Distribution of parasites in their second intermediate host, the cockle *Cerastoderma edule*: community heterogeneity and spatial scale

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ABSTRACT: We studied the spatial heterogeneity of infection of the marine bivalve *Cerastoderma edule* (cockle) by trematode parasites at 5 different scales in Banc d'Arguin, France. At the km-scale, the communities of trematodes differed among stations in terms of species composition and specific abundance. Infection heterogeneity was likely due to the occurrence of the definitive host and the conditions of transmission (water parameters). At the 100 m scale, trematode species richness was similar among stations, but specific trematode abundances differed. The most significant variable in terms of explaining trematode infection heterogeneity was the presence of the first intermediate host. At the 10 m scale, the distance at which parasite abundance diminished varied according to trematode species. When an intense source of trematode larvae (cercariae) emission was suspected, this abundance was maintained within ca. 20 m. Outside this area, parasite abundance progressively dropped. We found a higher aggregation in parasite distribution at the 100 m scale than at the 10 m scale using a community aggregation measure. At the cm-scale, the parasite species richness was similar in all cockles but parasite abundance differed in relation to distance when the parasites were shed by a sedentary host compared to a motile host. Observed differences of infection could also be due to intrinsic factors (life history, immune system) as suggested by an experimental infestation where cockles challenged in similar conditions displayed different parasite loads. We demonstrated that high heterogeneity of parasite infection in cockles should be taken into account in studies concerning the role of parasites in ecosystems.

KEY WORDS: Spatial heterogeneity · Aggregation pattern · Parasitism · *Cerastoderma edule* · Sampling scale

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INTRODUCTION

The influence of scale on processes that determine the distribution patterns of ecological populations and communities is well recognised, resulting in great variation of abundances at different spatial and temporal scales (Wiens 1989, Hui 2009). At broader scales, physical processes may dominate or dissipate biological effects that would be significant drivers of community structure at smaller scales. Fine-scale studies may reveal greater details about the biological mechanisms underlying patterns, but generalisations are more likely to emerge at broader scales (Wiens 1989). Understanding how species distribution (occupancy and spatial autocorrelation) and associations (multi-species co-distribution) change across scales is salient to depicting the pattern formation in population ecology and macroecology.

Because parasite species represent at least 40% of known species (Dobson et al. 2008), and because they can themselves influence spatial patterns of some free-living species (including their hosts; Mouritsen & Poulin 2005, 2010, Wood et al. 2007), an understanding of their distribution at different scales is important. With parasites, the notion of spatial scaling becomes more complex because both the hosts and their parasites need to be considered in the sampling design. On the other hand, hosts can be considered as habitats and

parasites as recruits that will settle in a special environment, i.e. tissues of target hosts. In this context, we can ask whether, as for free-living species, parasite community structure is also influenced by physical and climatic factors on a large scale and by biotic interactions on a small scale (Wiens 1989).

In coastal systems, trematodes are the most prevalent macroparasites of marine molluscs and may play a significant role in their population dynamics (Desclaux et al. 2004, Fredensborg et al. 2005, Thieltges 2006, Gam et al. 2009). Therefore, it is important to depict parasite community structure and consequently to assess specific parasite abundance in host populations. One difficulty with trematode parasites is the complexity of their life cycle. The presence or abundance of trematodes in 1 host depends on their presence/abundance in the 2 other hosts involved in the cycle and on transmission conditions between hosts. Indeed, typically, adult trematodes live and reproduce in the digestive tract of vertebrates, their definitive host. Their eggs are ingested by the first intermediate host unless they develop into miracidium larvae and penetrate the host, according to trematode species. Miracidium larvae develop into sporocysts or rediae that will provide cercariae larvae that are emitted in the water mass and swim towards the second intermediate host. They penetrate the tissues and wait (metacercarial stage) until the second intermediate host is predated by the definitive host in order to complete their life cycle.

Another major difficulty is that parasite distribution displays a species-dependent aggregative pattern (Mouritsen et al. 2003, Thieltges & Reise 2007, Faltynkova et al. 2008). Consequently, the significance of a given number of samples in order to describe a parasite community varies according to trematode species (Thieltges & Reise 2006). Therefore, we can expect that the community structure will change according to the investigated scale and that processes driving these communities will also vary with scale. Little is known about spatial heterogeneity in infection levels of second intermediate hosts and about the contributing factors at different spatial scales. Concerning bivalves as second intermediate hosts, a positive relationship between size or tissue weight and infection levels has been described and is usually ascribed to a higher filtration rate of larger individuals resulting in a higher exposure to infective stages (de Montaudouin et al. 1998, Mouritsen et al. 2003, Thieltges & Reise 2006). The distribution and abundance of the first intermediate host (the upstream host) seems to be important in determining infection levels in the second intermediate hosts (Thieltges & Reise 2006, 2007), but other factors also have an influence, including the density of the target host (Thieltges & Reise 2007), host condition (Mouritsen et al. 2003) and the ambient benthic com-

munity (Thieltges et al. 2008, Mouritsen & Poulin 2010).

A peculiarity in working with trematodes is also that variability at the small scale is largely due to another factor than for free-living fauna. Indeed, at the small spatial scale (cm to m), free-living species community structure can result from microhabitat heterogeneity and biotic interactions (Tanaka & Leite 2003). With parasites, the habitat may appear very similar at the small scale (1 host individual compared to a neighbouring host individual of the same species) but we can expect heterogeneity due to what we term 'intrinsic factors', such as immune system variability among individuals (Paul-Pont et al. 2010a).

In the present study, we compared parasite species richness, community composition and metacercariae abundance in the cockle *Cerastoderma edule* at 5 spatial scales to determine the significance of these parameters in relation to sampling scale.

MATERIALS AND METHODS

Sampling sites. Arcachon Bay is a 180 km macrotidal lagoon situated on the Atlantic south-west coast of France (44° 40' N, 1° 10' W; Fig. 1A). It connects with the Atlantic Ocean by a narrow channel. Secondary channels penetrate among large intertidal areas partly covered by *Zostera noltii* beds (Blanchet et al. 2004, Plus et al. 2010). Four stations (Maudit, Andernos, Lanton, Ile aux Oiseaux) were selected among mudflats in the inner part of the bay for a single sampling census while another station (Arguin) was also designed for the studies at a smaller spatial scale (Fig. 1B,C).

The main sampling site (Arguin) is a moderately sheltered intertidal sandflat at the oceanic part of the bay. The sediment is largely dominated by medium sands. Salinity remains high year-round (32 to 35), mean surface water temperature fluctuates between 9.5°C in winter and 21.1°C in summer, and sediment temperature varies from −0.2°C to 30°C. The benthic free-living fauna has previously been described (Bachelet & Dauvin 1993), as has the trematode parasite fauna of molluscs (de Montaudouin et al. 2000, 2003, Desclaux et al. 2002, Gam et al. 2009). Since 1972, Arguin (25 km^2) has been a national reserve with several marine bird species wintering, nesting or migrating.

Scale definitions. The aim of the study was to compare the structure of parasite communities in cockles in relation to the distance among these bivalves. In ecology, the notion of distance is not necessarily correlated with the geographic distance. Two different habitats may be within a short distance from each other but with such physical barriers that the communities will
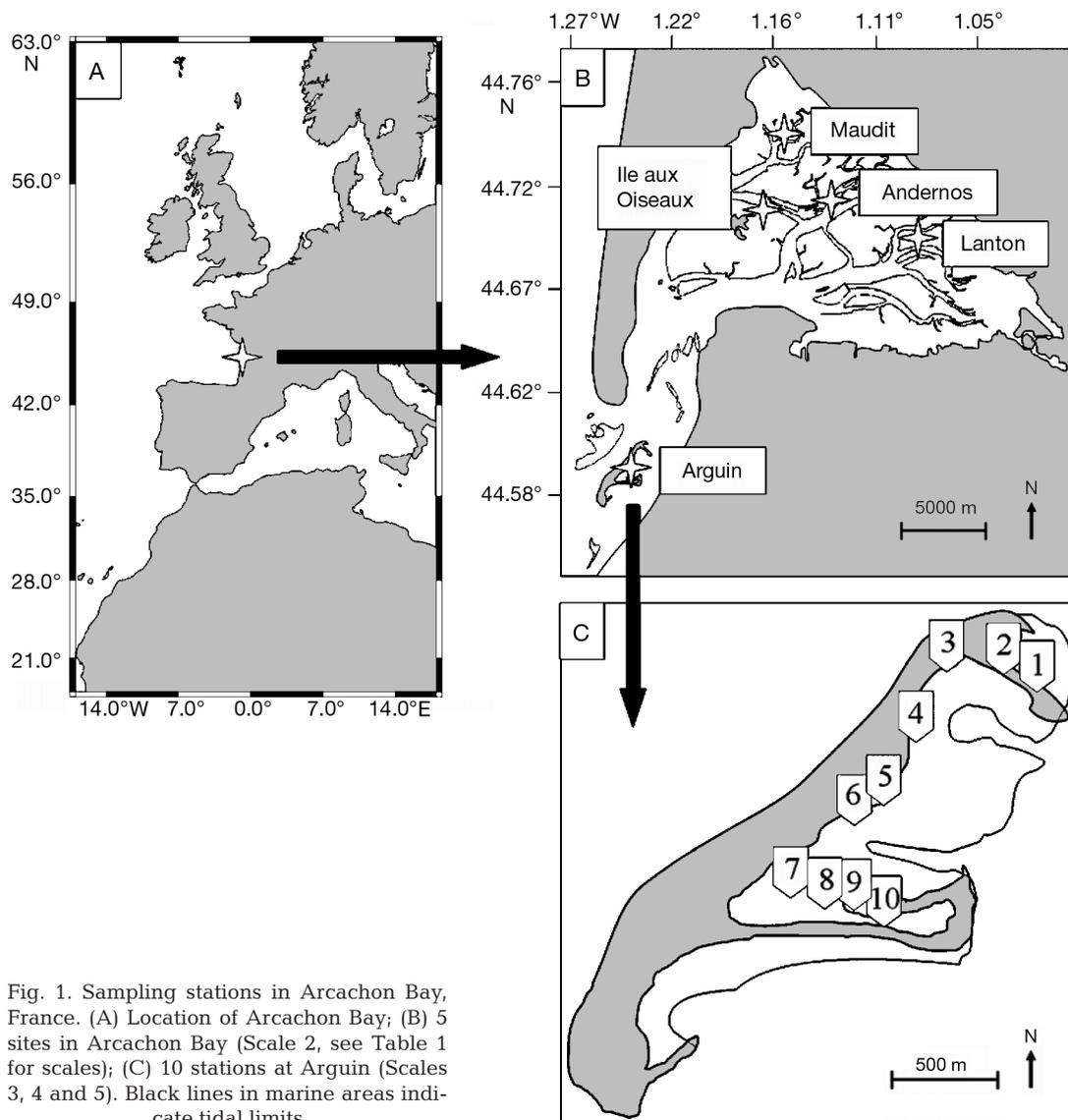
Fig. 1. Sampling stations in Arcachon Bay, France. (A) Location of Arcachon Bay; (B) 5 sites in Arcachon Bay (Scale 2, see Table 1 for scales); (C) 10 stations at Arguin (Scales 3, 4 and 5). Black lines in marine areas indicate tidal limits

be very different. In marine systems, water currents can also separate habitats independently of geographic distance. Conversely, homogeneous habitats may cover vast areas, with communities remaining similar along great geographic distance. Therefore, the different distances that we investigated corresponded to changes of environmental conditions, which we identified as key parameters in trematode infection of their second intermediate host. The global climate has a strong structuring impact on parasite communities, firstly due to the effect on free-living species that are also potential hosts for parasites. The present study was handled within a climatic biogeographic area (Arcachon Bay) and the studied heterogeneity was nested to that level, which we referred to as Scale 1 (usually, ×100 km; Table 1). All investigated

scales (2 to 5) were nested in Scale 1. In a decreasing hierarchical order, we considered the water body as the next structuring factor. The term 'water body' involved abiotic characteristics such as temperature, salinity and current velocity that impact on sediment parameters and related fauna. We selected different sites in Arcachon Bay with such different characteristics, but all with similar numbers of cockles. The parasite heterogeneity was referred to as Scale 2 and corresponded to a km distance (Table 1) because stations were separated by a minimum distance of 5 km and a maximum of 19 km. The next investigated scale (Scale 3) was within a water body and with similar sedimentary habitat (medium sands) but differed by some minor grain-size and hydromorphological patterns that had effects on benthic communities. This scale could

Table 1. Definitions used in this study in relation to major environmental characteristics. At each scale, 'x' signifies relative homogeneity of this factor (e.g. at Scale 3, general climate and water body characteristics are considered homogeneous while different potential host communities may display heterogeneity). Distance range: minimum–maximum distance (in m) between 2 sampled stations. N: number of dissected cockles per station. At Scale 5, distance range and N concern cockle individuals

Scale	Distance range (m)	No. of stations	N	Climate	Water body & hydrodynamics	Final host & benthic communities	First inter-mediate host
1 ($\times10^5$ m)							
2 ($\times10^3$ m)	5–19 ($\times10^3$)	5	15	x			
3 ($\times10^2$ m)	80–1440	10	15	x	x		
4 ($\times10^1$ m)	10	19	3	x	x	x	
5 ($\times10^{-2}$ m)	8–316 ($\times10^{-2}$)	2	15	x	x	x	x

also separate flocks of birds (potential definitive hosts) by favourite foraging or repository areas (Table 1). Arguin, a group of sandy banks at the entrance of Arcachon Bay, was selected with a set of 10 stations and a 100 m scale distance (Table 1). The only obvious difference among these stations was the emersion time, with a percentage of emersion increasing from Stn 1 (25%) to Stn 10 (40%). Because preliminary results showed parasite variability at Scale 3, especially among some stations, we investigated a smaller scale (Scale 4) through a sampling effort every 10 m between 2 stations (Stns 7 and 8). At that scale, the hypothesis was that the upstream first intermediate host was the major forcing factor explaining cockle infection. Finally, we determined parasite infection at the cm scale (Scale 5). In this case, we presumed that the parasite differences that could be detected were mainly due to intrinsic factors (e.g. cockle life history, immunity; Table 1).

Sampling procedure. The selected cockles were all from the 2006 cohort and were sampled in January and February 2007. Their mean shell length was within a 17 to 23 mm range at all stations. The sampling effort (number of stations, number of cockles and distance among stations or cockles) is summarised in Table 1. For the finest-scale analysis (Scale 5), at each station (Stns 7 and 8), a pole was planted in the sand, and the geographic position of the 15 nearest cockles was determined (N = 2 stations × 15 cockles). Each of these cockles was marked. The distance between 2 cockles ranged between 8 and 316 cm.

Simultaneously sampling at Scale 3 (10 stations at Arguin), we estimated cockle abundance (ind. m^{-2}) by sampling 6 quadrats (each 0.25 m^2) per site and sieving them through a 1 mm mesh. Prior to this campaign, we sampled the potential first intermediate hosts (*Scrobicularia plana*, *Hydrobia ulvae*, *Nassarius reticulatus*) in May 2006 just before cockle recruitment. This choice was based on previous studies performed at this site (de Montaudouin et al. 2000, Desclaux et al. 2002, Blanchet et al. 2003), and the

aim was to get a rough idea of the potential first intermediate host abundance. Unfortunately, this investigation was not performed at Stns 9 and 10 because they were added to the protocol later. The sampling effort depended on the target species. *S. plana* (bivalves) were sampled using a 15 × 15 cm quadrat dug 30 cm into the sediment (10 replicates per station, 5 mm mesh sieve). Gastropods *H. ulvae* were sampled by skimming off the first cm of sediment and sieving it through a 2.5 mm mesh sieve to select snails that had the potential length to be infected by trematodes (Blanchet et al. 2003). *N. reticulatus* (gastropods) were sampled by trapping in a net (1 cm mesh, baited with fresh flesh of the gastropod *Crepidula fornicata* and set for 20 mins), as described by Bachelet et al. (2004).

We measured all cockles to within 1 mm precision (shell length) and then opened them. The flesh was squeezed between 2 large glass slides and observed through a stereomicroscope. Trematode parasites were identified under the stereomicroscope (de Montaudouin et al. 2009) and counted. The distinction between *Himastla continua* and *H. quissetensis* was based on the number of oral spines (29 and 31, respectively) counted under the microscope after mechanical extraction of metacercariae. The parasite abundance was defined as the mean number of metacercariae per cockle, including infected and uninfected cockles (Bush et al. 1997). Due to strong heterogeneity of variance, comparisons among stations were performed 2 by 2 by a Kruskal-Wallis non-parametric test. Pearson's correlations were made among abundances of parasite species using the same first intermediate host, using the mean parasite abundance at each station.

At each station (Scales 2 and 3), surface sediment grain size was analysed through a wet sieve column, and organic matter content in the sediment was estimated by calcination (550°C, 2 h). Mean, maximum and minimum water temperature and salinity were reported in a previous study (Dang et al. 2010).

Measurement of parasite aggregation. The objective was to measure the pattern of parasite aggrega-

tion among hosts at 2 hierarchical scales as described by Boulinier et al. (1996). In the present case, the comparison of parasite abundance (for 7 parasite species) was performed among cockles within stations and cockles among stations at Banc d'Arguin. The mathematical procedure proposed by Boulinier et al. (1996) was followed and led to a set of parameters that allowed quantification of parasite aggregation. In summary, J measures the global aggregation of parasites among cockles, J_j measures aggregation within station j (Scale 4), and J_k measures aggregation resulting from differences in the numbers of parasites per cockle among stations (Scale 3). The difference between these measures, $J - J_k = EJ_j$, is the weighted average of the within-station measure of aggregation, J_j, and provides a measure of the global within-station aggregation of the different parasite species. To test for the significant departure of the aggregation measures from values corresponding to a random distribution of the trematodes within and among stations, chi-squared dispersion tests of between-station aggregation (χ^2_{bs}) and within-station aggregation (χ^2_{ws}) of parasites were performed (Boulinier et al. 1996).

Intrinsic heterogeneity of infection. At Scale 5 (cm), we hypothesised that the surrounding factors affecting trematode transmission success were equal for all cockles and that parasite structure variability among cockles should be due primarily to intrinsic factors. We experimentally tested this hypothesis.

A controlled infection of cockles was performed to estimate the intrinsic variability of infection. By controlling the maximum number of parameters, i.e. cockle shell length, temperature, salinity, number of parasite cercariae to which cockles were exposed, challenge duration and cockle density, the aim was to minimise environmental variability to detect intrinsic variability. Cockles were collected at Stn 1. They were immediately sorted according to their shell length in order to select 45 cockles within a narrow size range (mean ± SD: 24.2 ± 1.6 mm). Fifteen cockles (control) were dissected to verify that they were free of the parasite that was utilised in this experiment, *Himasthla elongata*. The 30 other cockles were used for the experiments at 16 and 22°C. These 2 temperatures were selected because they corresponded to the field temperature range during the study at Arguin. *H. elongata* cercariae were collected from infected periwinkles *Littorina littorea* sampled in the inner part of Arcachon Bay. Infected snails were kept at 15°C and fed with macroalgae (*Ulva* sp.). To induce cercariae emission, each snail was isolated in a dish with seawater

and exposed to light at about 24°C. The infection experiment was designed as a 1-factor experiment in which temperature was the fixed factor and the number of metacercariae was the dependent variable. For each temperature, 15 replicates were used. The experimental units consisted of small plastic square dishes (side = 55 mm; height = 25 mm) filled with 60 ml of seawater. Each cockle was incubated in each dish with 40 cercariae for 24 h. Cercariae were collected within less than 1 h after emission from periwinkles. After 24 h, cockles were dissected and parasites counted as previously described. The number of *H. elongata* metacercariae, reflecting the infection efficiency or infection success (fraction of the added cercariae found as metacercariae), was recorded and compared with Student's *t*-test. Additionally, we calculated the coefficient of variation (SD/mean) for each treatment. We then compared these variability indices to those calculated in the field at the cm scale (Scale 5)

RESULTS

Scale 2 ($\times 10^3$ m)

At this scale, water body and sediment characteristics among stations were different, especially among Banc d'Arguin (Stn 1), Maudit and the other sites (Table 2).

In total, 8 species of trematodes using cockles as a second intermediate host were found (15 cockles × 5 stations; Table 3). Parasite species richness differed among stations, from 4 species at Lanton to 7 species at Banc d'Arguin. Three species were common to all stations (*Himasthla interrupta*, *H. quissetensis* and *Diphterostomum brusinae*) while 2 species were exclusive to single locations *Meiogymnophallus minutus* at Banc d'Arguin and *H. elongata* at Lanton. The abundance of the dominant parasites also fluctuated among stations (Table 4). *H. quissetensis* was the most abundant at Ile aux Oiseaux (p < 0.001). Banc d'Arguin was also

Table 2. Environmental characteristics of 5 sampling sites (Scale 2 analysis, see Table 1 for scales). Mean values (annual range in brackets) are given

Site	Water temperature (°C)	Water salinity (psu)	Sediment median (µm)	Organic matter content in sediment (%)
Maudit	15.5 (2.7–26.6)	27.3 (4.4–35.7)	75	8.8
Lanton	15.6 (3.0–26.5)	26.7 (4.8–34.4)	100	9.6
Andernos	15.5 (3.0–26.5)	30.0 (18.5–34.5)	100	8.2
Ile aux Oiseaux	15.5 (3.0–26.5)	29.6 (12.1–34.8)	100	11.1
Arguin	15.7 (7.8–23.1)	34.2 (31.2–35.4)	330	2.2

Table 3. Trematode species found in the present study with different hosts necessary to accomplish their life cycle

Family	Species	1st intermediate host	2nd intermediate host	Definitive host
Echinostomatidae	*Himasthla quissetensis*	*Nassarius reticulatus*	*Cerastoderma edule*	*Larus argentatus*
Echinostomatidae	*Himasthla elongata*	*Littorina littorea*	*C. edule*	Larides
Echinostomatidae	*Himasthla interrupta*	*Hydrobia ulvae*	*C. edule*	Larides
Echinostomatidae	*Himasthla continua*	*Hydrobia ulvae*	*C. edule*	Larides
Echinostomatidae	*Curtuteria arguinae*	???	*C. edule*	???
Gymnophallidae	*Meiogymnophallus minutus*	*Scrobicularia plana*	*C. edule*	*Haematopus ostralegus*
Psilostomatidae	*Psilostomum brevicolle*	*Hydrobia ulvae*	*C. edule*	Birds
Zoogonidae	*Diphterostomum brusinae*	*Nassarius reticulatus*	*C. edule*	Fishes

Table 4. Mean metacercariae abundance of trematodes within samples of cockles *Cerastoderma edule* (*Hq*: *Himasthla quissetensis*; *Hc*: *H. continua*; *Hi*: *H. interrupta*; *He*: *H. elongata; Ca*: *Curtuteria arguinae*; *Mm*: *Meiogymnophallus minutus*; *Pb*: *Psilostomum brevicolle*; *Db*: *Diphterostomum brusinae*) and trematode species richness (SR) at the 5 sites of Arcachon Bay (Scale 2, see Table 1 for scales). For each trematode species, the average value for the whole bay is given and a comparison between stations was performed (Kruskal-Wallis test; similar stations are listed in brackets)

| Site	Hq	Hc	Hi	He	Ca	Mm	Pb	Db	SR
1. Arguin Stn 1	6.18	3.49	21.13	0.00	8.33	23.67	2.20	4.53	7
2. Andernos	7.10	2.37	0.13	0.00	1.40	0.00	0.00	1.73	5
3. Lanton	4.60	0.00	0.70	0.80	0.00	0.00	0.00	1.60	4
4. Ile aux Oiseaux	27.85	1.02	2.27	0.00	1.13	0.00	0.00	1.33	5
5. Maudit	5.13	0.00	0.93	0.00	0.07	0.00	0.13	1.60	5
Arcachon Bay	10.57	1.47	5.34	0.11	2.34	5.07	0.50	2.2	8
p (Kruskal-Wallis)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	
Multiple comparison	(1-2-3-5)	(1-2-4) (3-5)	(2-3-4-5)	(1-2-4-5)	(1-2-3-4-5)	(2-3-4-5)	(2-3-4-5)	(1-2-5) (3-4-5)	
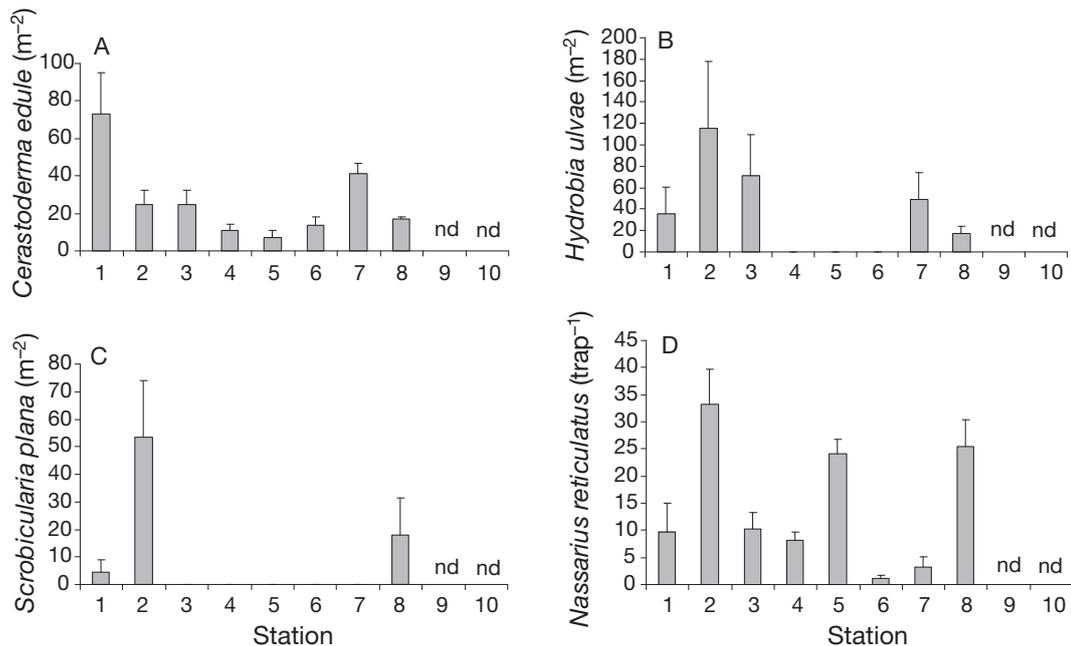
Fig. 2. Mean (+1 SE) abundance of (A) *Cerastoderma edule*, (B) *Scrobicularia plana*, (C) *Hydrobia ulvae* and (D) *Nassarius reticulatus* within 10 stations at Arguin. Abundance is in ind. m^{-2} except for *N. reticulatus*, for which abundance was calculated as the mean number of snails caught in 20 min in a baited trap (see 'Materials and methods'). nd: not determined

discriminated from the other sites by the dominance of *H. interrupta* (p < 0.001), *Curtuteria arguinae* (p < 0.05) and *Psilostomum brevicolle* (p < 0.001; Table 4).

Scale 3 (×10² m)

All stations had a similar sediment median (330 μm) except Stn 6, which was characterised by the presence of gravel (median diameter = 710 μm). The tidal level was different and progressively lower from Stn 10 to Stn 1, where the cockle habitat emerged 1 h 45 min

later at ebb tide. Adult cockle abundance ranged between 1 and 73 ind. m⁻² (Fig. 2A). First intermediate hosts were present at some or all stations (Fig. 2B–D). Abundance of parasite species using *Hydrobia ulvae* as the first intermediate host (*Himasthla continua*, *H. interrupta* and *P. brevicolle*; Fig. 3) were all positively correlated 2 by 2 (0.59 > Pearson's R > 0.20; p < 0.05). Conversely, abundance of parasite species using *Nassarius reticulatus* as the first intermediate host (*H. quissetensis* and *Diphterostomum brusinae*) were not correlated (R = −0.05; p = 0.56). There was no correlation between metacercarial abundance and their respec-
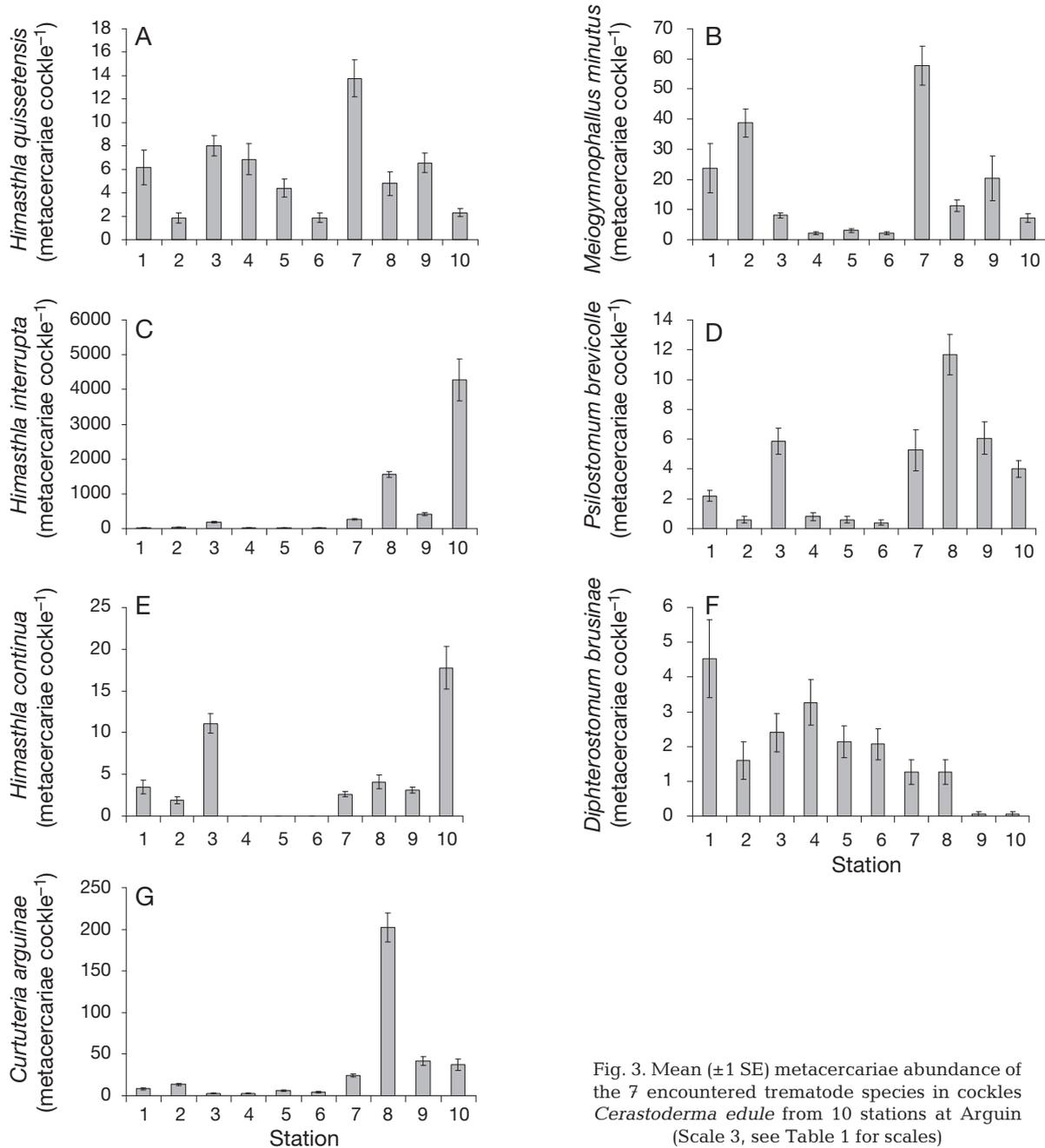

Fig. 3. Mean (±1 SE) metacercariae abundance of the 7 encountered trematode species in cockles *Cerastoderma edule* from 10 stations at Arguin (Scale 3, see Table 1 for scales)

tive first intermediate host abundance (p > 0.05). However, the absence of first intermediate hosts was related to the absence/scarcity of trematodes in cockles (e.g. *H. continua* and *H. interrupta* compared to *Hydrobia ulvae* at Stns 4, 5 and 6, and *Meiogymnophallus minutus* compared to *Scrobicularia plana* at Stns 3 to 6, though not Stn 7). Parasites totalled 7 species for 7 stations and 6 species for the other 3 stations (Fig. 3). Six parasite species were common to all stations. Conversely, the parasite abundance per trematode species fluctuated highly among stations. When comparing neighbouring stations (2 by 2, i.e. 80 m < distance < 480 m), parasite abundance dif-

fered (p < 0.05) in most cases and for most parasite species (Table 5). For example, Stns 1 and 2 were different for all parasite species although they were only 150 m apart. There were similarities among Stns 4, 5 and 6 (p > 0.05 for most parasite species). In terms of parasite species, *Diphterostomum brusinae* displayed the most homogeneous distribution (Table 5).

For the 7 parasite species, there was a significant aggregation among cockles within stations (χ^2_{ws}, p < 0.001; Table 6). Except for *Diphterostomum brusinae*, J_k was higher than EJ_j, meaning a higher aggregation among stations (χ^2_{bs}, p < 0.05) than among cockles within a station. For instance, aggregation of *Curtute-*

Table 5. Comparison of metacercariae abundance per trematode species in cockles *Cerastoderma edule* (*Hq*: *Himasthla quissetensis*; *Hc*: *H. continua*; *Hi*: *H. interrupta*; *Ca*: *Curtuteria arguinae*; *Mm*: *Meiogymnophallus minutus*; *Pb*: *Psilostomum brevicolle*; *Db*: *Diphterostomum brusinae*) between 15 cockles at neighbouring stations (Scale 3, see Table 1 for scales). ns: not significant; *p < 0.05; **p < 0.01; ***p < 0.001 (*U* value of the Mann-Whitney test)

Parasite	Station pair								
	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10
Hq	** (36)	*** (7)	*** (25)	ns (80)	** (46)	*** (0)	** (39)	ns (89)	** (40)
Hi	** (45)	*** (0)	*** (0)	ns (72)	ns (105)	*** (0)	*** (0)	*** (0)	*** (0)
Hc	* (56)	*** (1)	*** (0)	ns (112)	ns (112)	*** (0)	ns (85)	ns (98)	*** (6)
Ca	** (43)	*** (0)	ns (97)	ns (58)	ns (77)	*** (0)	*** (0)	*** (0)	ns (92)
Mm	** (39)	*** (15)	*** (12)	ns (92)	ns (93)	*** (0)	*** (9)	ns (105)	ns (80)
Pb	** (37)	*** (6)	*** (8)	ns (99)	ns (98)	*** (5)	*** (22)	*** (31)	ns (74)
Db	** (49)	ns (79)	ns (97)	ns (85)	ns (109)	ns (82)	ns (110)	** (43)	ns (112)

Table 6. Aggregation of 7 trematode species, parasites of *Cerastoderma edule*, at the within- and among-station scales between 10 stations at Arguin. J and J_k measure, respectively, the total and among-station aggregation of parasites; $EJ_j = (J - J_k)$ measures the weighted average of the within-station aggregation of the parasites among cockles. Chi-squared dispersion tests of among-station aggregation (χ^2_{as}) and within-station aggregation (χ^2_{ws}) are given. n: total number of cockles per trematode species; N: total number of stations; N_p: total number of stations with at least 2 cockles and with at least 1 parasitised; n_p: total number of cockles by stations with at least 2 cockles and with at least 1 parasitised; X_m: mean number of parasites cockle^{-1} (global mean); p: prevalence (percentage of stations with at least 1 parasitised cockle); CI: confidence intervals at 96% under the null hypothesis of no within- or among-nest aggregation; df_{as}: degrees of freedom for the χ^2 among stations (= N – 1). df_{ws}: degrees of freedom for the χ^2 within stations (= $n_p - N_p$). Trematode species: *Hq*: *Himasthla quissetensis*; *Hc*: *H. continua*; *Hi*: *H. interrupta*; *Ca*: *Curtuteria arguinae*; *Mm*: *Meiogymnophallus minutus*; *Pb*: *Psilostomum brevicolle*; *Db*: *Diphterostomum brusinae*. Significant aggregations at *p < 0.05; **p < 0.01; ***p < 0.001; ns: not significant

| Species	n	N	N_p	n_p	X_m	p	J	J_k	EJ_j	J_k/J	χ^2_{as}		df_{as}	χ^2_{ws}		df_{ws}
Hq	150	10	10	15	5.66	100	0.61	0.34	0.27	0.56	20.53	*	9	306.55	***	5
CI							(–0.12, 0.59)	(0.04, 0.35)	(–0.26, 0.44)							
Hc	150	10	10	15	4.41	70	2.00	1.49	0.51	0.74	58.14	***	9	203.10	***	5
CI							(–0.16, 0.59)	(0.05, 0.41)	(–0.35, 0.37)							
Hi	150	10	10	15	661.60	100	4.66	3.52	1.14	0.76	23297.06	***	9	21684.00	***	5
CI							(–0.02, 0.03)	(0.00, 0.00)	(–0.02, 0.03)							
Ca	150	10	10	15	34.35	100	3.24	2.81	0.44	0.87	965.36	***	9	945.36	***	5
CI							(–0.05, 0.16)	(0.00, 0.05)	(–0.07, 0.14)							
Mm	150	10	10	15	17.47	100	1.82	0.99	0.83	0.54	173.95	***	9	1746.90	***	5
CI							(–0.15, 0.24)	(0.01, 0.11)	(–0.19, 0.10)							
Pb	150	10	10	15	3.75	100	1.20	0.80	0.40	0.67	24.37	**	9	229.34	***	5
CI							(–0.24, 0.46)	(0.04, 0.48)	(–0.59, 0.30)							
Db	150	10	10	15	1.87	100	1.13	0.42	0.71	0.37	4.88	ns	9	259.41	***	5
CI							(–0.01, 1.43)	(0.11, 1.08)	(–0.45, 0.94)							
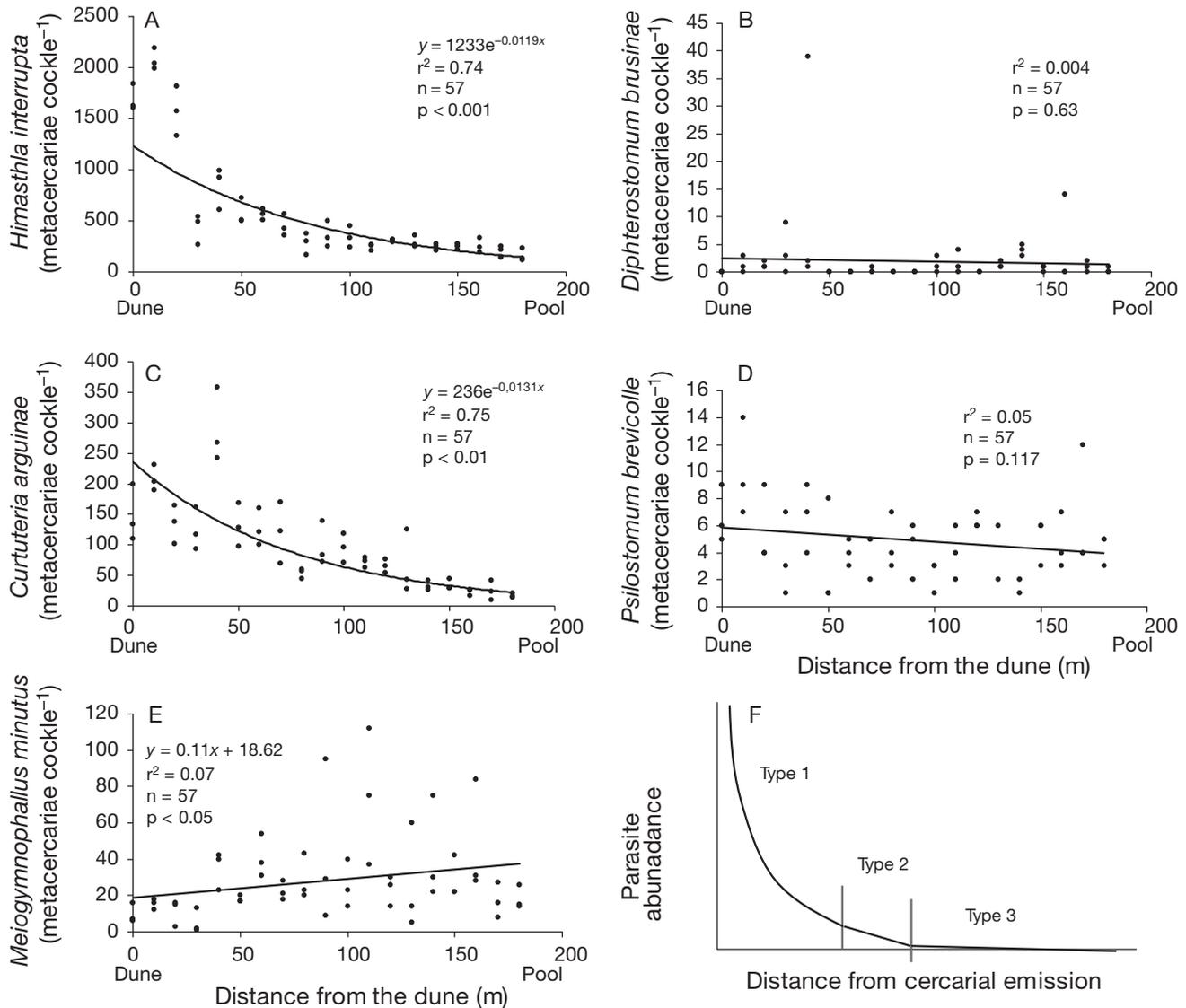
Fig. 4. (A–E) Relation between metacercariae abundance per cockle *Cerastoderma edule* and distance (in meters) between Stns 7 ('Pool') and 8 ('Dune') for 5 dominant trematode species (Scale 4, see Table 1 for scales). (F) Theoretical pattern of trematode metacercariae abundance according to location of cercarial emission. The left part is close to cercarial emission. There is a rapid exponential decrease of infection by cercariae with distance (i.e. dilution of cercariae in the water mass; Type 1, panels A and C). After this distance, the infection decreases slowly and linearly (Type 2, panel E). When cercariae become rare (i.e. far from emission or low emission), there is no more significant relation of infection with distance (Type 3, panels B and D)

ria arguinae metacercariae among stations explained 87 % (J_k/J) of the total aggregation of this parasite species among cockles.

Scale 4 (×10 m)

Three cockles were dissected every 10 m within a distance of 180 m. All stations were at similar tidal level and with the same sediment characteristics, but they were bordered by different habitats. One extremity of this transect (Stn 7) was near a pool while the

other was against a sand dune (Stn 8). The mean parasite species richness per station was equal (same 7 species). Concerning the 5 dominant parasite species, different scenarios were observed. In the case of *Himasthla interrupta* and *Curtuteria arguinae*, mean abundance in cockles displayed a clear exponential decrease from the dune to the pool ($p < 0.001$ and $p < 0.01$, respectively; Fig. 4A,C). When comparing the abundance of these 2 parasites, station by station (Tukey test), there was a significant drop in abundance between 2 stations within the first 20 m from the dune for *H. interrupta* and within the first 70 m from the

dune for *C. arguinae*. After this distance, parasite abundances remained similar. In contrast, *Meiogymnophallus minutus* metacercariae abundance in cockles (Fig. 4E) exhibited significant increase from the dune to the pool (p < 0.05), although a Tukey test did not reveal significant differences when comparing stations 2 by 2. *Diphterostomum brusinae* and *Psilostomum brevicolle* (Fig. 4B,D) displayed similar patterns of infection along the gradient (p > 0.05).

Scale 5 ($\times 10^{-2}$ m)

The fifteen cockles that were collected around each pole (Stns 7 and 8) all belonged to the 2006 cohort (mean ± SD shell length = 23 ± 1 mm). All cockles harboured the same 7 parasite species. For each of the 5 dominant parasite species, a correlation was performed between the difference of abundance of metacercariae between 2 cockles and their spatial distance for all possible combinations. For 4 parasite species (*Himasthla interrupta, Curtuteria arguinae, Diphterostomum brusinae* and *Psilostomum brevicolle*) there was no correlation (p > 0.05) at both Stns 7 and 8. Conversely, *Meiogymnophallus minutus* exhibited a positive correlation between both stations (p < 0.05; Table 7).

Table 7. Mean (and SD) metacercariae abundance per trematode species in cockles *Cerastoderma edule* (*Hq*: *Himasthla quissetensis*; *Hc*: *H. continua*; *Hi*: *H. interrupta*; *Ca*: *Curtuteria arguinae*; *Mm*: *Meiogymnophallus minutus*; *Pb*: *Psilostomum brevicolle*; *Db*: *Diphterostomum brusinae*) at Stns 7 and 8. The minimum and maximum distance between 2 cockles (Scale 5, see Table 1 for scales) and correlation (Spearman R; n = 105; **bold** values are statistically significant at p < 0.05) between abundance and distance between 2 cockles are shown

Trematode species	Mean	SD	SD/Mean	R
Stn 7 (Distance, cm: Min. = 9, Max. = 133)				
Hq	7.4	3.5	0.5	0.06
Hc	1.4	0.7	0.5	0.07
Hi	161.5	56.9	0.4	**0.23**
Ca	18.9	9.3	0.5	0.001
Mm	20.6	10.8	0.5	**0.21**
Pb	3.3	2.0	0.6	−0.11
Db	1.6	1.1	0.7	0.09
Stn 8 (Distance, cm: Min. = 8, Max. = 316)				
Hq	5.0	2.5	0.5	0.13
Hc	4.3	2.2	0.5	0.17
Hi	1611.2	246.4	0.2	0.17
Ca	150.2	42.1	0.3	0.08
Mm	11.3	5.2	0.5	**0.41**
Pb	7.7	2.9	0.4	0.16
Db	1.7	1.2	0.7	**0.24**

Intrinsic heterogeneity of infection

Cockles were experimentally infected by *Himasthla elongata* cercariae at 2 temperatures, 16°C and 22°C. The 15 control cockles were free of *H. elongata* metacercariae. There was a higher infection success at 22°C (32.1 ± 1.3 metacercariae, i.e. 80% of introduced cercariae retrieved in the cockles as metacercariae) than at 16°C (24.5 ± 0.8 metacercariae, i.e. 63%; Student's *t*-test, df = 28, *t* = −5.07, p < 0.001). The ratio 'SD/mean' (a variability index) was 0.12 and 0.16 at 16°C and 22°C, respectively. These values were much lower than those calculated in the field at the cm scale (Scale 5; Table 7).

DISCUSSION

The diagnosis of parasite abundance and species richness in cockles highlighted the high aggregative patterns of trematodes at all scales of observation (from cm to km) and for most trematode species. For free-living species, the effects of biotic processes at the fine scale are generally overridden by physical processes at a larger scale (Wiens 1989). Our study suggested that biotic processes are the major drivers to explain trematode parasite distribution within a wide range of scales (from cm to km). Indeed, the trematode community seemed closely related to the distribution of host species (that are themselves under the control of biotic and abiotic factors).

At the km scale, we found significant differences when we compared the trematode communities among the 6 investigated sites. The potential first intermediate hosts of these trematode species (*Hydrobia ulvae*, *Nassarius reticulatus* and *Scrobicularia plana*) were present at all of our investigated sites (Blanchet 2004), whereas possible trematode species in cockles (downstream hosts) were not present everywhere. Moreover, when species were shared among sites, trematode abundances in cockles were different, with species-dependent patterns. The density of cockles and/or their shell length could contribute to spatial infection heterogeneity (Thieltges & Reise 2006, 2007), but in the present study dissected cockles displayed similar lengths and abundance everywhere. Among possible drivers of trematode distribution, the presence of birds (= definitive hosts) should be explored (Hechinger & Lafferty 2005, Byers et al. 2008). Characteristics of the water body could also interfere with parasite transmission by miracidium or cercariae larvae and may explain heterogeneity at this scale. In addition, differences in sediment grain size may have an indirect effect by attracting benthic macrofauna that are potential first intermediate hosts (Skirnisson et al.

2004). Finally, ambient fauna can also act as 'distracters', or decoy organisms, for the transmission of parasites (Thieltges et al. 2008). These decoy organisms can be predators preying on cercariae, non-host filter feeders that filter cercariae but do not become infected or alternative hosts becoming infected by cercariae and distracting them from target hosts. In conclusion, neither trematode species nor trematode abundance in cockles from 1 station can represent the infection pattern at a large observation scale.

The 10 stations that were compared at Arguin (×100 m scale) were situated within the same water mass, and cockles were buried in similar medium sands albeit at different tidal levels. Six of 7 trematode species were retrieved in cockles but with significant differences in their abundance. Presence and density of potential first intermediate hosts were often related to the abundance of trematodes in cockles as observed elsewhere at this scale (Thieltges & Reise 2006, 2007), although counter examples exist with no correlation between both variables (Poulin & Mouritsen 2004, Hansen & Poulin 2006). It can be assumed that cercariae dispersal by swimming activity and by passive current drifts was sufficient to infect cockles from all areas (de Montaudouin et al. 1998). However, cercariae have short life spans which limit their dispersal (Mouritsen 2002, Pietrock & Marcogliese 2003, Thieltges & Rick 2006), and consequently, the proximity of the final host ensured higher infection success. In our study, the density of infected first intermediate hosts would have been a better descriptor than the density of potential first intermediate hosts only. However, this would have necessitated too many dissections of first intermediate hosts because of the very low parasite prevalence in these species (de Montaudouin et al. 2003, Desclaux 2003). Beyond the first intermediate host, other factors could explain infection heterogeneity. Cockles from different sites could display different resistance patterns as observed with other bivalves (Bryan-Walker et al. 2007, Paul-Pont et al. 2010a). However, there is strong evidence that the larval dispersion pattern of cockles results in individuals from the same genetic pool in the whole area (G. Bachelet unpubl. data). Finally, the tidal level did not provide clear results. Indeed, for *Himasthla interrupta*, the most parasitised cockles came from the stations at the lowest tidal level, suggesting a longer period of possible infection. Conversely, for 4 other trematode species, lower infections occurred at the lower level stations. The latter case has previously been observed, although reasons for its occurrence remain unclear (Poulin et al. 2000, Mouritsen et al. 2003); a possible reason is the distribution of the infected upstream first intermediate host (Curtis 2007).

At the 10 m scale, the only clear environmental difference was the proximity of a subtidal pool at 1

extremity (Stn 7). It is also possible that microcurrents or hydrodynamic turbulences occurred in this area and contributed to the aggregation of parasite larvae. However, in the latter case, we could expect that all trematode species would accumulate in cockles from a given site. In contrast, the distribution pattern of metacercariae in cockles between both stations depended on trematode species. We propose that the decline of parasite abundance with distance followed a negative exponential function due to dilution of cercariae larvae in a 3-dimensional space (i.e. the water body; Fig. 4F) with a maximum abundance near the site of parasite emission by the upstream host and a rapid drop of this abundance with distance (Type 1). When distance increases or when the parasite emission is lower (scarce host and/or low prevalence), the slope of the function giving the parasite abundance in relation to the distance decreases and the best model becomes a linear function (Type 2, Fig. 4F). At great distance from the cercariae emission, or when the parasite is rare, no relationship to distance can be observed (Type 3, Fig. 4F). At this scale, even though the parasite species richness was similar, parasite abundance was homogeneous only at a distance <20 m.

The last field investigation concerned the heterogeneity of infection at the cm scale. At this scale, parasite species richness was equal among cockles but abundance fluctuated. There was no relationship between the abundance of metacercariae of *Himasthla interrupta, Curcuteria arguinae*, *Diphterostomum brusinae* and *Psilostomum brevicolle* and the distance among cockles. The upstream hosts of these species are motile snails that can wander around and infect nearby cockles. Conversely, there was a significant relationship between the abundance of *Meiogymnophallus minutus* in cockles and the distance among cockles, highlighting the presence of hotspots of infection at this small scale that could be related to the presence of the non-motile upstream host, the bivalve *Scrobicularia plana.*

The test of aggregation allowed an estimation of the relative importance of aggregation among stations and within stations. Aggregation was observed at both scales but was stronger among stations than within station (except for *Diphterostomum brusinae*, which was rare and showed similar abundance among stations). This suggested that factors such as the presence of other hosts were more important than intrinsic factors (e.g. resistance of individuals, condition).

Experimental infection, although restricted to a single trematode species, provided information concerning the strength of intrinsic factors contributing to infection heterogeneity. The hypothesis was that all cockles were challenged with trematode cercariae in an identical environmental situation (fixed salinity,

temperature, substrate, cercariae availability). Experimental results highlighted an intrinsic variability that could be assessed as the ratio of the infection abundance standard deviation and the related mean (SD/mean). This value reached 0.12 to 0.16 according to temperature and was within the same range as that found elsewhere for *Himasthla elongata* (0.22; Wegeberg et al. 1999) and *H. quissetensis* (0.30; de Montaudouin et al. 2005). These ratios were lower than the infection variability among cockles calculated at the cm scale within Stns 7 (SD/mean = 0.48) and 8 (SD/mean = 0.50). Amongst factors that can explain these differences, general fitness driving individual filtration rate (= infestation route) can be discriminating (de Montaudouin et al. 1998). There is also strong evidence of the inter-individual heterogeneity of the immune system of cockles facing 1 or multiple stressors (Paul-Pont 2010, Paul-Pont et al. 2010b).

The present study highlighted the strong spatial heterogeneity of infection patterns of cockles as second intermediate hosts for trematode species. The homogeneity of the parasite community structure (species × abundance) was valid within ca. 20 m, which was considered the 'distance of significance'. In other words, the parasite diagnosis of cockles is representative of cockles that are distributed within a ~20 m radius. The size of this area of significance will depend on the homogeneity of the ecosystem being considered, particularly the distribution of other potential hosts.

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