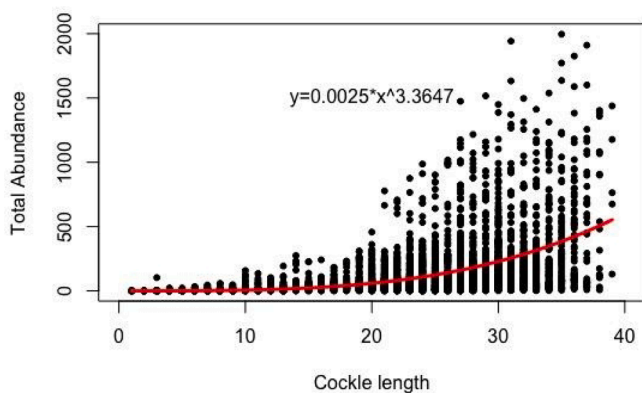


Fig. 2. Relationship between total abundance of metacercariae per cockle and cockle shell length (mm). Red line: non-linear regression following the equation: Total abundance of metacercariae = $0.0025 \times \text{shell length}^{3.3647}$.

scaling (n-MDS) and hierarchical cluster analysis (HCA) were used in combination to identify groups of years with similar parasite assemblages. The four main species were more or less constantly present during the entire period of time considered. We consequently expected specific abundances to be more sensitive to structural community changes than species richness. Hence no transformation was applied to parasite data in order to conserve the strong fluctuations in specific abundances. A resemblance matrix based on Bray-Curtis dissimilarities, well adapted to faunal data, was used to conduct the n-MDS and HCA. Cophenetic distances were computed to determine the best hierarchical clustering method. The average linkage (UPGMA) method was consequently used as it resulted in the highest correlation between the

original distances and the cophenetic distances. Groups were finally identified by cutting the resulting HCA-dendrogram at a resemblance level of 50 %. Field book notes, written between 1992 and 2013 (and referenced herein as de Montaudouin, pers. comm.), describing the kind of landscape (that is general landscape around (10–50 m) to the sampling station, e.g., bare sand beach, seagrass bed, oyster parks) and the dominant molluscs, were examined and compared with changes in parasite communities. Lastly, associations between parasite species were assessed by conducting a probabilistic species co-occurrence analysis (Veech, 2013).

Graphs and statistics were done using PRIMER v6 (Clarke and Gorley, 2006) and R 3.5.0 (R Core Team, 2020). Following R packages were used: dplyr (Wickham et al., 2021), nlshelper (Duursma, 2017), tibble (Müller and Wickham, 2021), lubridate (Grolemund and Wickham, 2011), reshape2 (Wickham, 2007), vegan (Oksanen et al., 2019), ggplot2 (Wickham, 2016), ggpubr (Kassambara, 2020), cowplot (Wilke, 2020), gridExtra (Auguie, 2017) and cooccur (Griffith et al., 2016).

2.3. Spatial monitoring of the trematode community

To examine the spatial variation of the trematode parasite community in cockles, sampling was carried out at 15 stations spread out on a latitudinal gradient along the intertidal flat of the Eastern side of Banc d'Arguin (Fig. 1). The stations were located in different types of environments. Stations 1, 2, 3, 4, 5, 7, 12, 13 and 15 consisted of bare sands, representing homogeneous substrate. Stations 8 and 10 were located in areas characterized by the presence of wild oysters, while stations 6, 9, 11 and 14 consisted of *Zostera noltei* seagrass beds, which, given the season (winter), exhibited low density of leaves. We considered these two latter sets of stations (6, 8, 9, 10, 11 and 14) as exhibiting substrate heterogeneity. Three sampling campaigns were carried out in winter 2021, before the period of cockle infection by trematode cercariae

(Thieltges and Rick 2006; Fermer et al., 2010; de Montaudouin et al., 2016).

2.3.1. Sampling procedure

2.3.1.1. Grain-size. The top layer (ca. 3 cm) of the sediment was sampled at each station on January 18, 2021. Sediment grain-size characteristics (median grain-size, percentage of silt and clay (% < 63 μm) were assessed after sieving pre-weighed dried sediment (desiccated at 60 °C for 48 h) through a wet column of sieves with decreasing apertures (1000, 500, 250, 125 and 63 μm). Percentage of organic matter in the sediment was determined after calcination (at 450 °C for 4 h) of a desiccated sediment sample. Calculations were carried out with GRA-DISTAT (Blott and Pye, 2001).

2.3.1.2. Cockle sampling. Sampling took place on February 25 and 26, 2021. Fifteen cockles from similar age-class (cohort 2019) were collected at each station by searching the top layer of the sediment by hand. Cockles were dissected, trematode parasites identified and counted following the same method as for the long-term monitoring. On occasion, samples were sent to the molecular biology platform to confirm trematode species identification. With fifteen cockles per station, a total of 225 cockles were hereby dissected and examined.

2.3.2. Data analyses

Three multivariate analyses were applied to the collected data: non-metrical multidimensional scaling (n-MDS), hierarchical cluster analysis (HCA) and SIMPER. n-MDS and HCA were used in combination to identify groups of stations based on their trematode parasite communities. Analysis of similarity percentage (SIMPER, Clarke, 1993) was used to assess which parasite species contributed most to observed differences in community composition and structure. Data consisted of a [7 species \times 15 stations] matrix of median abundances. The different species being mostly ubiquitous over all of the sampling area, no transformation was applied in order to conserve the strong fluctuations in specific abundances, as it was done for the chronological dataset. n-MDS and HCA were applied on Bray-Curtis dissimilarities matrices. Inspection of the cophenetic distances lead to applying the average linkage method (UPGMA) for the HCA. SIMPER was applied on the transformed or raw datasets. The three analyses were conducted on PRIMER (Clarke and Gorley, 2006).

Moreover, we searched for potential correlations between the identified groups of stations based on their parasite community structure and seven potential explanatory factors: (1) substrate heterogeneity, (2) in/out of the integral protection zone (IPZ), (3) cockle length (mm), (4) cockle sex, (5) median grain-size (μm), (6) proximity to oyster farm (m) and (7) station grouping according to geographical area. For the latter qualitative factor, stations were categorized in four groups according to their geographical/topographical position on the Banc d'Arguin: North (stations 1–4), Center North (5–7), Center South (8–12) and South (13–15). Analyses of similarities (ANOSIM, Clarke, 1993) were conducted for the four qualitative factors, i.e., factors 1, 2, 4 and 7 (using Bray-Curtis dissimilarities and 999,999 permutations), Mantel tests for the three quantitative factors, i.e., factors 3, 5 and 6, (using Euclidean distances and 999,999 permutations). These statistical tests were done with R 3.5.0 (R Core Team, 2020) using the vegan (Oksanen et al., 2019) and ade4 (Dray and Dufour, 2007) packages.

3. Results

3.1. Long-term monitoring of the trematode community

3.1.1. Evolution of trematode community from 1997 to 2013

For 183 sampling dates encompassing a total of 4,084 dissected cockles, ten trematode species belonging to five different families were

found infecting the common cockle *Cerastoderma edule* on Banc d'Arguin from 1997 to 2013. These species presented different life cycles which can include at least seven different benthic mollusc species as first intermediate hosts, three second intermediate host species as well as a variety of fish and water birds as final hosts (Table 1).

Median total abundance ranged from 0 to 187 metacercariae per “standardized” cockle (after size effect correction) with an average of 36 metacercariae/cockle over the entire period. Species richness showed variations in time with total absence of parasites in only 0.3 % of cockles and all ten species present simultaneously in 15 % of the 4,084 sampled cockles. The following results were achieved for the seven species using *C. edule* as second intermediate host. Present as metacercariae, abundances could hereby be assessed, in opposition to the three remaining species present as sporocysts in the cockle. The evolution of species-specific median abundances of metacercariae per cockle revealed strong fluctuations over time with temporal dynamics that differed from one species to another (Fig. 3). *Himastha quissetensis*, *Himastha interrupta*, *Gymnophallus minutus* and *Curtuteria arguinae* were the four dominant parasite species as they were present in the great majority of cockles (≥ 73 %) with moderate to high abundances. These four species still presented large fluctuations in metacercariae counts over time. Indeed, *H. quissetensis* experienced particularly high abundances during the years 2000, 2001, 2002 (median of 11–14 metacercariae/cockle). *H. interrupta* abundances peaked in 2004 with a median of 24 metacercariae/cockle, whereas *G. minutus* experienced the highest count in 2005 and 2006 with a maximum median abundance of 72 metacercariae/cockle. *C. arguinae* presented two more modest abundance peaks in 2003 and 2009 (respectively 18 and 19 metacercariae/cockle). The presence of the remaining three species, *Psilostomum brevicolle*, *Renicola roscovitus* and *Diptherostomum brusinae*, was much more unstable in time. Exhibiting sizably lower prevalence levels (respectively 51, 37, 43 %), these species also presented fluctuating abundances that varied considerably less compared to the four main species. *R. roscovitus* was present sporadically for limited periods, that is from 1997 to 2000 and again from 2009 to 2013, *P. brevicolle* showed an abundance peak in 2002, while *D. brusinae* was present in 2001 and 2002 and again in 2006. Towards the end of the monitoring period, more specifically in 2012 and 2013, a noteworthy increase in abundances can generally be observed for six out of the seven trematode species (not for *G. minutus*).

The n-MDS analysis (Fig. 4) showed differences in the composition of parasite assemblages in time. The low stress-value (<0.2) ensured a decent 2-dimensional representation. Seven groups of years could be distinguished at a similarity of 50 %, meaning that the parasite community at the sampling station exhibited seven different structures from 1997 to 2013. When two consecutive years were not grouped together, it could indicate that the parasite community underwent a change in its structure. Ten different periods could thus be identified and compared to field book notes which reported some major local environmental changes herein named “landscape” (Table 2, Fig. 5).

3.1.2. Species co-occurrence

The probabilistic species co-occurrence analysis resulted in 52 % of non-random species associations. Seven out of the 10 considered species presented at least one non-random association with another species (Fig. 6), with only positive interactions revealed. *D. brusinae* significantly co-occurred with all trematode species. In addition to these associations, *P. brevicolle* was positively associated with *C. arguinae*, *H. interrupta* and *G. minutus*, whereas *R. roscovitus* co-occurred with *C. arguinae*. All seven species using the cockle as second intermediate host were positively linked to at least one other species of the trematode community.

3.2. Spatial monitoring of the trematode community

3.2.1. Sedimentary environment

Analyses of surface sediment samples at each station revealed that

Table 1

Trematode species which appeared in this study (both temporal and spatial surveys) and their life-cycle.

	Family	Species	1st intermediate host	2nd intermediate host	Definitive host
Cockles as 1st intermediate host	Bucephalidae	<i>Bucephalus minimus</i>	<i>Cerastoderma edule</i>	<i>Pomatoschistus</i> spp.	<i>Dicentrarchus labrax</i>
	Gymnophallidae	<i>Gymnophallus choledocus</i>	<i>C. edule</i>	<i>Mugil cephalus</i>	Water birds
	Monorchidae	<i>Monorchis parvus</i>	<i>C. edule</i>	<i>C. edule</i>	<i>Diplopus</i> spp.
Cockles as 2nd intermediate host	Echinostomatidae	<i>Himasthla quissetensis</i>	<i>Tritia reticulata</i> <i>Tritia neritea</i>	<i>C. edule</i>	<i>Larus argentatus</i>
	Echinostomatidae	<i>Himasthla interrupta</i>	<i>Peringia ulvae</i>	<i>C. edule</i>	Laridae
	Echinostomatidae	<i>Curtuteria arguinae</i>	Unknown	<i>C. edule</i>	Unknown
	Gymnophallidae	<i>Gymnophallus minutus</i>	<i>Scrobicularia plana</i>	<i>C. edule</i>	<i>Haematopus ostralegus</i>
	Echinostomatidae	<i>Psilostomum brevicolle</i>	<i>Peringia ulvae</i>	<i>C. edule</i>	Laridae
	Echinostomatidae	<i>Diptherostomum brusinae</i>	<i>Tritia reticulata</i>	<i>C. edule</i>	Fishes
	Renicolidae	<i>Renicola roscovitus</i>	<i>Littorina littorea</i>	<i>C. edule</i>	Laridae
	Unknown	Metacercaria sp.	Unknown	<i>C. edule</i>	Unknown

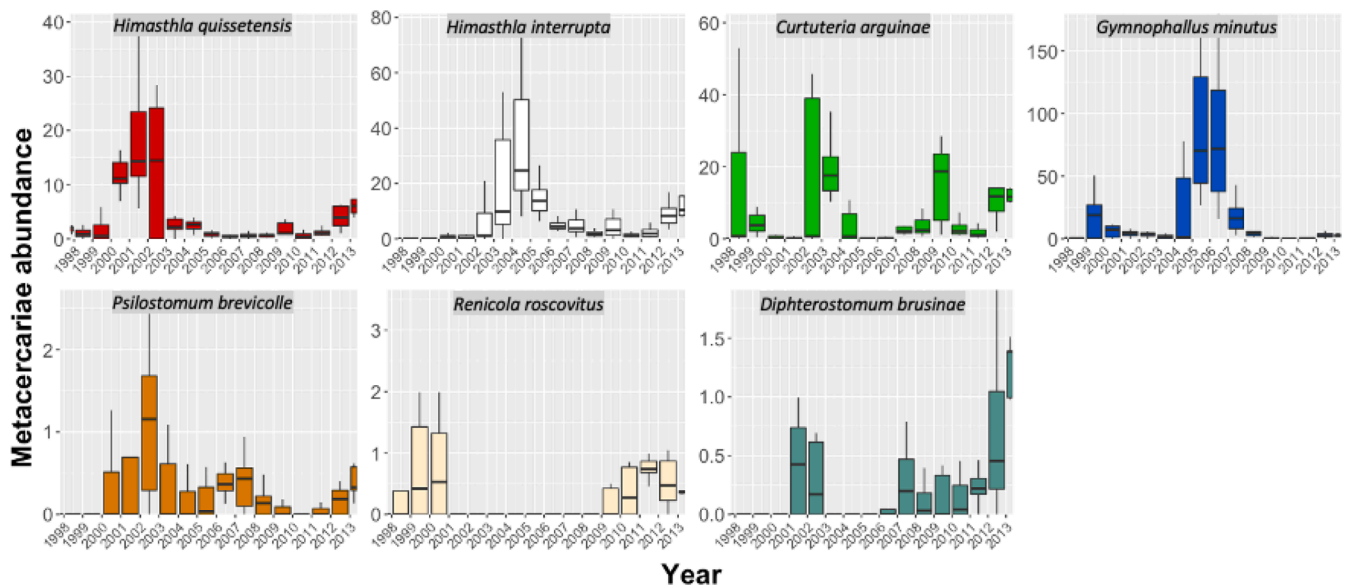


Fig. 3. Median abundance of the metacercariae (weighted) per trematode species and per standardized cockle from 1997 to 2013.

the general sedimentary environment of Banc d'Arguin in winter 2021 was dominated by medium, well sorted sands (median grain-size of 288 μ m). All stations (except station 11) exhibited a median grain-size of 289 μ m \pm 11 with relatively small portions of silt and clay (1.9 to 20.4 %) and low organic material contents (0.3 to 3.0 %) (Table 3). However, one station severely deviated from these values. Indeed, station 11 showed an exceptionally low sediment median (21 μ m) with high portions of silt and clay (82 %) and organic material (8.7 %).

3.2.2. Trematode assemblages

A total of 44,186 metacercariae were counted and identified as belonging to nine different trematode species. *Gymnophallus minutus* was by far the most abundant species in terms of both prevalence (96 % of sampled cockles were infected by it) and abundances (highest observed intensity in one cockle: 2,368 metacercariae) (Table 4).

Overall, five of the nine species, namely *G. minutus*, *C. arguinae*, *D. brusinae*, *H. quissetensis*, *H. interrupta* exhibited high prevalences as they had infected over two thirds of the cockles (prevalence \geq 72 %). The "unknown species" was a rather rare and unimportant species infecting 5 % of the cockles. It presented large metacercariae with approximately 350–450 μ m diameter which were mostly located in or near the digestive gland. *B. minimus* and *M. parvus* which use the cockle as first intermediate host showed very low prevalence levels (respectively 4 % and 1 %). The non-parametric multidimensional scaling

analysis (n-MDS) showed differences in the composition of trematode communities according to the sampling station (Fig. 7). The low stress-value (<0.2) ensured a decent 2-dimensional representation. Four groups of stations could hereby be distinguished (Fig. 7, Table 5). Group I consisted of two stations located on bare sand (3 and 4). These stations displayed the lowest species richness and total abundances. A median of 5 species were identified for a median total abundance of 7 metacercariae at each station. The SIMPER analysis revealed that these two stations were characterized by remarkably low *G. minutus* abundances (100 % contribution with a median of 3 metacercariae/cockle), thus dissociating them from the other sampling stations. Group II encompassed four stations (9, 10, 11 and 12) located in all three different observed environments with different substrate heterogeneity features. These stations were dominated by *G. minutus* and *C. arguinae* which were particularly abundant (269 and 102 metacercariae/cockle). Indeed, the two species contributed to 77 % and 19 % of the group's differentiation with the other groups. It was characterized by a very abundant (550 metacercariae/cockle) and rich (7 species) yet uneven trematode community. Group III was made up of stations 1 and 14 which were respectively located in bare sands and seagrass patches. Composed of 6 trematode species contributing to a median total abundance of 69 metacercariae, this group was strongly dominated by *G. minutus* (12 metacercariae/cockle) but more importantly by *C. arguinae* (50.5 metacercariae/cockle) which contributed to 89 % of the differences between

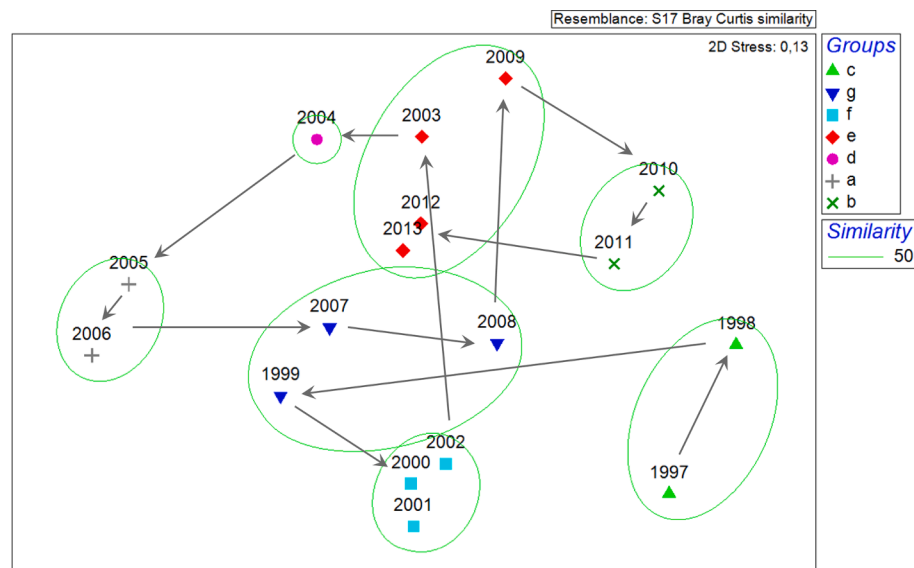


Fig. 4. Results of n-MDS analysis based on median trematode species abundances (weighted and untransformed) per cockle per year. The green circles indicate the station grouping revealed by the cluster analysis when setting the cut at a similarity level of 50%. Arrows are meant to help visualize the transition from one year to the next (or tightly packed groups of years).

Table 2

Comparison of trematode data between the groups of years determined by n-MDS and cluster analysis. SR: species richness. For group correspondence, see Fig. 4.

Years	Field notes	Median SR	Median total abundance	Dominance	nMDS group
1997–1998	Proximity of oyster parks	3	2	<i>H. quissetensis</i>	c
1999	Storm, new station (100 m aside) close to oyster parks	4	24	<i>G. minutus</i> <i>C. arguinae</i>	g
2000–2002	Proximity of oyster parks	5	20	<i>H. quissetensis</i>	f
2003	Oyster park dismantling	4	24	<i>C. arguinae</i> <i>H. interrupta</i>	e
2004	Silt and clay enhancement	4	29	<i>H. interrupta</i>	d
2005–2006	<i>Z. noltei</i> settlement and expansion	5	81	<i>G. minutus</i>	a
2007–2008	<i>Z. noltei</i> bed expansion	6	16	<i>G. minutus</i> <i>C. arguinae</i>	g
2009	<i>Z. noltei</i> bed expansion	5	23	<i>C. arguinae</i> <i>H. interrupta</i>	e
2010–2011	Max density followed by disappearance of <i>Z. noltei</i> bed	5	5	<i>C. arguinae</i>	b
2012–2013	<i>Z. noltei</i> disappeared: bare sand beach	7	30	<i>C. arguinae</i> <i>H. interrupta</i>	e

this group and the remaining. Group IV consisted in seven stations presenting different substrate and environmental features (stations 2, 5, 6, 7, 8, 13, 15). While the median species richness was high, metacercariae were moderately abundant (58 metacercariae/cockle). These stations were characterized by low *C. arguinae* (6 metacercariae/cockle) and moderate *G. minutus* (35 metacercariae/cockle) abundances as well as the highest median count in *H. interrupta* (8 metacercariae/cockle). Accounting for respectively 67 %, 12 % and 9 %, these species were responsible for forming this separate group of stations. The SIMPER analysis confirmed that the four most abundant species, i.e., *G. minutus*, *C. arguinae*, *H. quissetensis* and *H. interrupta*, which mainly characterized the four groups of stations, were consequently responsible for the major differences between groups.

3.2.3. Factors explaining the spatial variability in the trematode community assemblages

The trematode community matrix appeared to be significantly and strongly correlated ($p < 0.001$ and $R = 57\%$) to only one of the seven tested factors, the geographical area of the Banc d'Arguin in which a given sampling station was located.

3.2.4. Species co-occurrence

The co-occurrence analysis revealed no non-random trematode species associations.

4. Discussion

4.1. Temporal variability of the trematode community

The trematode community was not stable over time and underwent nine structural changes with different species sequentially dominating the community. When comparing the temporality of these changes to the observed evolution of the sampling station's general landscape, concordances do appear (de Montaudouin, pers. comm.). Indeed, the six observed landscape changes were accompanied by changes in the trematode community structure.

In 1997, when the cockle/trematode monitoring started, the sampling site was in medium sands, but few meters from oyster parks. *Himasthla quissetensis* dominated the community. This correlated well with the landscape at the time as the species' first intermediate host, the netted dog whelk (*Tritia reticulata*), a mobile scavenger strongly attracted by such environments rich in living or dying organic tissue provided by the high oyster biomass (Blanchet, 2004), was relatively abundant during that time with 42 ind.m^{-2} until 2001.

The storm of January 1999 led to a small change in location (ca. 100 m), as cockles were buried by sediment. The environment thus changed slightly, which coincided with a change in structure for the trematode community. The community drastically changed from a low diversity and abundance community to one strongly dominated by *Gymnophallus minutus* and a total abundance over 10 times greater. The oyster park dismantling starting in 2002 was also accompanied by a change in the parasite community one year later. The netted dog whelk population

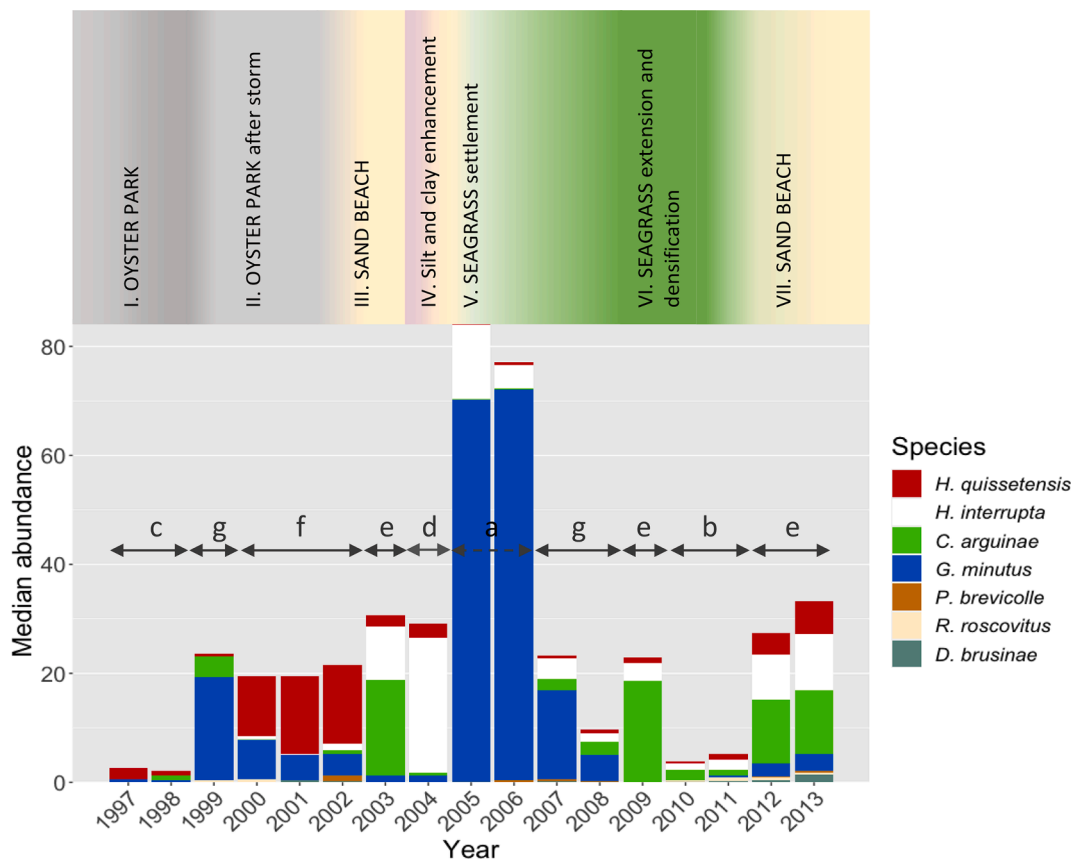


Fig. 5. Median abundance per year of each of the seven trematode species present as metacercariae infecting cockles from 1997 to 2013. The different community structures and periods identified previously are indicated by black arrows (see Table 2 for the letters' signification). The changes of environment are indicated in the upper part of the figure.

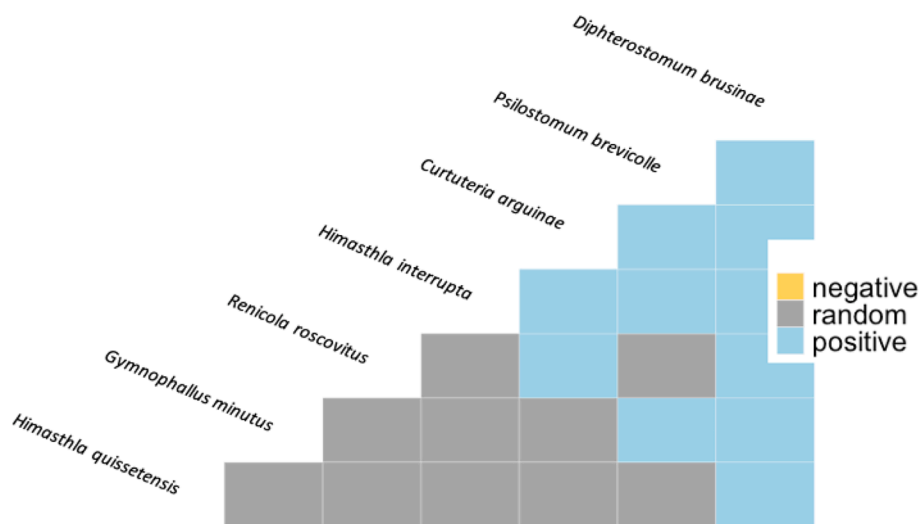


Fig. 6. Results of the species co-occurrence analysis. Species co-occurrence plot performed by the cooccur-package representing the negative, random or positive species associations.

dropped down to 15 ind.m⁻², which could explain the concomitant drop in 2003 of the *H. quissetensis* population in cockles, its second intermediate host. The community was then no longer dominated by *H. quissetensis* but rather by *Curtuteria arguinae* (unknown first and final intermediate hosts) and to a lower extent *Himasthla interrupta*. The latter first intermediate host is the mudsnail *Peringia ulvae* whose occurrence and abundance are highly and unpredictably fluctuating (Do, 2012). The

progressive silt and clay enhancement from 2004 (2.5%) until 2006 (6%) coincided with a change in community in 2005 and 2006, which became exceptionally abundant, and largely dominated by *G. minutus*. Absent up until then, high abundances in *Scrobicularia plana*, the first intermediate host of this parasite species, were observed (96 ind.m⁻²) during these two years (de Montaudouin, 1995; de Montaudouin et al., 2000). Known to prefer muddy environments, the area probably became

Table 3
Sedimentary environment at the sampling stations.

Station	Environment description	Sediment median (µm)	Silt & clay (%)	Organic material content (%)
1	Bare sand	292	2.6	0.3
2	Bare sand	286	5.9	0.8
3	Bare sand	292	1.9	0.3
4	Bare sand	296	2.0	0.4
5	Bare sand	297	2.9	0.5
6	Seagrass bed	288	6.9	1.1
7	Bare sand	288	3.3	0.4
8	Bare sand with sparse seagrass and wild oysters	290	5.4	0.7
9	Seagrass bed	278	12.9	1.8
10	Wild oyster reef in oyster farming area	256	20.4	3.0
11	Seagrass bed	21	82.3	8.7
12	Bare sand	290	2.9	0.4
13	Bare sand	295	2.5	0.5
14	Seagrass bed	279	14.9	2.0
15	Bare sand	283	5.6	0.7

Table 4
Comparison of observed abundances and prevalence levels between the encountered trematode species. Prevalence is defined as the percentage of cockles infected with a particular parasite species. *B. minimus* and *M. parvus* were not present as metacercariae (species using *C. edule* as first intermediate host). Their abundances could not be assessed.

Species	Mean abundance (metacercariae/cockle ¹)	Max. abundance (metacercariae/cockle ¹)	Prevalence (%)
<i>G. minutus</i>	121 ± 267	2368	96
<i>C. arguinae</i>	61 ± 132	766	88
<i>D. brusinae</i>	3 ± 4	21	79
<i>H. quissetensis</i>	4 ± 6	39	75
<i>H. interrupta</i>	7 ± 11	59	72
<i>P. brevicolle</i>	0	4	9
<i>M. sp.</i>	0	1	5
<i>B. minimus</i>	–	–	4
<i>M. parvus</i>	–	–	1

attractive for this species, thus settling in. With the settlement and densification of the seagrass bed, *S. plana* became less abundant (50 ind. m⁻², de Montaudouin and Lanceleur, 2011) and its associated trematode species *G. minutus* numbers decreased. The *Zostera noltei* seagrass bed attaining a large surface and maximum density as of 2009 (Do et al., 2011), the trematode community changed the same year with an increase of *C. arguinae*. The cycle of this species being yet to be determined to this day (Desclaux et al., 2006), we do not know if it coincides with an increase in abundance of its first intermediate host. The disappearance of seagrass in 2011 corresponded to a restructuration of the trematode community in 2012. Indeed, the cockles then located on a large sand beach were infected by a more diversified and moderately abundant trematode community, which resembled the community of 2003, when the landscape also consisted in a sand beach. The six changes in landscape were thus accompanied by changes in the trematode community, occurring with an eventual lag of one year. This might be attributed to the population dynamics of *Cerastoderma edule*, the parasite community changes most probably appearing in the new cohort of the cockle population, free of metacercariae belonging to the previous trematode community. Nevertheless, the trematode community exhibited a total of nine structural changes, that is two more than the landscape. In 2000, within the landscape “oyster park after storm”, the community underwent another change evolving from a *G. minutus* to a *H. quissetensis* dominated community. Perhaps the community was disturbed by the storm but recovered quickly, evolving to a moderately abundant community linked to the presence of the oyster parks as previously

Table 5
Description of the trematode communities in the groups of stations identified by n-MDS analysis. Stations composing the group; median abundance of the five main trematode species (metacercariae/cockle); median of the total species richness (SR) at each station; median total abundance of metacercariae per cockle.

Group	I	II	III	IV
Stations	3, 4	1, 14	9, 10, 11, 12	2, 5, 6, 7, 8, 13, 15
<i>G. minutus</i>	2.5	12	269	35
<i>C. arguinae</i>	0.5	50.5	101.5	6
<i>D. brusinae</i>	0.5	2.5	3	3
<i>H. quissetensis</i>	0	1.5	4.5	2
<i>H. interrupta</i>	1	0	3	8
Median total SR	5	6	7	7
Total abundance	6.5	69	550	58

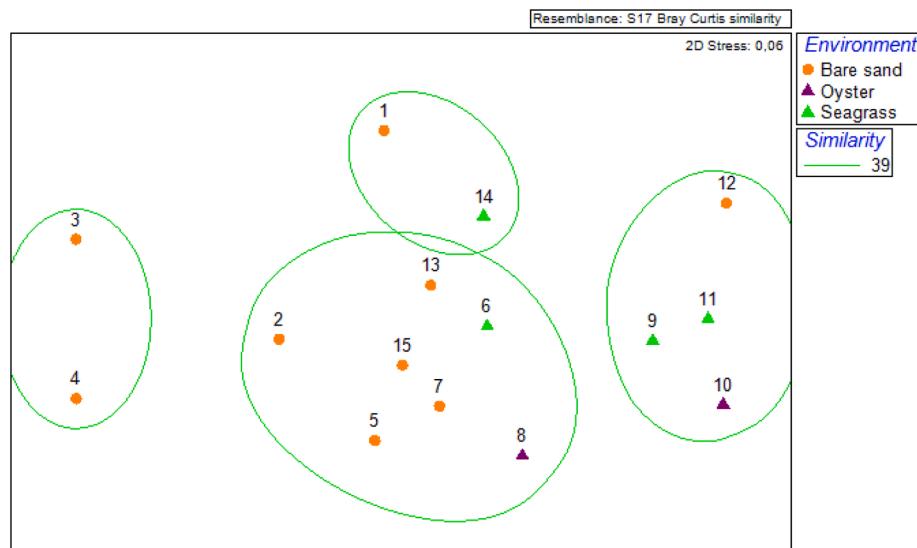


Fig. 7. Results of the n-MDS analysis based on median specific abundances per station of the seven trematode species using *C. edule* as second intermediate host. The green circles indicate the station grouping revealed by the cluster analysis when setting the cut at a similarity level of 39 %.

described. In 2004, within the landscape “sand beach”, *C. arguinae* crashed, while *H. interrupta* peaked and dominated the community for a limited period (2004). *P. ulvae*, the parasite species’ first intermediate host, presented a density of 8000 ind.m⁻² until 2005 and 4000 ind.m⁻² in the following years (de Montaudouin et al., 2003; Do et al., 2011). Thus, unlike the other two main parasite species, *H. interrupta*’s sudden domination could not be related to an abundance increase of its first intermediate host. Lastly, within the landscape “seagrass” and during the first transitional year to “sand beach”, in 2010, the trematode community became radically unabundant, whilst preserving its relatively high diversity. Consequently, it seems that trematode communities were linked to the landscape. However, this factor does not solely explain the evolution of the trematode community structure. Latter appears to also be sensitive to finer, more subtle changes than only those we, human beings, can observe. Among cryptic factors which can explain variations of the trematode community structure, the dynamics of final hosts can be determinant (Smith, 2001; Hechinger and Lafferty, 2005). However, no reliable data about bird or finfish were available.

4.2. Spatial variability of the trematode community

Eight out of 15 known trematode species infecting the common cockle *Cerastoderma edule* on the Eastern Atlantic coast (de Montaudouin et al., 2009) were present in the cockles sampled on Banc d’Arguin in winter 2021. The ninth species was a yet unidentified trematode species which has only been found in Arousa, Spain to this day (de Montaudouin et al., 2021). The various communities that these species formed at the different stations seemed to be explained most by the station’s geographical area in Banc d’Arguin. Indeed, the proximity between stations influenced their similarity in regard to parasite infection. However, the differences of communities between areas were certainly related to the effect of an “influencing station/habitat”. Taking a closer look at stations 9 to 12 for instance, these four stations were different in terms of substrate but were similar in terms of trematode communities because they were close to each other: indeed, the high parasite abundance and species richness of this Group III was related to stations 9–11 which displayed high substrate heterogeneity.

The free-living stages of these parasites (cercariae) from these stations were able to reach the surrounding cockles, even if those were in naked sand (station 12). In contrast, stations 3 and 4 (group I) were similar in terms of parasite community (geographic proximity), but with low metacercarial abundance and species richness because none of these stations of naked sand could stimulate parasite density and/or diversity.

The next question is why heterogeneity of the substrate could stimulate trematode diversity and abundance. As suggested in the temporal monitoring, this heterogeneity may attract first intermediate hosts. Numerous authors have found a close link between the abundance of upstream as well as downstream final hosts and infection patterns in intermediate hosts (de Montaudouin and Lanceleur, 2011; Hechinger et al., 2007; Hechinger and Lafferty, 2005; Smith, 2001; Thieltges and Rick, 2006; Thieltges and Reise, 2007). Besides one could hypothesize that oyster farms attracted, more than in other areas, an important number of oystercatchers (*Haematopus ostralegus*), *Gymnophallus minutus*’ final host. While feeding on abundant associated mussels and cockles, they could have released many miracidia into the environment through their feces, infecting the first intermediate host, *Scrobicularia plana*, which in turn emitted *G. minutus* cercariae in the area, greatly infecting the closely located cockles. It is possible that the distribution of final host and/or of first intermediate host individuals were unevenly distributed along the intertidal flats of Banc d’Arguin and that the hydrodynamics of the surrounding waters were insufficiently strong for an effective homogeneous dispersal of the miracidia and/or cercariae over the entire sand bank. Supplementary data of the abundance and proximity of both first intermediate and final hosts to the different sampling stations would be necessary to verify this hypothesis. In addition, it is possible that the strong effect of the geographical factor overruled and

hid the effect of less evident but not irrelevant factors such as substrate heterogeneity, closely correlated to benthic macrofauna heterogeneity (Edgar, 1999), in turn found to be linked to parasite community structure as shown by Hechinger et al. (2007).

4.3. Drivers of trematode community structure

Indeed, as previously described in this study (§ 4.1.), parasite fluctuations indicated variations in first intermediate host abundances for two of the four main trematode species, which could have been driven by the changes in landscape. Our results hereby partially support the assumption that the presence and abundance of upstream first intermediate host species (such as *Tritia reticulata*, *Peringia ulvae* or *Scrobicularia plana* in this study) drive the pattern of parasite infection in downstream second intermediate host species (herein the cockle). If this relationship were to be true for the majority of the parasite species, the trematode community structure could be considered as an indicator of first intermediate host species population variations. Latter exhibiting various habitat preferences and specificities which can find expressiveness at the landscape level, trematode communities could by extension be indicators of certain landscape/habitat variations not detectable by simple observation or even by using benthos descriptors. However, some authors observed no correlation between first intermediate host abundances and parasite patterns in downstream hosts (Mouritsen et al., 2003).

If upstream host distribution and landscape changes can explain infection patterns to some extent, our spatial and temporal studies also revealed trematode community variability that appeared independent of these drivers. Many other factors can influence infection patterns, such as host condition (Mouritsen et al., 2003; Poulin and Mouritsen, 2003) and density (Thieltges and Reise, 2007) or the ambient benthic community (Thieltges et al., 2008; Mouritsen and Poulin, 2009). Various abiotic conditions can also affect the trematode infection (Pietrock and Marcogliese, 2003). Warming sea temperatures are expected to modify trematode infection patterns as parasite transmission has been proven to closely depend on sea temperature (Mouritsen, 2002; Thieltges and Rick, 2006). However, intrinsic factors and abiotic conditions are considered to be the same over all of Banc d’Arguin and thus do not apply at the scale of our study. This is consistent with Wiens (1989) review stating that at smaller scales drivers of ecological processes are more biotic than physical. Though sediment grain-size can have an indirect effect on trematode communities by influencing the benthic macrofauna communities which include potential first intermediate hosts (Skirnisson et al., 2004), the sedimentary environment at the sampling stations generally exhibited similar sediment medians. Indeed, medium sands dominated in almost all stations, aside from station 11 located in seagrass, for which sediment samples presented a median of 21 µm. Seagrass beds are known for reducing current velocity and facilitating the deposition and accumulation of fine sediment (Bos et al., 2007; Hansen, 2012; van Katwijk et al., 2016). It can therefore be expected that the median grain-size is inferior to that at stations without seagrass. Nonetheless, this value is extremely low, not similar to sediment medians of the other seagrass stations and does not reflect the observations made on site while sampling. It seems we collected only surface sediment made up of very fine particles whilst failing to sample the underlying coarser sediment. Sediment grain-size is therefore excluded as a driving factor. Submersion time of a given site can considerably affect infection patterns (Gam et al., 2008; Correia et al., 2021). However, in this study, stations are considered as presenting similar submersion periods. Consequently, the factor which can be considered as the most important is the distribution and abundance of upstream and downstream host species which are tightly related to the landscape. Though the co-occurrence analysis applied on data from our spatial study revealed no non-random species association, probably due to the insufficient number of stations (Veech, 2013), results from our temporal study highlighted the fact that parasite species co-occur when

they share the same first intermediate host. Indeed, *Himasthla quissetensis* and *Diptherostomum brusinae* (*T. reticulata* as first intermediate host), as well as *Himasthla interrupta* and *Psilostomum brevicolle* (*P. ulvae* as first intermediate host), were positively associated. Yet, some species co-occurred without sharing upstream hosts. Perhaps, these associations can be explained by co-occurrence of various final hosts attracted by similar landscapes.

Our study suggests the possibility that trematode communities might be potential indicators of more or less “cryptic” changes in the environment. However, this was observed for changes in time but was less obvious in space. It is unclear to which scale of environmental changes trematodes are actually sensitive. Furthermore, while substrate heterogeneity did not directly structure the trematode assemblages as it is well documented for benthic communities (Andrew, 1993; Balazy and Kuklinski, 2017; Blanchet et al., 2004; Kuklinski et al., 2006), it still appeared to play a role for the trematode communities. The sensitivity of trematodes might consequently be of a different nature than that of benthic fauna, which could be very interesting when applied to ecosystem status evaluation. Indeed, similar to benthic fauna, several studies have underlined the potential usefulness of trematodes as indicators of ecosystem biodiversity, health and recovery (Hechinger et al., 2007; Aguirre-Macedo et al., 2011; Miura et al., 2019).

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Ethics approval

All applicable institutional and/or national guidelines for the care and use of animals were followed.

CRediT authorship contribution statement

Leslie Stout: Data curation, Writing – original draft. André Garenne: . Xavier de Montaudouin: Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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