Effect of trematode parasites on cockle *Cerastoderma edule* growth and condition index: a transplant experiment

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ABSTRACT: We quantified the effect of trematode parasites in cockles through a 303 d transplant experiment. Cockles were sampled in 3 different sites on the southwest coast of France characterized by 3 different trematode communities, i.e. Royan (RO): high *Meiogymnophallus minutus* abundance near cockle heart, South Arguin (SA): high *Himasthla interrupta* abundance in the mantle margin, and North Arguin (NA): almost free of trematodes. Sampled cockles were individually tagged and placed in different cages at Banc d'Arguin, Arcachon Bay. At the end of the experiment, RO cockles had lost their trematodes, highlighting the fact that trematode lifespan can be short, while SA cockles maintained their trematode load. When cockles were the second intermediate host, there was no effect of site of origin on cockle condition index and survival. The effect on growth rate was mostly due to initial cockle length. Within each treatment, the only significant effect of trematode infection on growth was found for *H. interrupta* at SA. The 23% growth deficit observed could be due to the disruption of cockle shell synthesis in the mantle margin. During the transplant experiment, cockles were also infected at an exceptionally high rate by *Bucephalus minimus*, a trematode which invades most of the tissues and uses the cockle as a first intermediate host. Surprisingly, there was an effect on the growth rate and condition index of cockles at only one of the sites under study. This field experiment succeeded in quantifying the moderately negative effect of trematode parasites on the growth and condition of their host and suggested the existence of interactions with as yet unknown factors.

KEY WORDS: *Cerastoderma edule* · Parasitism · Growth · Mortality · Condition index · Transplant experiment

INTRODUCTION

Parasites are omnipresent and are important drivers in interpreting population dynamics of many marine species (Thomas et al. 1997). Their role is not restrained to the host level but can have implications on a broader scale, i.e. population and community levels (Lauckner 1987, Thomas et al. 1997, Mouritsen & Poulin 2002). For example, parasites can alter food web stability (Thompson et al. 2005, Lafferty et al. 2006), but also represent a potential path of energy flow in benthic systems (Thieltges et al. 2008). Among parasites, trematodes constitute a zoological group of helminths that specialize in mollusc infections. In their complex life cycle, trematodes can infect molluscs as the first or second intermediate host. Along the northeast Atlantic coast, one of their preferred mollusc host species is the edible cockle

Cerastoderma edule. A recent census listed 16 species associated with trematodes, from Norway to South Morocco, including the British Isles (de Montaudouin et al. 2009). In the first intermediate host, the parasite reproduces asexually and invades most host tissues, resulting in dramatic effects such as castration or death. This is the sporocyst or rediae stage, leading to the emission in the water body of thousands of new parasite propagules called cercariae. The parasite prevalence at this stage (i.e. percentage of infected hosts) is generally low, and the effect at the population scale is small. However, under certain environmental conditions (often remaining unidentified), prevalence reaches dramatically high values and is correlated with mass mortality (Jonsson & André 1992). Cercariae that are shed by the first intermediate host have only a few hours to infect the second intermediate host and to settle as a latent stage called the metacercariae. The effect on these second intermediate hosts seems less deleterious, since metacercariae do not reproduce in the host tissues: they wait for their host to be predated upon by the final host, a vertebrate, in which sexual reproduction takes place. However, it is considered that a high number of metacercariae, alone or in combination with other stressors like oxygen depletion and extreme temperatures, may provoke effects at the host population scale (Mouritsen & Jensen 1997, Wegeberg & Jensen 1999, Desclaux et al. 2004).

Estimation of the effects of trematode load on the population dynamics of marine species remains poorly documented and restricted to effects at the individual scale. For molluscs, most studies to date deal with gastropods that act as first intermediate hosts, and different trends were observed in different studies: depending on the host species, parasitism stunted growth (Sousa 1983, Curtis 1995, Huxham et al. 1995, Curtis et al. 2000), stimulated growth ('gigantism') (Rothschild 1936, Mouritsen & Jensen 1994, Zakikhani & Rau 1999), or had no effect (Anderson 1971, Mouritsen et al. 1999).

For marine bivalves, few studies have taken place on this subject, and for those that have, their execution has encountered some problems. Due to the low prevalence of trematodes in bivalves acting as first intermediate hosts, most investigations have concentrated on the interaction between metacercariae and bivalves as second intermediate hosts. An attempt was made to assess the loss of cockle production due to trematodes based on mortality calculations (Gam et al. 2009). Mortality was estimated by following a cohort, the drop in parasite infection being considered as parasite-dependent mortality (Anderson &

Gordon 1982, Kennedy 1984, Desclaux et al. 2004). The impact of metacercariae on cockle production and elimination was −35 and −25%, respectively. However, in these calculations, the effects of parasites on fundamental primary parameters of population dynamics, such as mortality, shell growth and/or flesh growth, were not taken into account. In order to avoid the methodological limitation of just sampling parasitized and non-parasitized individuals with no control of infection rate and other potential factors, Thieltges (2006a) experimentally infected mussels *Mytilus edulis* with *Renicola roscovita* cercariae shed by periwinkles, transplanted mussels in the field, and subsequently observed a growth deficit in these mussels. However, it was a short-term transplantation (10 wk). In another study, a similar design was performed with juvenile cockles, but also within a short-term transplant trial (1 mo) (Wegeberg & Jensen 2003). The authors observed a decline in growth rate in infected cockles, but introduced an artefact by unintentionally starving cockles during the laboratory trial. In another study, these authors demonstrated an interaction between metacercariae load and oxygen depletion on cockle mortality, but within a very short observation period (35 h experiment) (Wegeberg & Jensen 1999). Another problem with an experimental approach is that the infection success and response of the host can be modulated by the relationship between metacercariae dose and host body mass, introducing confounding methodological effects (Poulin 2010). Two studies demonstrated the effects of trematodes on bivalve growth when they act as first intermediate hosts. Lim & Green (1991) measured *Macoma balthica* shell growth rate using ring increments and correlated it to metacercariae number, considered here as a proxy for infection of the bivalve as a first intermediate host (due to the fact that the 3 involved trematode species utilize *M. balthica* as first and second intermediate hosts). This population was heavily infected as a first intermediate host, but the correlation between metacercariae infection and sporocyst infection was never demonstrated. Finally, the most definitive study concerns mussel *Perna perna* along the South African shore (Calvo-Ugarteburu & McQuaid 1998). The authors individually tagged the mussels, which were naturally infected at a high prevalence as first and second intermediate hosts. Unfortunately they did not count metacercariae, and they related the growth deficit that they observed in infected mussels to the parasite presence only.

Our main objective was to evaluate the effect of trematode infection on cockle. Then, we selected 3 areas in our study region with cockle populations dis-

playing differing characteristics in terms of trematode infection. Considering the experimental design, we expected that different trematode communities would lead to contrasting effects on cockle fitness (as the second intermediate host) and that trematodes using cockles as the first intermediate host would have a detrimental effect, based on the broad consensus that bivalves are severely impacted at this stage (Jonsson & André 1992, Calvo-Ugarteburu & McQuaid 1998, da Silva et al. 2002, Baudrimont et al. 2006, Thieltges 2006b, Lajtner et al. 2008, Dubois et al. 2009).

MATERIALS AND METHODS

Our primary aim was to measure the effect of trematode infection (as the second intermediate host) on cockle growth, condition index and survival, informed by the approaches taken in previously published investigations. Then, we selected 3 cockle subpopulations displaying contrasting characteristics in terms of trematode infection. One cockle subpopulation (at Royan; RO) had a particularly high abundance of *Meiogymnophallus minutus*, a trematode located near the cockle heart in infected animals; another subpopulation had a particularly high abundance of *Himasthla interrupta* (at South Arguin; SA), a trematode species that settles in the mantle margin; and a third subpopulation was almost free of trematodes (at North Arguin; NA) (Fig. 1). One unexpected positive and rare development during the study was that a high prevalence of infection by *Bucephalus minimus*, a trematode species using the cockle as the first intermediate host, occurred during the transplant experiment. That was a unique opportunity to measure the effect of this parasite, considered detrimental to cockle health, on a sufficient number of cockles of which original shell length was known. Condition index was also compared with non-parasitized cockles.

Sites

The 3 sites (Fig. 1) were selected due to their previously observed variation in trematode loads (de Montaudouin & Lanceleur 2011, X. de Montaudouin pers. obs.). Sites 1 and 2, NA and SA, are part of Banc d'Arguin National Reserve, Arcachon Bay, France
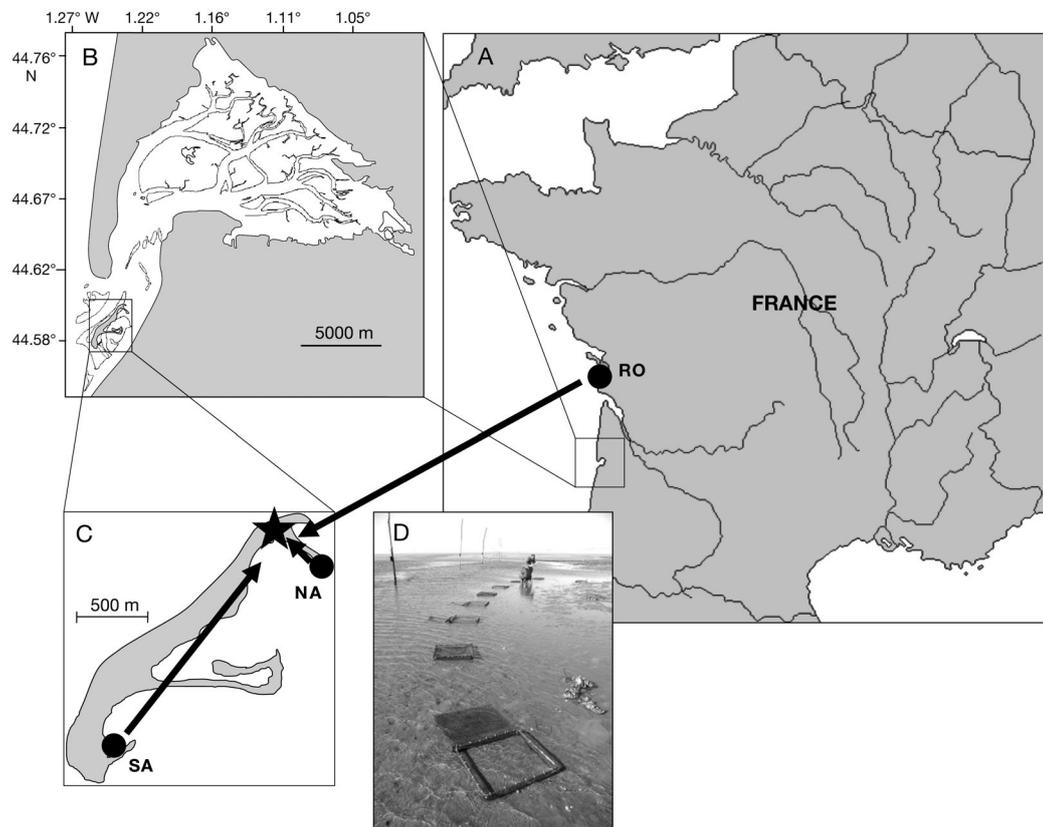

Fig. 1. Sampling stations (●) and destination site for the transplant experiment (★). (A) Location of the Royan site (RO), France. (B) Banc d'Arguin in Arcachon Bay, France. (C) South Arguin site (SA), North Arguin site (NA; where cockles were almost free of trematodes), and transplant site (★), to which all cockles were transplanted in cages. (D) Transplant site

(44.60° N, 1.25° W). The sampling sites were situated in moderately sheltered intertidal sandflats at both extremities of the bank. In both places, sediments are largely dominated by medium sands. Salinity remains high year-round (23 to 35). The mean surface water temperature fluctuates between 9.5°C in winter and 21.1°C in summer. The sediment temperature was measured during the experiment (data were collected regularly with an infrared optic shuttle and transferred to PC with BoxCar Pro v3.51) and exhibited wider fluctuation, from 4.5°C in March and December to 30°C in June. The trematode fauna in molluscs in Banc d'Arguin was recently described (de Montaudouin & Lanceleur 2011). Royan is the major town near Site 3 (RO), located in the sheltered lagoon of Bonne Anse (45.68° N, 1.20° W). This is a 7.5 km² tidal flat. The cockle population is found in medium sands (median grain size = 350 µm). At high tide, the lagoon is influenced by the Gironde estuary and the coastal Atlantic waters: sea temperature varies annually between 10 and 23°C, while salinity ranges between 26 and 32 (sometimes less significant when freshwater discharge occurs). The receiving site was Site 1, NA.

Transplant experiment

Cockles from the 3 sites were placed within 0.25 m² cages constructed to prevent emigration and immigration of adult cockles and to limit predation. Each enclosure consisted of a 50 × 50 × 25 cm metal frame meshed with 10 mm plastic net, buried 20 cm and projecting 5 cm above the sediment. In February 2008, 9 enclosures were installed in a single line parallel to the shoreline at 0.9 m above chart datum, i.e. at mean low water level, corresponding to mean emersion times of ca. 20%. The enclosures were regularly spaced with 2 m between each. Enclosures were cleared of all adult bivalves, then assigned to 3 treatments: (1) NA origin (N = 100 cockles, shell length ± SD = 22.7 ± 2.1 mm), (2) SA origin (N = 100 cockles, shell length = 21.9 ± 1.3 mm) and (3) RO origin (N = 100 cockles, shell length = 17.2 ± 1.8 mm). All cockles previously marked with an individual number and measured prior to transplantation. Three replicates of each treatment were established in a regular design by alternating each origin.

Cockle analysis

Prior to the transplant experiment, 10 adult cockles per site were analysed. Cockles were opened, the flesh was squeezed between 2 large glass slides, observed through a microscope (de Montaudouin et al. 2009) and trematodes counted. The parasite abundance is defined as the mean number of metacercariae per cockle, including infected and uninfected cockles, and the prevalence is the percentage of infected cockles (Bush et al. 1997). At the completion of the experiment, 303 d later, 10 cockles per cage were dissected as previously described (i.e. 30 cockles per site). Along with this screening, all remaining cockles were also dissected, but only to detect the presence of *Bucephalus minimus*. When a cockle was infected by this trematode species, the condition index of the cockle was measured as well as the condition index of an uninfected cockle of a similar shell length from the same cage. The condition index was defined as flesh dry weight (in mg)/shell weight (in g).

The total surviving cockles were also counted (to calculate survival rate), and shell lengths were measured. Shell growth rate was deduced from a von Bertalanffy growth function in order to take into consideration the initial shell length, which varied among sites: growth rate (mm d^{-1}) = [ln(final shell length) − ln(initial shell length)]/303 d.

Data analysis

The effect of site origin (and consequently of trematode load) on final total metacercariae number per cockle, *Curtuteria arguinae* abundance per cockle, cockle growth and cockle condition index was tested with a 2-way nested ANOVA, with site origin as a fixed factor and cage as a nested random factor (Sokal & Rohlf 1981). Prior to analysis, homogeneity of variance was verified with a Cochran test. Normality was assumed. Abundance of the 4 dominant trematode parasites in cockles, *Himasthla quissetensis*, *H. interrupta*, *Curtuteria arguinae* and *Meiogymnophallus minutus*, was compared between the beginning and the end of the experiment for the 3 sites of origin. Due to the amount of analyses (12 Student's *t*-tests), a significance level of $\alpha = 0.05/12 = 0.004$ was applied (Bonferroni correction). The effect of site origin on cockle survivorship was determined with a 1-way ANOVA (with origin as the fixed factor) after verifying homogeneity of variance. Correlations between parasite metacercariae number and cockle growth rate relative to each site of origin were calculated using the Pearson parametric correlation test. All these analyses were performed with cockles that were not the first intermediate host for *Bucephalus minimus*.

The effect of *Bucephalus minimus* on cockle growth and condition index was quantified for each site treatment with a Wilcoxon non-parametric test for dependent samples in order to compare 2 by 2 parasitized and non-parasitized cockles with similar shell length. All statistics were performed with Statistica version 6.1 (StatSoft).

RESULTS

Infection patterns in cockles as second intermediate hosts

As expected from previous observations, trematode infection of cockles from different sites, screened before the transplant experiment commenced, differed (Fig. 2A,B,C). After 303 d at NA (Fig. 2D), cockle trematode communities were partly modified, with 4 major observations (Fig. 2): (1) all cockles became infected by *Curtuteria arguinae*, with no significant difference due to origin and cages (Table 1). The mean abundance was 192 metacercariae per cockle (100% prevalence) as opposed to 0–1 metacercariae per cockle at the beginning of the trial (Student's t-test, p < 0.001 in each site of origin). (2) The mean abundance of *Meiogymnophallus minutus*, initially 1882 metacercariae per cockle at RO (100% prevalence, Fig. 2C) dropped to 2 metacercariae per cockle at the end of the survey (73% prevalence) (Student's t-test, df = 35, p < 0.001) (Fig. 2F). (3) For SA, the mean abundance of *Himasthla interrupta* remained stable at a mean of 227 metacercariae per cockle (Student's t-test, t = 2.44, df = 29, p = 0.021 [α = 0.004 after Bonferroni correction]), and prevalence was maintained at 100% (Fig. 2B,E). (4) The other trematode species, including *Himasthla quisseten-*
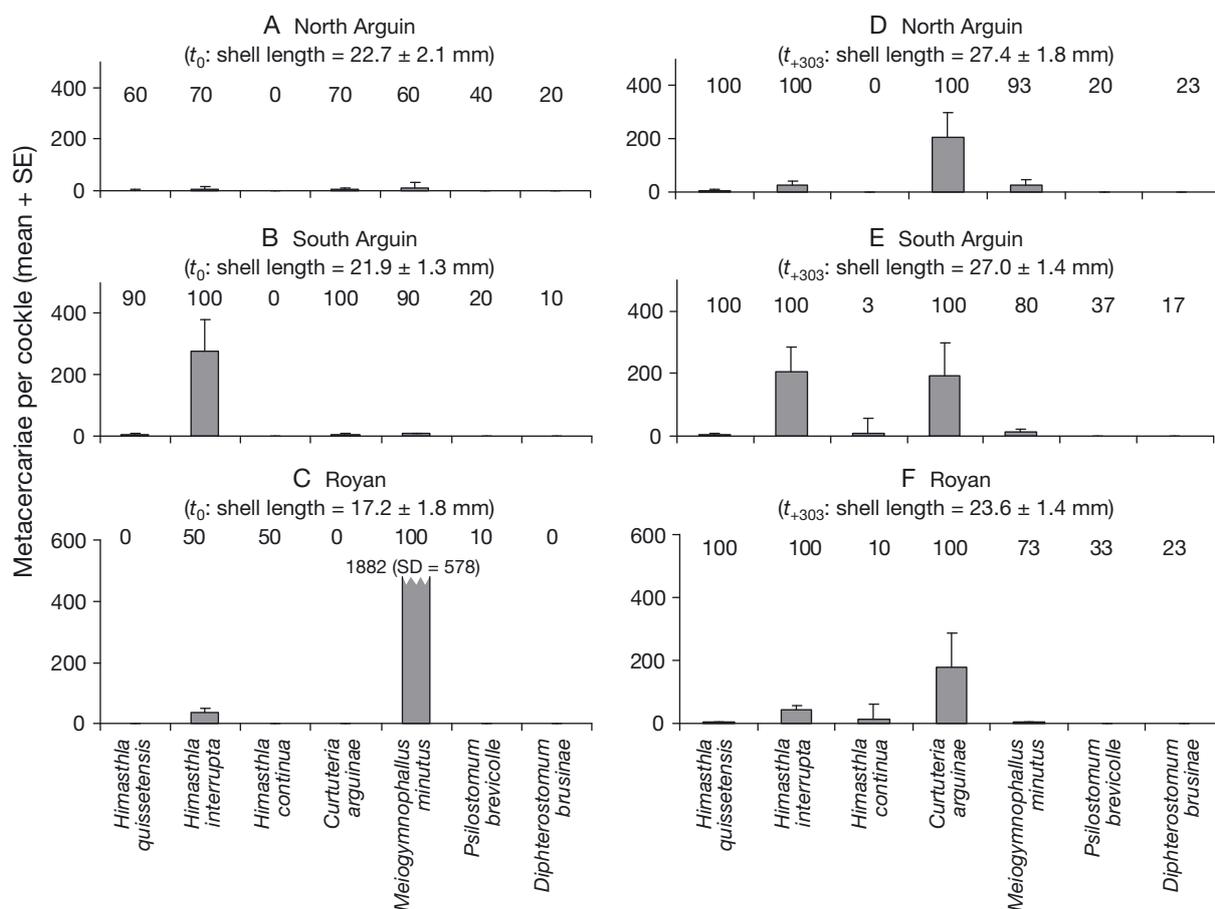

Fig. 2. *Cerastoderma edule.* (A,B,C) Initial and (D,E,F) final parasite abundance in cockles from the 3 different sites (mean + SD metacercariae number per cockle) and for the 7 trematode species using cockles as second intermediate host. Mean + SD cockle shell lengths are mentioned. t_0: initial time; t_{+303}: time after 303 d. Prevalence (%) is given for each species along the tops of the panels

sis, remained at a low abundance, and the final infection pattern, i.e. a significantly higher metacercariae infection in cockles from SA cages compared to cockles from the 2 other origins, was due to *C. arguinae* and *H. interrupta* (Fig. 2D,E,F, Table 1).

Table 1. Effect of site of origin on cockle survivorship (1-way ANOVA) and effect of site of origin and cage on total metacercariae number per cockle, *Curuteria arguinae* metacercariae per cockle, cockle condition index and cockle growth rate (2-way nested ANOVA). For pairwise comparison results (Tukey's test), see Fig. 3. RO: Royan site; NA: North Arguin site; SA: South Arguin

Variable	Effect	df	F	p
Metacercariae	Site	2	26.3	<0.001
(no. per cockle)	Cage(Site)	6	0.9	0.466
	Error	65		
Curuteria arguinae	Site	2	0.524	0.616
(metacercariae	Cage(Site)	6	0.459	0.836
per cockle)	Error	65		
Survival (%)	Site	2	4.266	0.070
	Error	6		
Condition index (‰)	Site	2	4.3	0.069
	Cage(Site)	6	5.3	<0.001
	Error	89		
Growth rate	Site	2	461.5	<0.001
	Cage(Site)	6	0.2	0.981
	Error	255		

Effects of parasites in cockles as second intermediate hosts

Cockles infected by *Bucephalus minimus* were not taken into account. Cockles from NA and RO had a similar level of infection, while cockles from SA displayed a higher metacercariae abundance (Fig. 3A, Table 1). There was no significant difference in the survival rates of cockles in cages in relation to their origin (Fig. 3B, Table 1). The overall survival rate was 37%. Condition index of cockles was not significantly different among origin sites (66‰) but there was a significant difference among cages (Fig. 3C, Table 1). The difference in condition index among cages was neither related to the position, as a gradient between the 2 ends of the row of cages, (Pearson correlation, N = 9, r = 0.55, p = 0.13) nor to cockle survival (N = 9, r = 0.35, p = 0.36).

There was a significant difference in growth rates in relation to origin (p < 0.001) (Fig. 3D, Table 1): the higher rates were encountered in cockles from RO and the lowest in cockles from NA . When correlating the number of metacercariae in cockles with cockle growth rate for each trematode species and origin, the only significant correlation was found for *Himasthla interrupta* in cockles from SA (Table 2). This relationship was not due to a correlation between number of *H. interrupta* metacercariae and initial cockle
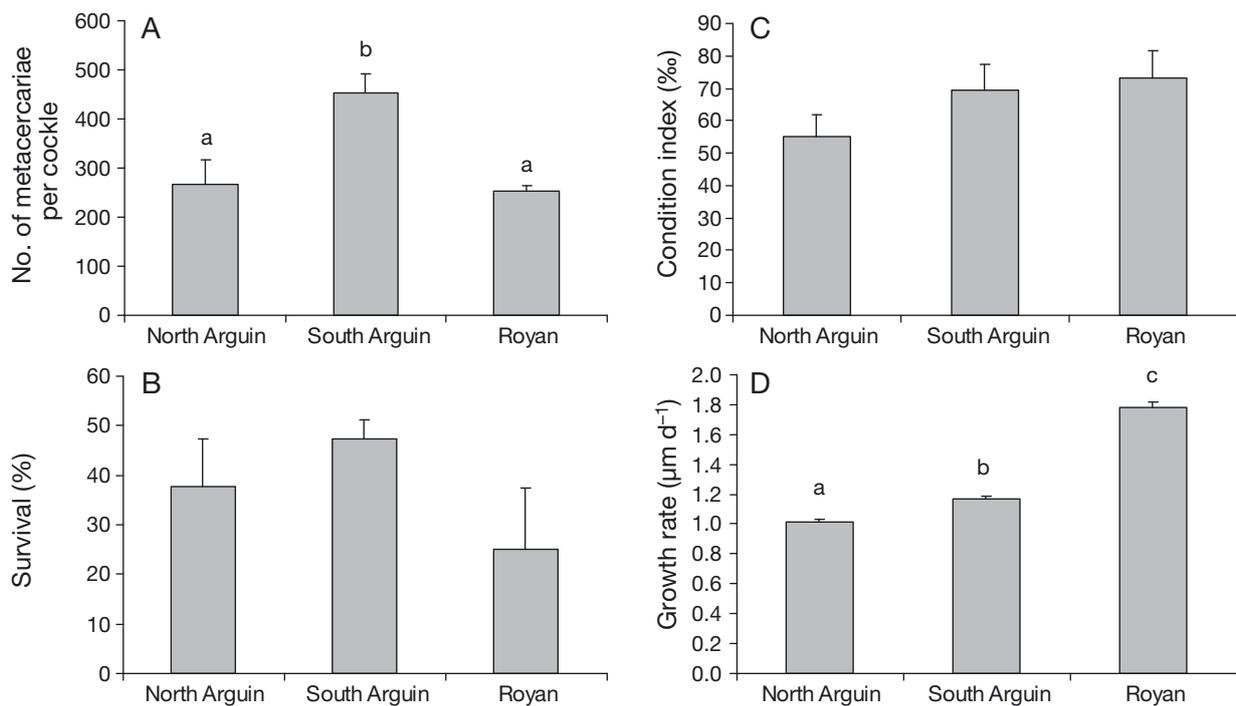

Fig. 3. *Cerastoderma edule.* Mean + SD of (A) metacercariae abundance, (B) survival, (C) condition index and (D) growth rate of cockles from the 3 different origins, at the completion of the experiment (+303 d). Cockles harbouring *Bucephalus minimus* were excluded from these analyses. Letters a–c show results that are significantly different (Tukey's test, p ≤ 0.05)

length (Pearson correlation, N = 30, r = 0.03, p = 0.89) or final cockle length (N = 30, r = −0.13, p = 0.51).

When pooling all cockles (except the individuals that were infected as a first intermediate host), mean growth increments per shell length class varied between 7.7 and 13.0 mm (within the 15–25 mm cockle length range). There was variation in growth of cockles among shell length classes, with a mean of 8 mm, a minimum of 3 mm (for the 20 mm shell length class) and a maximum of 14 mm (for the 22 mm shell length class) (Fig. 4).

Table 2. Correlation between parasite metacercariae number and cockle growth rate according to site of origin. Significant correlations are in **bold** (p ≤ 0.05)

Site of origin	Parasite species	df	r	p
North Arguin	*Himasthla interrupta*	24	0.11	0.60
	Himasthla quissetensis	24	0.09	0.68
	Curtuteria arguinae	24	0.03	0.88
	Meiogymnophallus minutus	24	−0.19	0.36
South Arguin	*Himasthla interrupta*	**19**	**−0.42**	**0.05**
	Himasthla quissetensis	19	−0.14	0.55
	Curtuteria arguinae	19	−0.33	0.14
	Meiogymnophallus minutus	19	0.03	0.89
Royan	*Himasthla interrupta*	25	−0.22	0.26
	Himasthla quissetensis	25	0.15	0.45
	Curtuteria arguinae	25	−0.22	0.28
	Meiogymnophallus minutus	25	0.16	0.42

Effects of parasites in cockles as first intermediate hosts

Prevalence of *Bucephalus minimus* in cockles at the end of the experiment was not significantly different among the different origins (ANOVA, $F_{2,6}$ = 0.55, p = 0.605) with an average of 17%, compared to 0% in control cockles at the beginning of the experiment.

The growth rate at 303 d was compared by a paired test between cockles of similar shell length with or without *Bucephalus minimus* (Fig. 5A). At NA, median shell growth was not significantly different between infected and uninfected cockles (Wilcoxon test, N = 16, z = 1.19, p = 0.230). At SA, shell growth rate was 12% higher in uninfected cockles than in infected cockles (Wilcoxon test, N = 32, z = 2.32, p = 0.020). At RO, median shell length increments were not significantly different between infected and uninfected cockles (Wilcoxon test, N = 11, z = 0.53, p = 0.590).

The effect of *Bucephalus minimus* on condition index varied among cockles of differing origins (Fig. 5B). It was significantly higher in infected cockles at NA (62 versus 53‰) (Wilcoxon test, N = 16, z = 1.91, p = 0.050), it was significantly higher in uninfected cockles at SA (67 versus 61‰) (Wilcoxon test, N = 32, z = 2.45, p = 0.014) and it was not significantly different in both situations at RO (78‰) (Wilcoxon test, N = 11, z = 0.44, p = 0.660).
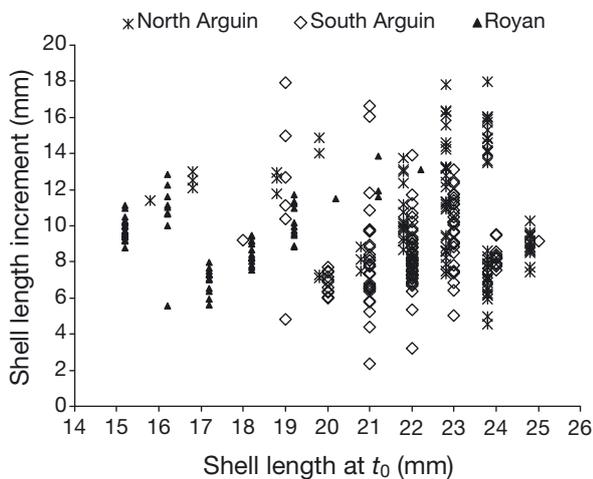

Fig. 4. *Cerastoderma edule.* Shell length increment during the 303 d transplant experiment in relation to initial shell length and according to site of origin. Cockles harbouring *Bucephalus minimus* were excluded from these analyses. t_0: initial time
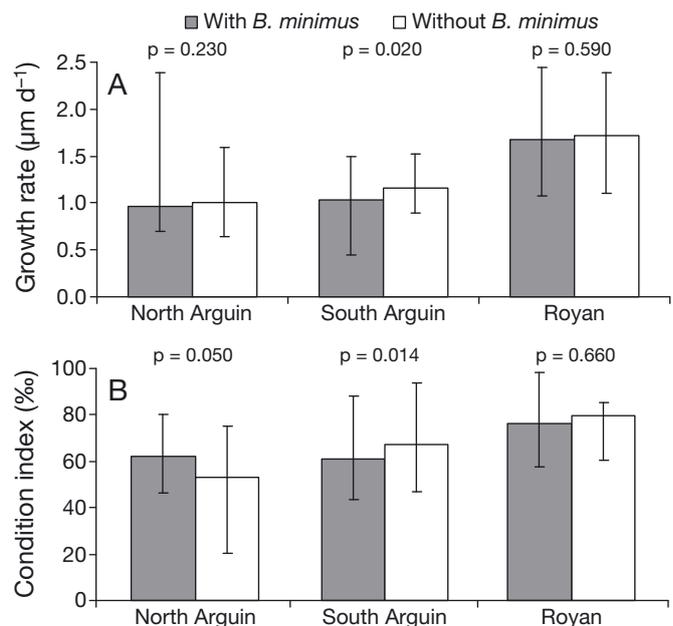

Fig. 5. *Cerastoderma edule.* (A) Growth rate and (B) condition index at the end of the experiment, with and without *Bucephalus minimus.* p-values of Wilcoxon test for dependent samples are specified, and bars extend between minimal and maximal values

DISCUSSION

This 303 d transplant experiment contributed to a better knowledge of trematode/cockle dynamics through 4 major findings concerning: (1) the dynamics of parasites; (2) the variability of cockle growth; (3) the effect of metacercariae on cockle (second intermediate host) fitness; and (4) the effect of trematode sporocysts on cockle (first intermediate host) fitness.

Parasite dynamics

During this transplant experiment, infestation by cercariae and mortality of metacercariae were observed. Infestation was not a surprise, considering that part of the experiment was conducted during the summer infection period and in a site where trematodes are potentially present (Desclaux et al. 2004, Gam et al. 2009, de Montaudouin & Lanceleur 2011). However, the infection remained very low for most trematodes using the cockle as a second intermediate host. The only significant occurrence was due to *Curtuteria arguinae*, with a mean abundance of 192 metacercariae per cockle. This is a rather severe infection compared to other studies (Desclaux et al. 2006, de Montaudouin et al. 2009), but it did not distort our analysis due to a similar prevalence of infection in all cages. More instructive information was obtained from the observed decrease of metacercariae in cockles from SA and RO. For cockles originating from SA, *Himasthla interrupta* metacercariae abundance at the end of experiment remained at the original level. Conversely, for RO, *Meiogymnophallus minimus* completely disappeared from cockles (−99.9%), precluding the hypothesis that the more infected cockles died. No metacercariae were hyperparasitized, as observed elsewhere (Carballal et al. 2005, Gam et al. 2008), and environmental conditions at Arguin are potentially suitable for infection to develop, as this parasite is present in the transplant area (de Montaudouin & Lanceleur 2011). Although transplantation can be a general explanation for *M. minimus* mortality, this study found that the lifespan of the metacercariae can be shorter than that of their host, and therefore one must be careful in always attributing a decrease in metacercariae in the host to parasite-dependent mortality (Anderson & Gordon 1982, Kennedy 1984, Desclaux et al. 2004, Gam et al. 2009).

Cockle growth variability

Growth variability among individuals of the same species is often attributed to biotic factors such as competition (Jensen 1993) or abiotic factors such as emersion time (Jensen 1992). In the present study, rigorous selection of cockles was undertaken to ensure that there was no variation in any parameters, with the only major difference being the site of origin. Because abundances alone are not sufficient to characterize bivalve populations with large individual size, the percentage of available surface area covered by cockles in the cages is calculated (Jensen 1985). In the present case, occupancy was <10% of available surface, i.e. below the potential threshold above which growth rates are reduced by intraspecific competition (de Montaudouin & Bachelet 1996). Individual tagging allowed quantification of growth variability and showed how high it was, independent of the site of origin (and so from parasite infection). Within 10 mo, the length increment varied by a factor of up to 5. This result explains how difficult cohort monitoring can be and highlights the inter individual variability that occurs, which was recently demonstrated in terms of individual vulnerability to parasites and in immune systems (Paul-Pont et al. 2010, de Montaudouin & Lanceleur 2011).

Effects of metacercariae on cockle (second intermediate host) fitness

There was no effect of the site of origin (or subsequent trematode load) on cockle survival within the transplant experiment. Predation by birds and fish was not possible, as a top grid was placed on all the cages. Therefore, trematode load alone did not result in cockle mortality. For the RO treatment (high *Meiogymnophallus minimus* abundance), we do not know how long the trematodes remained in the cockles, since they were absent by the end of the experiment. However, these non-encysted metacercariae are usually considered to be relatively harmless (Fermer et al. 2011) even though long-term monitoring gave them a significant (but moderate) responsibility for an observed reduction in cockle production (Gam et al. 2009). At SA, *Himasthla interrupta* abundance reached the highest values found within the whole cockle range in the north Atlantic without any effect on host mortality (de Montaudouin et al. 2009), as already observed in a 1 mo study that experimentally infected cockle juveniles with this trematode species (Wegeberg & Jensen 2003). Additionally, no effect on

cockle condition was found among treatments. These results demonstrated that cockles may accommodate high concentrations of metacercariae without exhibiting any severe detrimental effects under standard environmental conditions (Wegeberg & Jensen 1999). However, Calvo-Ugarteburu & McQuaid (1998) observed lower condition index in mussels *Perna perna* infected by the trematode *Proctoeces* sp., but only before spawning. The particularly large size of these metacercariae could explain this impact. Unfortunately, the authors correlated condition index to the presence of metacercariae only, whereas the number of metacercariae (abundance) is the most important variable (Desclaux et al. 2004).

Although cockle growth rate was calculated using the von Bertalanffy growth function, the effect on initial length was obviously the most important factor explaining growth performance: the smallest cockles at the beginning of the experiment displayed the highest growth rate. When investigating the correlation between growth rate and the number of metacercariae in cockles within each site, the only significant result concerned *Himasthla interrupta* in SA. The growth deficit between the less infected cockles and the most infected cockles reached 23%, which is of the same order of magnitude to that observed in *Renicola roscovita* infected and uninfected mussels (Thieltges 2006a). In the latter case, the author suggested various causes of the observed reduced cockle growth: tissue destruction and local inflammation, representing a physiological burden, the sustained energy demand required for immunological defenses, and the lower performance of the palps (the site of infection) in feeding activity. In our case, it is important to note that metacercariae accumulate in the outer folds of the mantle margin, which is involved in the secretion of the shell and consequently in growth (Ruppert & Barnes 1995). Therefore, *H. interrupta* invasion in the mantle could disrupt shell synthesis and alter host growth. However, the correlation was based on 21 cockles and only 17% of the variance in cockle growth was explained by the number of *H. interrupta* metacercariae.

Effects of sporocysts on cockle (first intermediate host) fitness

According to the broad consensus in previous studies that bivalves are severely impacted when acting as the first intermediate host (Jonsson & André 1992,

Calvo-Ugarteburu & McQuaid 1998, da Silva et al. 2002, Baudrimont et al. 2006, Thieltges 2006b, Lajtner et al. 2008, Dubois et al. 2009), we expected a dramatic effect of *Bucephalus minimus* on cockle growth and condition index. Surprisingly, these effects were moderate. A possible explanation is that the infection developed in the latter part of the experimental period. Moreover, since the bucephalid sporocysts infiltrate the gonad of their hosts, eventually replacing it, distinction between gonad tissue and parasite tissue is difficult and makes condition index calculation unreliable (Calvo-Ugarteburu & McQuaid 1998). In previous studies, meticulous dissection and separation of parasite sporocysts from cockle flesh allowed the calculation of an average value of 20% as representing the amount of parasitized tissues compared to whole flesh (dry weight) in these infections (Baudrimont et al. 2007, Dubois et al. 2009). Finally, the initial number of metacercariae (much higher at SA and RO than at NA) could have interacted with *B. minimus* presence. In previous studies, such interactions were observed with other factors such as oxygen depletion (Wegeberg & Jensen 1999), tidal level (Lim & Green 1991) or spawning period (Calvo-Ugarteburu & McQuaid 1998).

The role of trematode parasites in cockle dynamics is often overlooked or underestimated, due to the difficulty in determining the impact of these parasites, the complexity of their interaction with other factors, and the subsequent impact on the host. Parasite ecologists have pointed out numerous possible effects of these parasites, but it is difficult to assess this relationship and its cost to the host. The present study succeeded in quantifying the effect of trematode parasites on some population dynamics parameters of cockles such as survival, growth and condition index. It contributes to the few studies that assess the indirect effects of trematodes on bivalves. The ability of some trematode species to manipulate the phenotype of their intermediate hosts, enhancing predation probability (i.e. mortality) has been documented (Poulin 1995), but quantification of these effects remains rare. A positive relationship was observed between parasite intensity in the New Zealand cockle *Austrovenus stutchburyi* and the cropping intensity of their foot by fishes when trying in vain to burrow (Mouritsen & Poulin 2003a,b). However, our experiment also demonstrated that the effects of trematodes on their bivalve hosts depended on the parasite species present and also certainly on environmental factors, requiring further investigations of many other parasite-host systems.

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