

## ORIGINAL ARTICLE

# Impact of trematodes on the population structure and shell shape of the estuarine mud snail *Hydrobia ulvae* from a Southern European estuary

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## Keywords

Digenean; epibionts; gastropod intermediate host; Mondego Estuary; parasitism; shell morphology; shell size.

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## Abstract

The occurrence of trematodes within the gastropod *Hydrobia ulvae* was studied in two areas (a *Zostera noltii* bed and a eutrophic area) in the Mondego Estuary, Portugal. The aim was to assess trematode infections across snail age classes, as well as to investigate any influence of parasitism on the shell shape and size of these gastropods. In the *Z. noltii* bed, infection occurred in snails with shell height of 1.5–6 mm, but a higher prevalence was seen in intermediate size classes (3–5 mm). Infected individuals were never found among the elder gastropods. In the eutrophic area, infection was commonly observed within small individuals (1.6–2.5 mm), as large individuals were rarely found. The warm temperate climate in this coastal system may cause infected specimens to die before they can grow to larger dimensions, in contrast to Northern European populations. The frequency of snails with thin, distorted and corroded shells was higher among infected than non-infected snails. Infected individuals were frequently colonized by epibionts that may have additional detrimental effects and probably promote shell erosion. Infection with trematodes and their influence on snail size needs consideration in the evaluation of parameters related to population dynamics and population structure. If the implications of parasite infection are ignored, inadequate decisions about ecosystem management may be taken, resulting mainly from erroneous assessment of population structure, age composition and ecological longevity. The role of parasites is especially important to consider for systems under environmental stress.

## Introduction

Digenean trematodes are common parasites of marine organisms (Lauckner 1980; de Montaudouin *et al.* 2003). They typically have complex indirect life cycles, with obligatory alternation between sexual and asexual reproduction. Several trematode species use gastropods as their first intermediate hosts, where they reproduce asexually, producing rediae or sporocysts that later will give rise to free larvae (cercariae) in large numbers. Redial stages are known to be more damaging to the host than the sporocysts, since they feed directly on the snail's tissues,

whereas sporocysts feed by absorbing nutrients (Probst & Kube 1999; Sorensen & Minchella 2001).

Parasites may potentially harm their hosts, depending on the intensity of infection or the way they interact with their hosts. Trematodes can influence their snail hosts' survival, reproduction, growth and behaviour (Huxham *et al.* 1993; Mouritsen & Jensen 1994; Gorbushin 1997; Probst & Kube 1999; Mouritsen & Poulin 2002; de Montaudouin *et al.* 2003; Fredensborg *et al.* 2005). Infected snails are generally more vulnerable than uninfected ones to adverse environmental factors, such as desiccation, osmotic stress, oxygen deficiency, and tem-

perature fluctuations (Huxham *et al.* 1995; Jensen *et al.* 1996; Shinagawa *et al.* 2001; Marcogliese 2008). Eventually, the combined effect of trematode infections and extreme environmental conditions may cause high mortality rates, with potential implications for the host population (Jensen & Mouritsen 1992; Mouritsen & Jensen 1997; Mouritsen *et al.* 2005).

Trematode rediae and sporocysts normally develop in the digestive gland and gonad complex of the snails (Gorbushin 1997; Probst & Kube 1999). Parasites established in the gonads may lead to castration of the host, by tissue destruction, space competition and reallocation of resources or other chemical mechanisms (Hall *et al.* 2007; Lafferty & Kuris 2009). In these cases, the energy that was destined for gastropod reproduction may be diverted to the production of parasite offspring (Lafferty & Kuris 2009) or to somatic growth of the snail, leading to gigantism (Mouritsen & Jensen 1994; Gorbushin 1997; Probst & Kube 1999).

There is still debate as to whether such enhanced growth is a parasite adaptation to enhance the available space for parasite biomass and increased cercarial production (McCarthy *et al.* 2004), or whether it is an adaptive host response that may improve future reproductive output if the host survives the infection (Minchella 1985). Enhanced growth might also be a passive side-effect of parasitic infection. The observed relationship between snail size and prevalence is often explained by the fact that larger, and hence older, snails have been exposed to potential infection for a longer time. Increased food consumption rate with snail size may also enhance the possibility of ingesting trematode eggs, thus leading to infection (de Montaudouin *et al.* 2003).

Trematodes are known to influence not only the growth rates of their snail hosts, but also host shape (Krist 2000; McCarthy *et al.* 2004; Levri *et al.* 2005; Thieltges *et al.* 2009). Morphological variation in snail individuals is determined by a combination of genetic and environmental factors that influence development (Poulin & Thomas 1999; Krist 2000; Alda *et al.* 2010). A variety of factors such as substratum, diet, food availability, and parasitism may impact shell morphology (Fernandez & Esch 1991; Probst & Kube 1999). Alterations induced by parasites span the intracellular to organism levels (Miura *et al.* 2006). Given the different degrees of impact that rediae and sporocysts inflict on snail hosts (Probst & Kube 1999; Sorensen & Minchella 2001), it is expected that they would also have dissimilar effects on host shell morphology and size.

*Hydrobia ulvae* is host to a variety of digenean trematodes (Deblock 1980; Huxham *et al.* 1995; Field & Irwin 1999; de Montaudouin *et al.* 2003; Bordalo *et al.* 2011). It is considered a significant link in estuarine food webs

(Cardoso *et al.* 2002, 2005) with great importance for the functioning of ecosystems in which they are present (Lillebø *et al.* 1999; Cardoso *et al.* 2002, 2005). This mud snail is one of the key species in macrobenthic intertidal communities of the Mondego Estuary (Portugal) (Cardoso *et al.* 2002, 2005). In this system, *H. ulvae* recruits four times a year (March, June, July and September) and its life span varies from 16 to 20 months (Cardoso *et al.* 2002, 2005). Trematode species composition within this mud snail population and density patterns of infected individuals have been addressed before (Bordalo *et al.* 2011). In the present study, it was intended to assess how parasites influence the size and morphology of these gastropods and accordingly how they might interfere with analyses of population structure, thus potentially influencing studies of their population dynamics. Consequently, the main goals were: (i) to evaluate whether parasite prevalence increases with size/age, and (ii) to investigate any alterations in host morphology connected with parasitism.

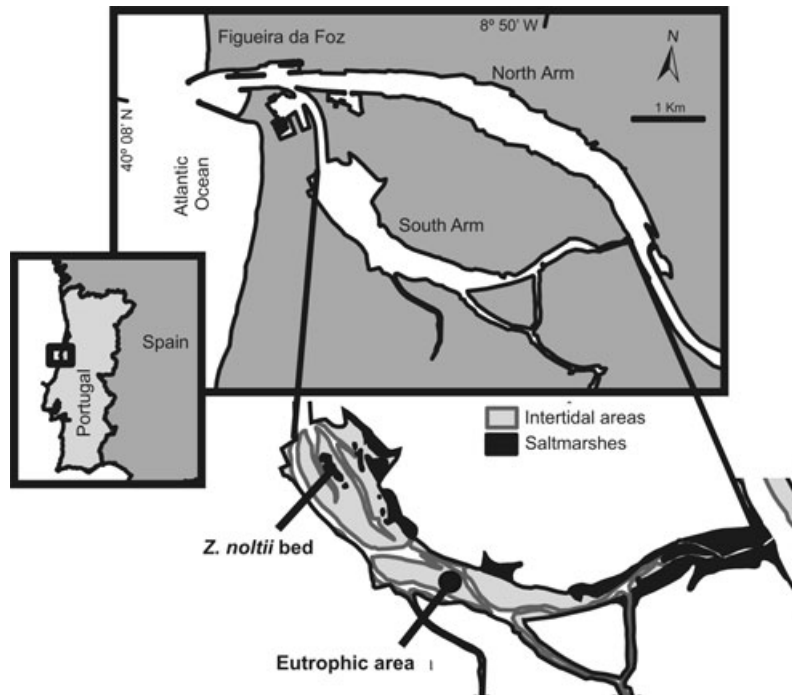
## Material and Methods

### Study site

The Mondego Estuary is a small coastal system (1072 ha) located in a warm temperate region on the central western coast of Portugal (40°08' N, 8°50' W). It comprises a north and a south arm, separated by Murraceira Island (Fig. 1). The north arm is deeper and constitutes the main navigation channel, being frequently dredged. The south arm is shallower and is characterized by large areas of exposed intertidal flats during low tide. Despite being relatively less impacted by human activities, the south arm is particularly susceptible to eutrophication due to its shallow depth, high water residence time, and elevated nutrient loading (Martins *et al.* 2001; Lillebø *et al.* 2005).

Two sampling areas in the south arm were regularly sampled, representing dominant habitats: (i) *Zostera noltii* bed: a mud flat covered with the seagrass *Z. noltii*, situated downstream, and (ii) eutrophic sand flat area: located upstream, in the inner section of the south arm, where *Z. noltii* disappeared decades ago as a result of eutrophication-induced seasonal green algal (*Ulva* sp.) blooms (Bordalo *et al.* 2011).

The nearby Coimbra forecasting station of the Portuguese Institute of Meteorology provided the monthly precipitation and atmospheric temperature data. Mean air temperature patterns vary according to the warm temperate climate of the Mondego region, increasing from spring (mean: 17 °C) to summer (mean: 21 °C) and decreasing in autumn (mean: 14 °C), towards winter (mean: 12 °C). In the summer, maximum air temperatures



**Fig. 1.** Location of the sampling stations along the south arm of the Mondego Estuary.

frequently reach values near 40 °C; temperature in intertidal pools can be higher than 30 °C (Bordalo *et al.* 2011).

#### Sampling programme and laboratory work

Macrobenthos was sampled between January 1993 and September 1995. This period has already featured in several studies (Cardoso *et al.* 2002; Ferreira *et al.* 2004; Jensen *et al.* 2004; Pardal *et al.* 2004; Dolbeth *et al.* 2007), including examinations of *H. ulvae* population dynamics (Lillebø *et al.* 1999; Cardoso *et al.* 2002) and an analysis of trematode diversity in the mud snails (Bordalo *et al.* 2011). Samples were taken in a haphazard manner each month during morning low tides, using six cores of 141 cm<sup>2</sup> at each station. Cores were taken to a depth of 20 cm in both the *Zostera noltii* bed and the eutrophic area. The resulting material was washed on a 500-µm-mesh sieve and preserved in 4% buffered formalin. For every sampling date at each sampling area, temperature and salinity were measured in low tide pools by means of a portable conductivity meter (model WTW v330i) with a standard TetraCon<sup>®</sup> 325 conductivity cell (Weilheim, Germany).

*Hydrobia ulvae* population structure was previously determined by recognition of cohorts from successive dates (Cardoso *et al.* 2002). In the present study, *H. ulvae* specimens collected in the sampling cores were sieved using a 1-mm mesh, to remove immature, and thus uninfected, juveniles (Probst & Kube 1999). Adult

specimens retained on the sieve mesh were sub-sampled using a Folsom splitter (Wildco<sup>®</sup>, Yulee, FL, USA) for a total of at least 300 individuals from each monthly sample. Replicates with smaller numbers of individuals were screened completely. *Hydrobia ulvae* individuals were measured for shell width, dissected, and inspected for parasites, which were identified according to Deblock (1980). It was only possible to identify infections with mature cercariae and therefore immature infections were not considered. The shell was removed by crushing it with a pair of tweezers and later cleaned in distilled water. Maximum shell width was measured instead of shell height because in most individuals the apex was missing or damaged. Therefore, shell height (mm) was estimated according to the following equation: shell maximum width = 0.4369 × shell height + 0.2091 ( $N = 339$ ,  $r^2 = 0.97$ ) (Lillebø *et al.* 1999). The relative proportion of infected individuals within each cohort was calculated to follow the evolution of their prevalence over time.

The shell of each specimen from the *Z. noltii* bed was described with respect to shape and state, including corrosion marks, epibionts, ballooning of the whorls, thinning of the shell, asymmetrical development of the spire and shell fragility (assessed while crushing the mud snails with tweezers for parasite inspection). It was impossible to give a similarly detailed description of the shells of preserved snails from the eutrophic area, as a large fraction of these shells were found to have

deteriorated after storage in formalin and because of their generally small size.

### Data analysis

A Scheirer–Ray–Hare (SRH) test was used as a non-parametric equivalent of a two-way ANOVA with replication (Dytham 2003) to test for differences in shell size of snails infected by trematodes from the most abundant families (Microphallidae, Haploporidae and Heterophyidae) between the two sampling areas (factors: site and trematode family).

Differences between the frequencies of shell shape abnormalities in relation to infection were examined by a chi-square test, including whether they were infected by trematode species whose life cycles include redia or just sporocyst larval stages. Statistical analyses were performed using IBM® SPSS® Statistics 20 (Armonk, NY, USA).

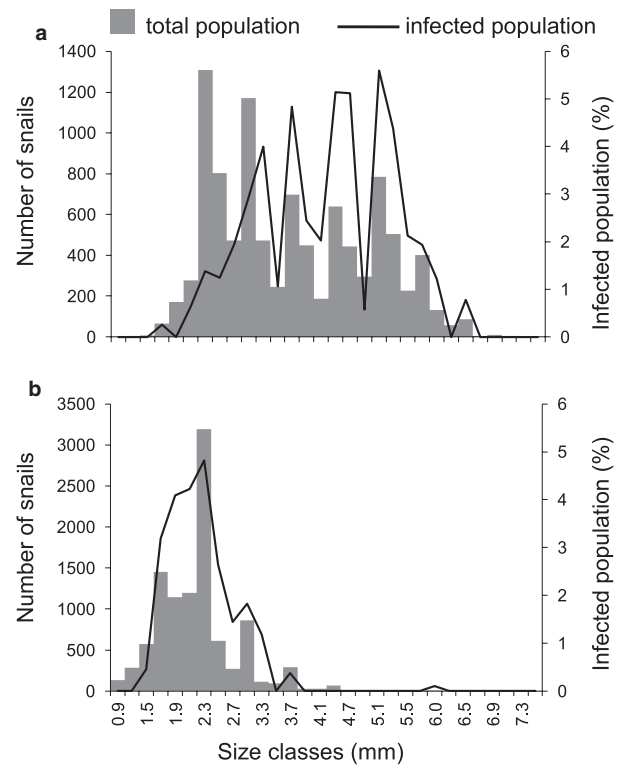
## Results

### Trematode fauna in relation to *Hydrobia ulvae* size and shape

A total of 14,260 *Hydrobia ulvae* specimens from the *Zostera noltii* bed and 11,014 individuals from the eutrophic area were examined. The population structure differed between the two sampling areas (Fig. 2). In the *Z. noltii* bed, the population was characterized by the presence of a wide range of size classes, whereas in the eutrophic area, mostly juveniles were found (Fig. 2).

Trematode species from five families were found within *H. ulvae* from the Mondego estuary: Microphallidae, Haploporidae, Heterophyidae, Notocotylidae and Echinostomatidae. Species from the first family produced only sporocysts, whereas species from all other families also had a redial stage. Neither double nor multiple infections were found. Only mature cercariae, within rediae or sporocysts, could be identified and therefore infections with immature larval stages were not considered in this analysis.

Digenean trematodes were found in specimens with shell heights of between 1.6 and 7.0 mm. In the *Z. noltii* bed, the proportion of infected individuals peaked in the intermediate size classes (3–5 mm), whereas at the eutrophic area they were more frequently found between 1.6 and 2.5 mm (Fig. 2a,b). Furthermore, the sizes of infected *H. ulvae* in the *Z. noltii* bed were significantly higher than those found in the eutrophic area (SRH test,  $P < 0.05$ ; Table 1, Fig. 3) in accordance with the different population structures observed in the sampling areas (Fig. 2a,b). On the other hand, the size of infected *H. ulvae* did not differ among trematode families (SRH test,



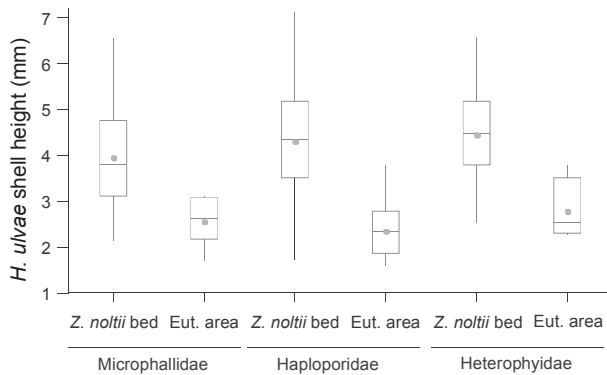
**Fig. 2.** Size structure of the *Hydrobia ulvae* population (absolute values of inspected individuals) and prevalence of infection (%) throughout size classes at (a) *Zostera noltii* bed and (b) eutrophic area.

**Table 1.** Results of a Scheirer-Ray-Hare (SRH) test with site (*Zostera noltii* bed or eutrophic area) and trematode family (infected with Microphallidae, Heterophyidae or Haploporidae) as fixed factors, using shell size as a dependent variable.

Source of variation	SS	SS/MS <sub>Total</sub>	df	P-value
Site (factor)	3043.06	7.57	1	0.01
Family (factor)	71.29	0.18	2	0.92
Size * family (interaction)	105.05	0.26	2	0.88

$P > 0.05$ ; Table 1, Fig. 3). There was no interaction between the two factors: trematode families and sampling areas (SRH test,  $P > 0.05$ ; Table 1, Fig. 3).

By tracking the evolution of the cohorts in the *Z. noltii* bed, it was observed that the percentage of infected individuals was relatively constant in median age/size individuals, and usually decreased in the larger size classes (Table 2a, Fig. 2a). In some cohorts (C2, C3, C4, C5, C9, C10, C11, C12) the infection decline occurred during or following the summer period, whereas in C7 and C8 infected individuals were found almost up until the disappearance of the cohorts. In these cases, the cohorts did not achieve their largest sizes during the summer period.



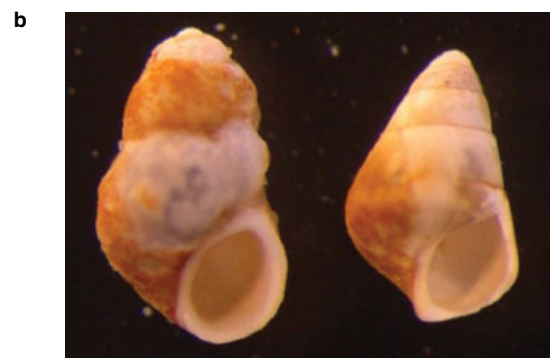
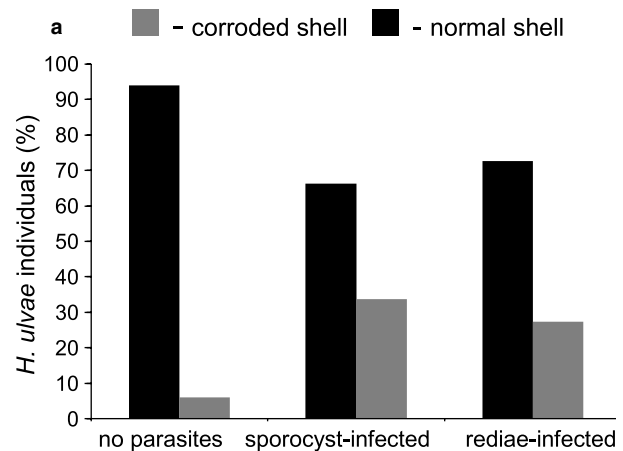
**Figure 3.** Box plot of infections in *Hydrobia ulvae* individuals by the most abundant trematode families Microphallidae, Haploporidae and Heterophyidae, in two sampling stations in the Mondego Estuary, *Zostera noltii* bed and eutrophic area, showing the shell sizes in which the infection is present. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles, respectively. The grey circles indicate mean values.

In the eutrophic area, infection appeared in young individuals that corresponded to the major stock of potential trematode hosts (Fig 2b). In practically all cohorts from this site, infection lasted until the disappearance of snails from this area, which occurred relatively early (Table 2b, Fig 2b) when they reached a shell length of approximately 3 mm (Fig. 2b). No obvious patterns were evident here, as infections in this area were scarce (Table 2b).

The frequency of abnormal shell shapes was significantly higher among infected than uninfected individuals ( $\chi^2_1 = 160.845$ ,  $P < 0.05$ ; Fig. 4) but there were no significant differences in the frequency of abnormal shells between snails infected with trematode species whose life cycles included rediae or those with just sporocyst larval stages ( $\chi^2_1 = 0.813$ ,  $P > 0.05$ ; Fig. 4).

## Discussion

In several previous studies of snail–trematode systems, an increased prevalence in the largest size classes was observed (Rothschild 1936; Mouritsen & Jensen 1994; Huxham *et al.* 1995; Gorbushin 1997; Field & Irwin 1999; Gorbushin & Levakin 1999; Probst & Kube 1999; de Montaudouin *et al.* 2003) (Table 2). In the present study, there was no evidence of larger individuals having more prevalent infections when compared with the rest of the population. Instead, infections were observed more often within the intermediate size classes. The impact of infection on the snail host is strongly dependent on its life history (Gorbushin 1997). In contrast to similar stud-



**Figure 4.** (a) Proportion of regular and irregular shells of *Hydrobia ulvae* in non-infected individuals and individuals infected with sporocysts and rediae, in the *Zostera noltii* bed, in the Mondego Estuary, from January 1993 to September 1995. (b) Shells of two *H. ulvae*, one infected with *M. subdolum* (left) and the other not infected (right). Both have the same width (1.2 mm) but the infected mud snail has a shell height of 2.5 mm whereas the non-infected snail measures 2.3 mm.

ies from systems at northern latitudes (Rothschild 1936; Mouritsen & Jensen 1994; Huxham *et al.* 1995; Gorbushin 1997; Field & Irwin 1999; Gorbushin & Levakin 1999; Probst & Kube 1999), infected snails from the present system may not live long enough to become 'giant'. These individuals commonly inhabit shallow-water pools formed during low tide, where temperatures may reach above 30 °C during summer, much higher than the values found in northern coastal systems. Considering the low thermal tolerance of debilitated infected snails (Jensen *et al.* 1996; Marcogliese 2008), an increased mortality of *Hydrobia ulvae* would be expected in the summer. These individuals might not outlive this season and consequently, the infected fraction of the population may not surpass 6–7 mm within a 16- to 20-month life span of the total population. The larvae living inside the snail inflict serious injuries in the tissues as well as



**Table 2.** Prevalence of infection within each cohort of *Hydrobia ulvae* (calculated as the proportion of infected individuals in relation to the number of individuals that belong to a given cohort) in the *Zostera noltii* bed and eutrophic area. The shaded areas represent the evolution of the cohort. The first column corresponds to the year of recruitment of the cohort. The second line of the column corresponds to the month of recruitment of the cohort (red – March, blue – June, green – July, yellow – September).

		Month																								
Year	Cohort	F93	A93	J93	M93	A93	J93	M93	A93	F94	J94	M94	A94	M94	J94	S94	O94	N94	D94	F95	M95	A95	M95	J95	S95	
92	C1	0.38																								
92	C2	0.38	0.55	0.43																						
92	C3	0.49	0.33	0.02																						
92	C4		0.33	0.02																						
93	C5		0.25																							
93	C6																									
93	C7																									
93	C8																									
94	C9																									
94	C10																									
94	C11																									
94	C12																									
95	C13																									
95	C14																									

		Month																								
Year	Cohort	F93	M93	A93	J93	M93	A93	J93	M93	A93	F94	J94	M94	A94	M94	J94	S94	O94	N94	D94	F95	M95	A95	M95	J95	S95
92	C3	0.24																								
92	C4	1.67	0.10																							
93	C5		0.07	0.20																						
93	C6		0.07	0.20	0.50	0.36	0.11																			
93	C7				0.54	0.23	0.31	0.30																		
93	C8				0.11																					
94	C9																									
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94	C11																									
94	C12																									
95	C13																									
95	C14																									
95	C15																									

pathological changes (Probst & Kube 1999). Moreover, keeping in mind that increased water temperatures usually promote a higher cercarial output (Mouritsen 2002; Poulin 2006; Marcogliese 2008; Studer *et al.* 2010) and that larger snails produce more of these free-living larval stages (Miura *et al.* 2006; Poulin 2006), very high temperatures may especially affect larger specimens. Hence, the deleterious effects of parasites may interact with extreme environmental conditions, leading to the death of the host (Jensen & Mouritsen 1992; Meissner & Bick 1999; Mouritsen 2002). Both stress factors influence the life history of these organisms, and both should be considered when analysing population structure. In this study, these effects were observed at low prevalence values (<6%). In highly infected snail populations (Table 3), parasites may be responsible for an even more noticeable variation in shell size, which might generate mistaken interpretations of the population structure, with strong repercussions in its further analysis.

The two sites studied represent distinct habitats that provide different environmental conditions for the mud snails, with implications for their population structures, which in turn have an influence on infection. Trematodes are found preferentially within adult individuals, as adult snails have mature gonads and a larger size, providing more soft tissue for parasite development and more energy resources (Probst & Kube 1999; Graham 2003; Miura *et al.* 2006). The *Zostera noltii* bed yielded an abundant and well age-structured mud snail population (Lillebø *et al.* 1999; Cardoso *et al.* 2005) and therefore considerable availability of potential hosts in this area. Infection in the *Z. noltii* bed was observed in almost all of the recognized cohorts, but was most obvious in the middle size/age classes. The largest/oldest size classes were represented mainly by uninfected specimens, probably because infected individuals died sooner than uninfected individuals, for the reasons mentioned above. On the other hand, in the eutrophic area, where the mud snail

population was dominated by juveniles, potential hosts were in short supply, so trematodes have no alternative but to infect small individuals in this location.

Shell shape modifications are frequently observed following trematode infection (Krist 2000; McCarthy *et al.* 2004; Hay *et al.* 2005). Infected mud snails often had thinner shells, with some distortions and with epibionts on the surface. According to Mouritsen & Bay (2000), trematode-infected individuals are more likely to become fouled by epibionts, probably because the parasites interfere with the anti-fouling mechanisms developed against epibiosis, mainly the production of toxic secondary metabolites. It may also be possible that the epibionts weaken the immune system of the snail, so that consequent infection is more likely (Mouritsen & Bay 2000). Parasitism may influence host morphology by altering rates of growth in one dimension but not in another (Levri *et al.* 2005), resulting in asymmetric shapes deviating from the 'normal' phenotype. Parasites may obtain profit from alterations in host shell shape (McCarthy *et al.* 2004), as the ballooning of some whorls may result in an increase in the total volume inside the shell, giving more room for the parasite and thus enhancing its cercarial production. Nonetheless, there is competition for space within the shell between host and parasite tissues (Sorensen & Minchella 2001). Alterations in the shape are visible if the parasites have a long period of time to grow within the snail (Krist 2000). For that reason, some infected individuals had a normal shell shape, in the case of early infections, when the effects of parasitism were still not evident. In addition, the parasite may not interfere with shell shape if it is not affecting growth rate, or not inflicting its maximum effect on the host (Krist 2000). A small proportion (6%) of non-infected mud snails also had an irregular shell shape, possibly due to a natural deficiency in the snail's defence mechanism not connected with parasitism. The thinner shells that frequently occurred in the infected individuals could be explained by the high energetic

**Table 3.** Prevalence data from studies of digenean trematodes in *Hydrobia ulvae* populations from other European localities. The presence or absence of a relationship between size and infection is indicated.

Author	Study site	Mean prevalence (%)	Max prevalence (%)	Min prevalence	Relationship with size
Fish & Fish (1974)	Dovey Estuary	2.8	3.8	1.4	n.a.
Huxham <i>et al.</i> (1995)	Ythan Estuary, Scotland	–	16.8	1.5	> 6.1 mm all infected
Sola (1996)	Bidasoa Estuary, Spain	6.5	–	–	n.a.
Field & Irwin (1999)	Belfast Lough, N. Ireland	12.6	25.5	–	Presence
	Ythan Estuary, Scotland	13.5	24.8	–	–
de Montaudouin <i>et al.</i> (2003)	Arguin, Arcachon Bay, France	–	16.0	0.0	> 5.1 mm all infected
	La Canellete, Arcachon Bay, France	–	6.0	0.5	Absence
	Lette Douce, Arcachon Bay, France	–	5.1	1.3	Absence

n.a., not applicable; indicates that such a relationship was not mentioned.

demand of the parasites that diverts important resources away from shell maintenance. This is probably due to the fact that the construction of shell material is energetically costly (Brusca & Brusca 2002). Accordingly, these snails are more likely to be crushed than others, being indeed more vulnerable than the non-infected snails.

Infected *H. ulvae* from the Mondego Estuary displayed a similar morphology irrespective of trematode type, with no size or shape differences being found. It was expected that redial and sporocyst infections might have different outcomes for the snail host, but instead a generalized response to infection was found. According to Sorensen & Minchella (2001) trematode species with redia larvae were expected to induce gigantism in their snail host more than those with sporocysts alone. Rediae are known to impose more physical damage on the host tissues than sporocysts because they feed directly on the host. Considering that rediae have a higher impact on the host snail, differences were expected to emerge regarding the degree of shell shape modification. In the present study, the results showed that trematode species induced changes in shell morphology of the snail host. However, no significant difference was found between species asexually reproducing by redial and sporocyst larval stages.

As the shell is a gastropod's main defense, alterations in shell shape or size may alter the effectiveness of the shell in protecting against predators (Krist 2000) or in competing with other individuals. As a result, alterations in shell morphology are likely to have fitness consequences for the snail host. Parasites may also play an important role in the phenotypic variation within the population, this role being increasingly important with higher prevalence. The influence of parasites on the population structure of the snail host can have implications in studies of population dynamics. Erroneous interpretation of the size frequency of the individuals of a given population will compromise understanding of age structure, individual growth rates, ecological longevity (by the recognition of cohorts, through size frequency analysis) and secondary production. Such errors may consequently influence the overall ecosystem investigation. Digenean trematodes arise as a cryptic factor that can interfere with the management of ecosystems, especially those that are under environmental stress, when their presence, quantity and implications are ignored.

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