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Primary Research Paper

Infection characteristics of a trematode in an estuarine isopod: influence of substratum

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Abstract

The estuarine isopod *Cyathura carinata* is a second intermediate host to microphallid trematodes, which use mud snails *Hydrobia* spp. and shorebirds as respectively first intermediate and final hosts. To identify processes responsible for infection patterns observed in *C. carinata*, a short-term microcosm experiment was conducted with both macroinvertebrates and one of their common parasites – *Maritrema subdolum*. Fine sand collected from two different shallow water sites was used to test if sediment type could affect infection rates. After 7 days at 25 °C, *C. carinata* from the substratum with the highest proportion of particles < 125 μm were more surface active and obtained significantly more *M. subdolum* individuals than isopods from the other sediment type. No parasite-induced effects on the hosts were found during this short-term experiment. The distribution pattern of microphallid cysts and mesocercariae inside the isopods revealed that *M. subdolum* cercariae primarily penetrated through the pleopods and afterwards located themselves in the middle-posterior region of the host's body. Even if it was not possible to identify the factor responsible for the observed infection patterns (cercariae production and/or host behaviour), the results of this experiment indicate that small-scale factors, such as differences in substratum and associated features, may have considerable impact on infections of host populations.

Introduction

In marine shallow water ecosystems, many invertebrates are hosts to a rich and diverse trematode fauna. In particular, prosobranch snails and either crustaceans or bivalves are used as first and second intermediate hosts by digenean trematodes (Lauckner, 1980, 1983; Meyers, 1990). These parasites are widespread in estuaries and coastal lagoons where their final hosts (water birds) congregate. It is known that parasites may have an impact on physiology (Meissner & Bick, 1999) and behaviour (Thomas et al., 1998; McCarthy et al., 2000; Combes, 2001) of their host specimens. However, population consequences have not yet been sufficiently explored. Recent evidence suggests that trematodes may determine the dynamics of their invertebrate host populations, with long-lasting community consequences (Jensen & Mouritsen, 1992; Meissner & Bick, 1997; Mouritsen & Jensen, 1997; Mouritsen et al., 1997, 1998; Meissner, 2001). Some digenean are known to promote trophic transmission by manipulating their intermediate hosts' behaviour (Lafferty & Morris, 1995; Thomas et al., 1998; Combes, 2001). So, considering digeneans potential to affect invertebrate populations, identification of ecological factors that influence spatio-temporal dynamics of parasites is an important issue.

High prevalence and intensity patterns of microphallid trematodes were observed in the isopod

Cyathura carinata from the Mondego Estuary, western coast of Portugal (Jensen et al., 2004). The trematode *Maritrema subdolum* is frequent within the isopod and also within its first intermediate host, the mud snail *Hydrobia ulvae* (Jensen et al., 2004). So far, trematodes have only been reported from *C. carinata* a few times (Reimer, 1963; Schulenburg & Wägele, 1998; Schulenburg et al., 1999; Jensen et al., 2004). Therefore, studies examining host effects or factors influencing infection characteristics are needed. Spatial patterns of parasites in *C. carinata* (Jensen et al., 2004) could be related to changes in sediment composition. So, cercariae production may depend on the availability of food resources for the snail host, such as bacteria and microalgae. The behaviour of the second intermediate host may also vary with sediment properties and thus their exposure to cercariae. To study both *M. subdolum* infection patterns and parasite effects on *C. carinata*, a small-scale short-term experiment was established with substrata from two different shallow water sites. Behaviour, survival rate, infection characteristics and parasite distribution within the hosts' body were the response variables analysed.

Materials and methods

Specimens' collection and storage

H. ulvae snails were collected at the Mondego Estuary. In laboratory, these gastropods were checked individually for cercariae shedding in small petri dishes, at 20 psu and 24 °C, under constant light conditions. Cercariae were identified to species level, according to Deblock (1980). Infected *H. ulvae* were sorted and kept separately in small aquaria at 15 °C.

C. carinata individuals were also collected in the same estuary, at a site where the population is abundant and almost non-parasitised. Collected specimens were kept in laboratory at 20 psu and 15 °C.

Sediment collection and treatment

Sediment was collected from two Danish estuaries (Haurvig, Ringkøbing Fjord and Aggersund, Limfjord). The two substrata differed visually in colour: one was brown whilst the other was light yellow. Sediment was sieved through a 0.5 mm mesh to remove macrofaunal individuals. At the end of the experiment, organic matter content and granulometric properties were determined (Holme & McIntyre, 1984). Unfortunately, most of the organic matter was washed away during the sieving process. The two substrata had similar median grain size (≈210 μm). However, one substratum contained a lower proportion of the grain size fraction < 125 μm than the other (9.4% within the brown substratum vs. 18% within the yellow one).

Experimental design

Twenty plastic containers were used as experimental aquaria. Each one had a 4 cm layer of sediment and 1 l of brackish water (≈22 psu). Brown substratum was used in half of the containers and yellow substratum in the other half. The aquaria were kept in a 25 °C room with a 12 h light/darkness cycle. A lid to diminish evaporation covered all containers. To prevent oxygen depletion, air was supplied through a glass pipette, 15 min each 4 h of light and each 2 h of darkness. The aquaria were placed randomly to avoid gradient effects. Experimental animals were introduced 24 h after the system was established. They were carefully selected to secure identical sizes in each container.

Five *H. ulvae* snails were introduced in each aquarium. They were enclosed in a net cylinder (1 mm gauged mesh, 35 mm in diameter), placed in the center of every container, to prevent their escape from the sediment-water system. Each sediment treatment consisted of five aquaria containing *M. subdolum* infected *H. ulvae* (treatments) and five other with *Cryptocotyle* infected snails (controls). *Cryptocotyle* do not use *C. carinata* as their second intermediate host, but encyst on fish instead. This was preferable rather than using non-infected *H. ulvae* because it is difficult to prove a non-infected state of living snails (Curtis & Hubbard, 1990). Afterwards, 16 *C. carinata* were added to each aquarium, corresponding to a density of 1304 ind m^{-2}, equivalent to the natural conditions when the experiment was performed (Pardal et al., 2002; Ferreira et al., 2004). The isopods could freely pass through the net container enclosing the snails.

The experiment ran for 7 days. Temperature, salinity and animal activity were daily checked. The number of isopods swimming, crawling on the surface or extruding the burrow were registered every morning, as well as the presence of tracks on the sediment of all aquaria. At the end, *H. ulvae* were removed from the containers, and each group was kept isolated in Petri dishes and stored at 15 °C. The aquaria were emptied and live *C. carinata* were counted. Afterwards, they were placed at 6 °C until dissection. All specimens were sexed, measured and inspected under a stereomicroscope for the presence, number and location of parasites. *M. subdolum* were found inside *C. carinata*, both as mesocercariae (tailless cercariae, moving in the host's tissues) and as recently formed metacercariae (encysted form of the parasite).

Cercarial emergence

To estimate cercariae shedding rates, *H. ulvae* snails were incubated again in the 25 °C room, after having recovered for one week at 15 °C. After 48 h under the same environmental conditions as the ones used during the experiment, each group of snails was removed to a clean Petri dish containing only brackish water. After 1 h, they were re-established in their original dish. The cercariae produced were stained with neutral lugol and counted under a stereomicroscope. The same procedure was repeated on the two following days.

Data analysis

Statistical analyses were performed using the MINITAB 10.2 software package. All data were inspected for violation of required assumptions and, if so, proper transformations or non-pararametric tests were used (Zar, 1996).

Results

Infection rates and survival

During the 7 days experimental period, significantly more *C. carinata* specimens from the yellow than from the brown substratum became infected with *M. subdolum* (yellow substratum: 67.1 ± 7.5% vs. brown substratum: 22.2 ± 5.1%, mean ± SE; *t*-test: $t_8 = -4.95, p < 0.01$) (Fig. 1a). No infections were observed in isopods from containers with *Cryptocotyle* infected snails. *M. subdolum* was also significantly more abundant within infected *C. carinata* from the yellow substratum (yellow substratum: 31.0 ± 4.6 parasites vs. brown substratum: 7.2 ± 2.9 parasites; mean ± SE; *t*-test: $t_8 = -4.41, p < 0.01$) (Fig. 1b). A maximum of 12 trematode larvae were found in a single individual.

Pooling together the 3 h of *H. ulvae* post-experimental shedding, snails from the brown substratum produced 438 cercariae (median), varying from 267 to 896, whereas those from the yellow substratum produced 1086 cercariae (median), varying from 186 to 2013. This difference was not significant (Mann–Whitney $U = 6$, $p > 0.05$). Neither was there a significant correlation between the infections obtained and the number of cercariae produced in each aquarium (Spearman's rho $= 0.333$, $p > 0.05$, $n = 10$).

C. carinata survival rates were higher on the brown substratum (98.8 ± 1.3% for control and

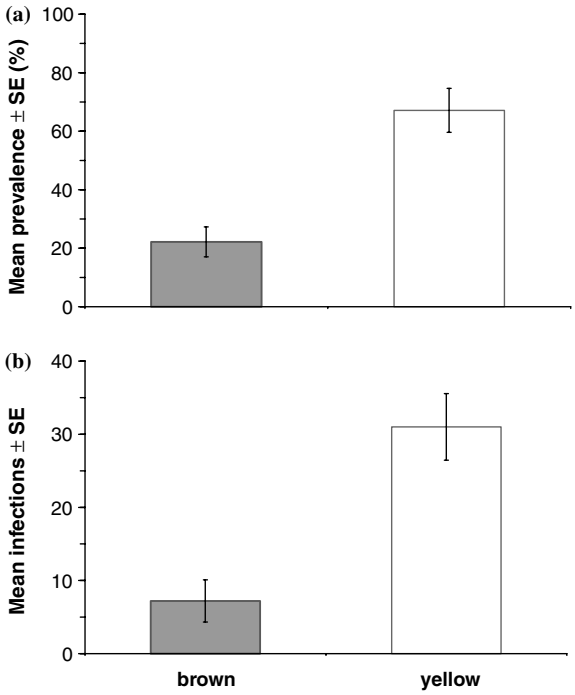

Figure 1. (a) Prevalence of *M. subdolum* in *C. carinata* and (b) intensity (mean ± SE) of the total number of infections found in each replicate of the brown and yellow substrata aquaria.

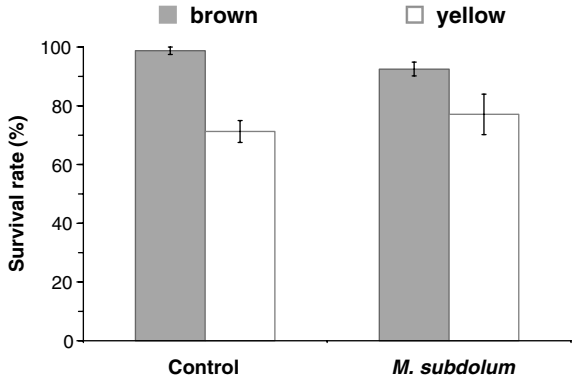

Figure 2. Survival rate (mean ± SE) of *C. carinata* as a function of substratum and parasite treatment (control: isopods incubated with *Cryptocotyle* infected snails).

92.5 ± 2.3% for *M. subdolum* treatment; mean ± SE) than on the yellow one (71.3 ± 3.8% for control and 77.2 ± 6.9% for *M. subdolum* treatment; mean ± SE) (Fig. 2). A two-way ANOVA revealed a significant effect of substratum type ($F_{1,16} = 26.98$, $p < 0.001$), but there were no parasite effects ($F_{1,16} = 0.00$, $p > 0.05$) nor any interaction between the two factors ($F_{1,16} = 2.17$, $p > 0.05$). No correlations were established between survival rates and the numbers of infections acquired by all *C. carinata* within *M. subdolum* treatment aquaria ($r_3 = -0.268$, $p > 0.05$ for the brown substratum and $r_3 = -0.447$, $p > 0.05$ for the yellow one).

Parasite location within C. carinata

Most mesocercariae were found in the posterior body segments (Fig. 3). The pleon accommodated 70% of the mesocercariae and 44% of all cysts. The head region did also contain a considerable fraction of mesocercariae (8.6%). Still, most metacercariae were found scattered along the body cavity of the pereon (52% in the total). Although dead *C. carinata* individuals were removed and examined daily, no trematodes were found within them. The rapid decomposition of dead isopods can destroy the mesocercarie present in the host tissues. This could have underestimated the total numbers of infections obtained. In the yellow substratum, numerous nematodes where found inside the remaining exoskeletons of dead *C. carinata*. They may also have contributed to the absence of mesocercariae in dead isopods.

Host behaviour

In the beginning, it was possible to observe a higher activity of isopods from the yellow substratum containers (Fig. 4a). However, it declined with time, ceasing after the fifth day. At the same time, sediment tracks appeared from the fourth day onwards, mainly within the brown substratum aquaria (Fig. 4b). Tracks were made during darkness, being perfectly visible in the morning and fading throughout the day, showing that *C. carinata* was more surface active at night.

A one-way ANOVA was performed to test if the intensity of microphallid larvae differed between males, ovigerous and non-reproductive females (respectively, 2.1 ± 0.5, 3.2 ± 1.0 and 3.4 ± 0.4 parasites, mean ± SE). However, there were no significant differences between them ($F_{2,50} = 0.95$, $p > 0.05$), neither between the frequency of infected and non-infected specimens (8 males, 5 ovigerous and 47 non-reproductive females infected with *M. subdolum* vs. 6, 9 and 66 non-infected individuals, respectively; χ^2-test: $\chi^2_2 = 0.222$, $p > 0.05$). Also, no relation between *C. carinata*'s cephalic length and infection intensity was found ($r_{58} = 0.079$, $p > 0.05$).

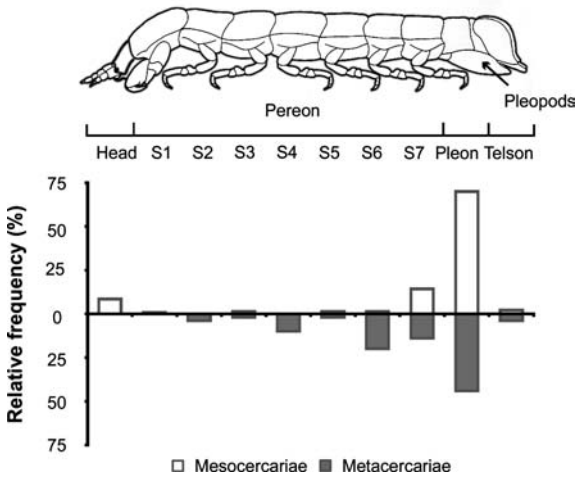

Figure 3. Distribution of meso ($n = 140$) and metacercariae ($n = 50$) of *M. subdolum* in different body parts of infected *C. carinata* ($n = 60$). S1–S7 are the seven segments of the pereon region of the isopod's body.

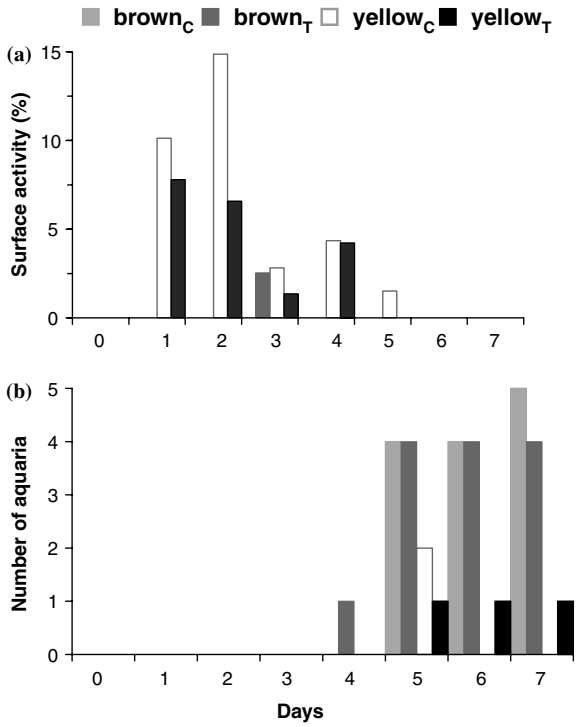

Figure 4. Surface activity of *C. carinata* in the brown and yellow substrata aquaria. (a) Relative frequency of isopods swimming, crawling on the surface or extruding the burrow, during light hours. (b) Number of aquaria with isopod tracks on the sediment's surface, made during darkness. (**C**, control; **T**, *M. subdolum* treatment).

Discussion

Prevalence and intensity of *M. subdolum* were higher within *C. carinata* from the yellow than from the brown substratum. Variation in either cercariae production and/or isopods' behaviour in relation to substratum type could be responsible for the observed pattern.

H. ulvae from the yellow substratum seemed to produce more cercariae than those from the brown type aquaria, promoting higher infection rates. The higher proportion of particles < 125 μm from the yellow substratum could indicate the presence of more food for the snails, as they ingest the small grains and their associated microalgae. Nevertheless, data did not support an impact of shedding-rates on the numbers of infections acquired in *C. carinata*. Post-experimentally estimated short-term rates may not necessarily reflect cercariae production during the experimental period (Curtis & Hubbard, 1990). They are normally subjected to considerable variation within and between snails (Théron & Moné, 1984). Sediment contamination by heavy metals or other pollutants have been proved to affect the survivorship and/or transmission capacity of digenean cercariae (MacKenzie, 1999; Morley et al., 2003). Still, the sediment used in the experiment was collected from brackish water sites inhabited by dense and varied stocks of invertebrates. Furthermore, the sites are situated far away from any industrial plants that potentially could be sources for contaminants.

M. subdolum is frequently found in *C. carinata* from the Mondego estuary (Jensen et al., 2004), but this experiment revealed that it has a low transmission success. Based on the post-experimentally estimated shedding rates, it is plausible to assume that many thousands of cercariae were released in each aquarium. Nevertheless, only a maximum of 42 infections was found in one of the experimental *C. carinata* populations. Behavioural traits may contribute to this. *M. subdolum* cercariae swim in the bottom layer of the water column, (Mouritsen, 2002), what may be interpreted as an adaptive strategy to reach bottom-dwelling crustaceans (Meissner & Bick, 1997; Mouritsen & Jensen, 1997; Mouritsen et al., 1998; Mouritsen, 2001). *C. carinata* is a predator (Wägele, 1981; Ólafsson & Persson, 1986) that spends most of the time hiding and waiting for a prey to pass above its burrow. As *M. subdolum* distribution within the *C. carinata*'s body indicated that pleopods were the main infection path, cercariae have to be drawn inside the isopod's burrow, through very weak ventilation currents created by it (own observation). When caught on the pleopods, the cercariae crawl towards the host's body and penetrate the cuticula. Therefore, even if *M. subdolum* is able to infect *C. carinata* and metacercariae have been found inside it (Reimer, 1963; Schulenburg & Wägele, 1998; Schulenburg et al., 1999; Jensen et al., 2004), this isopod does not seem to be its most adequate host. The low parasite transmission observed in this experiment suggests that it may be difficult for cercariae swimming in the boundary layer to locate a host that remains hidden most of the time.

C. carinata from the yellow substratum aquaria were frequently visible on the surface during the first days. This higher activity implied a greater

exposure to cercariae. For instance, swimming involves more intensive strokes of the pleopods, which will attract cercariae. Although no mesocercariae were found on pereopods, *M. subdolum* cercariae can attach to them as the isopod crawls on the surface (own observation). *C. carinata*'s head was another susceptible body part, since it is frequently exposed above the sediment when they sense a possible prey nearby. Therefore, isopods from the yellow substratum had a higher probability to become infected than those from the brown substratum, which mostly remained hidden in their burrows. Still, tracks were found in the mornings of the last days, as an indicator of excursions during darkness. Hunting for food could explain this nocturnal activity. Although all *C. carinata* behaved similarly, burrowing immediately when first inserted in the containers, the higher daytime activity on the yellow substratum suggests that there were some unfavourable conditions associated with this sediment.

In the present short-term experiment, no differences could be established between controls and *M. subdolum* treatments regarding survival rates. Nonetheless, high infection intensities might still have some consequences for *C. carinata*, like it has been reported for the amphipod *Corophium volutator* (Mouritsen & Jensen, 1997; Jensen et al., 1998; Meissner & Bick, 1999). No metacercariae were ever found inside *C. carinata*'s head and the encystment occurred only in pereon and pleon segments. So, no ganglia control mechanisms over the isopod's behaviour appear to be exerted by *M. subdolum*, as has been reported for *Microphallus papillorobustus* using *Gammmarus* sp. (Amphipoda) as intermediate hosts (Thomas et al., 1998; Combes, 2001). Although there is no clear explanation for the lower survival rates registered on the yellow substratum, it can be speculated that dense stock of nematodes may have contributed to it. They were found inside all dead isopods from the yellow sediment. The nematodes started always by feeding on the tissues of the posterior end of the dead isopod's body and their action made it difficult to identify cysts within those host individuals.

In conclusion, similar experiments should be carried out over a longer period, in order to identify parasite consequences for the biology and population dynamics of *C. carinata*. Although this isopod can be found naturally parasitised by *M. subdolum* (Jensen et al., 2004), the current work revealed that the infection is not easily made. Still, this study proved that slight alterations of environmental conditions (such as minor differences in sediment composition) might have several implications (e.g. alterations in food resources, macroinvertebrates' behaviour), which may cause profound variations in the digenean infection patterns (e.g. cercariae shedding rates, transmission efficiency).

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