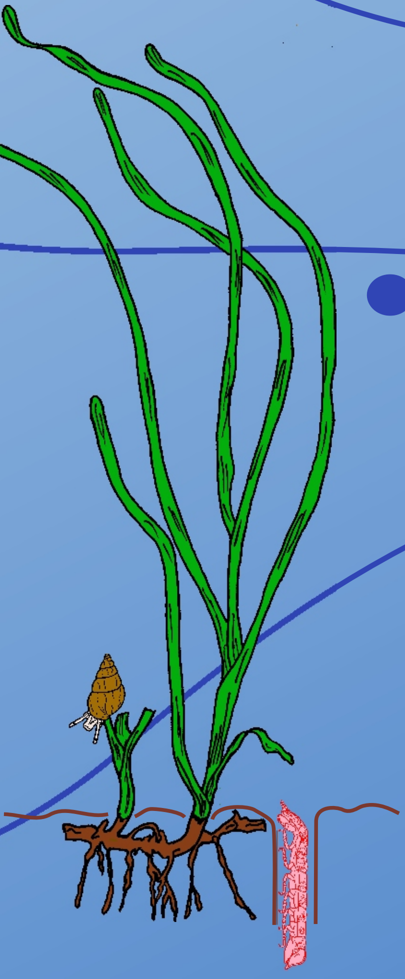
Long-term survey of key macrobenthic species in a temperate eutrophic estuary and the influence of trematode parasites



Susana Margarida de Freitas Ferreira

Coimbra, 2005

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Long-term survey of key macrobenthic species in a temperate eutrophic estuary and the influence of trematode parasites

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Photos from the book marker in sequential order from top to bottom: 1 - *Hydrobia ulvae* (Gastropoda); 2 & 3 - *Maritrema subdolum* (Digenea); 4 & 5 - *Microphallus claviformis* (Digenea); 6 & 7 - *Levinseniella brachyosoma* (Digenea); 8 - *Cyathura carinata* (Isopoda); 9- flamingos in an abandoned salina; 10, 11 & 12 - *Zostera noltii* bed; 13, 14 & 15 - eutrophic area; 16 - a functional salina in the Mondego Estuary.

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Resumo

Um dos principais objectivos deste trabalho é identificar e investigar a influência de tremátodes digenéticos na dinâmica populacional do isópode estuarino *C. carinata*, para além de descrever padrões temporais de prevalência e intensidade destes parasitas nas populações dos seus hospedeiros intermediários. Pretende-se também estudar o desenvolvimento de *C. carinata* ao longo de um período de quase 10 anos, num estuário eutrofizado, o estuário do Rio Mondego, onde foi implementado um programa de reabilitação ambiental.

A presente dissertação será apresentada em vários capítulos, correspondentes a diferentes manuscritos em preparação, submetidos ou já publicados em revistas científicas.

Inicialmente, é feita uma breve introdução à necessidade de desenvolver estratégias de gestão ambiental, com vista a uma utilização sustentável dos recursos costeiros. Nesta secção, são mencionados alguns dos tipos mais frequentes de pressão ambiental em zonas costeiras, nomeadamente processos de eutrofização. Neste cenário são abordados alguns dos problemas que afectam o estuário do Mondego, no qual o isópode *C. carinata* é uma das espécies-chave das comunidades bentónicas intertidais, e faz-se uma primeira abordagem à possível influência de tremátodes digenéticos.

O primeiro capítulo é dedicado à identificação de tremátodes digenéticos que infectam *C. carinata* (segundo hospedeiro intermediário) e à descrição dos padrões destes parasitas em dois locais chave do estuário: um banco de *Zostera noltii* e uma zona arenosa eutrofizada. Duas espécies de tremátodes foram dominantes: *Maritrema subdolum* e uma espécie *Levinseniella* sp. ainda não descrita, sendo esta última a mais abundante. Geralmente, a prevalência destes parasitas culminou durante os meses de Inverno, quando as aves migratórias limícolas eram numerosas no estuário. Os isópodes do banco de *Z. noltii* alojaram mais infecções por espécime do que os da zona arenosa. Uma maior densidade de gastrópodes *Hydrobia ulvae* > 2 mm (primeiro hospedeiro intermediário) e uma fraca abundância de *C. carinata* naquele local foram apresentados como os motivos mais prováveis para justificar o padrão observado.

O segundo capítulo refere-se a uma pequena experiência conduzida com o tremátode *M. subdolum* e ambos os seus hospedeiros intermediários: *H. ulvae* e *C. carinata*. Os resultados indicaram que factores de pequena escala, como diferenças de substrato e características associadas, poderão ter um impacto considerável nas infecções das populações de hospedeiros. *C. carinata* de um substrato de areia fina, com uma maior proporção de partículas < 125 μm, foram mais activos à superfície e alojavam mais larvas de *M. subdolum* do que os isópodes de outro tipo de sedimento, também de areia fina. Durante esta experiência de curto prazo, não foram observados quaisquer efeitos induzidos pelos parasitas nos seus hospedeiros. A distribuição de *M. subdolum* demonstrou que as cercárias penetraram em *C. carinata* através das estruturas de ventilação deste isópode e que se alojaram depois na zona médio-posterior do corpo do hospedeiro.

O terceiro capítulo relata uma experiência laboratorial para avaliar o impacto de tremátodes Microphallidae na sobrevivência, crescimento e fecundidade de *C. carinata*. Durante quase 12 semanas, isópodes infectados e não infectados foram mantidos a 25ºC. Independentemente dos mecanismos envolvidos, os tremátodes Microphallidae demonstraram que podem ter consequências graves, afectando negativamente a sobrevivência, o crescimento e a fecundidade dos seus hospedeiros. Para espécies com desenvolvimento directo, como *C. carinata*, a impossibilidade de reprodução induzida por estes parasitas pode contribuir para flutuações de abundância. Por este motivo, recomenda-se que os parasitas sejam reconhecidos como um factor importante que influencia as populações de hospedeiros em sistemas costeiros.

O quarto capítulo é dedicado à identificação de tremátodes digenéticos que também infectam o gastrópode *H. ulvae* no estuário do Mondego. Os parasitas encontrados pertenciam às famílias Haploporidae, Plagiorchiidae, Echinostomatidae e Microphallidae. As prevalências mais elevadas verificaram-se no

Outono/Inverno (4.3%) e na Primavera (3%), diminuindo depois até ao Verão (1%). Estes valores corresponderam entre 800 a 5 440 gastrópodes infectados por m^2. Estes parasitas foram encontrados em *H. ulvae* de classes de comprimento de concha entre 2.0 e 5.9 mm. Todos os gastrópodes > 5.9 mm não estavam infectados. Espécimes de *H. ulvae* parasitados por trematódes apresentaram geralmente conchas estreitas, fáceis de esmagar, com voltas largas e pronunciadas, incrustadas por epibiontes.

O quinto capítulo descreve as características de infecção e estratégias de transmissão usadas pelos dois trematódes mais abundantes em *C. carinata*: *M. subdolum* e *Levinseniella* sp.. Duas experiências de curto prazo foram executadas para comparar a eficiência de transmissão destes parasitas a partir de *H. ulvae* para *C. carinata*. Ambos as espécies de trematódes penetraram nos isópodes através dos pleópodes e alojaram-se na zona médio-posterior do corpo do hospedeiro. Estes dois parasitas mostraram diferentes estratégias no que respeita à produção e dispersão de cercárias. *M. subdolum* produziu elevadas quantidades de cercárias capazes de nadar, enquanto *Levinseniella* sp. gerou poucas cercárias que rastejavam à superfície do sedimento. A estratégia desenvolvida por *Levinseniella* sp. revelou-se mais eficaz para infectar *C. carinata*, um macroinvertebrado infaunal bastante inactivo.

O sexto capítulo foca a dinâmica populacional de *C. carinata* no estuário do Mondego, desde 1993 até 2002. As medidas de mitigação introduzidas desde 1998 (visando a recuperação de bancos de *Z. noltii* ameaçados por processos de eutrofização) contribuíram para uma população mais estável na área eutrofizada, onde este isópode é mais abundante. *C. carinata* revelou-se bastante resiliente à ocorrência de *blooms* macroalgais e fortes descargas fluviais derivadas de situações de cheia, ainda que estes eventos causassem a dispersão de indivíduos para outras áreas do estuário. Apesar da melhoria da qualidade ambiental no estuário, *C. carinata* não conseguiu estabelecer-se nos bancos de *Z. noltii*, contrariamente a outras espécies-chave (*e.g. H. ulvae* e *Scrobicularia plana*) que prosperam em todas as áreas intertidais amostradas.

Finalmente, segue-se uma discussão geral sobre a influência de trematódes Microphallidae na presença irregular de *C. carinata* no banco de *Z. noltii*, onde o gastrópode *H. ulvae* é uma espécie dominante e todos os elementos para o desenvolvimento do ciclo de vida daqueles parasitas se encontram presentes. As características de infecção e efeitos destes parasitas nos seus hospedeiros macroinvertebrados são também sumariados. A possível recuperação do coberto de *Z. noltii* original é discutido, assim como as possíveis consequências para a população de *C. carinata*. Face aos resultados obtidos ao longo deste trabalho, sugere-se que respostas inesperadas de diferentes populações relativamente à modificação de habitat, tais como relações parasita-hospedeiro, devem ser também ser consideradas em gestão ambiental.

Abstract

One of the main purposes for the present work is to identify and investigate the influence of digenetic trematodes on the estuarine isopod *Cyathura carinata*, plus to describe temporal patterns of prevalence and intensity of these parasites in the populations of their intermediate macroinvertebrate hosts. It is also intended to assess the population dynamics of *C. carinata* through a period of almost 10 years, in a eutrophic estuary, the Mondego Estuary, located in the mid-Atlantic coast of Portugal, where an environmental rehabilitation programme was implemented.

This work will be presented in several chapters, corresponding to different manuscripts being prepared, submitted or already published in scientific journals.

Initially, there is a brief introduction to the need of developing management strategies, with the purpose of achieving a sustainable utilisation of coastal resources. In this section, some of the most important types of environmental pressures in coastal zones are mentioned, namely eutrophication processes. In this scenario, some of the problems that affect the Mondego Estuary are addressed. *C. carinata* is presented as one of the intertidal macrobenthic key-species and there is a first approach to the possible role of digenetic trematodes.

The first chapter is dedicated to the identification of digenetic trematodes infecting *C. carinata* (second intermediate host of these parasites) and to the description of trematode patterns from two key sites in the estuary: a *Zostera noltii* bed and an inner eutrophic sand flat. Two trematode species were dominant: *Maritrema subdolum* and a hitherto unknown *Levinseniella* species, the latter being the most frequent one. Generally, the prevalence of both species peaked during winter months, when migratory water birds occurred in the estuary. Isopods from the *Z. noltii* bed harboured more infections per specimen than those from the sand flat. A much higher density of *Hydrobia ulvae* mud snails ≥ 2 mm (first intermediate host) and a low abundance of *C. carinata* were indicated as the most probably motives for this pattern.

The second chapter refers a short-term microcosm experiment, conducted with the microphallid trematode *M. subdolum* and both of its intermediate hosts: *H. ulvae* and *C. carinata.* Results indicated that small-scale factors, such as differences in substratum and associated features, may have considerable impact on infections of host populations. *C. carinata* from a fine sand substratum, with a higher proportion of particles < 125 μm, were more surface active and hosted significantly more *M. subdolum* parasites than isopods from another fine sand sediment type. There were no parasite-induced effects on the hosts during this short-term experiment. *M. subdolum* distribution within *C. carinata* showed that the cercariae primarily penetrated through the isopod's ventilation structures and afterwards located themselves in the middle-posterior region of the host's body.

The third chapter is related to a laboratory experiment performed to assess the impact of microphallid trematodes on the survival, growth and fecundity of *C. carinata*. For nearly 12 weeks, infected and non-infected isopods were kept at 25ºC. Regardless of the mechanisms involved, microphallids showed that they may have severe consequences, through negative effects on survival, growth and fecundity of their hosts. For species with direct development, such as *C. carinata*, parasite induced reproduction failure may contribute to temporal fluctuations of abundance. Consequently, it was recommended to include parasites as an important factor influencing host populations from shallow-water ecosystems.

The fourth chapter is dedicated to the identification of digenetic trematodes also found within *H. ulvae* mud snails from the Mondego Estuary. These parasites belonged to the families Haploporidae, Plagiorchiidae, Echinostomatidae and Microphallidae. Higher prevalence occurred in autumn/winter (4.3%) and again in spring (3%), decreasing until 1% in summer, which corresponded to 800 - 5 440 infected mud snails per m^2. Digenetic trematodes were found in mud snails from size classes between 2.0 and 5.9 shell height and those > 5.9 mm were non-infected. The parasitised *H. ulvae* had generally thin shells, very easy to crush, with large and round whorls, fouled by epibionts.

The fifth chapter regards the infection characteristics and transmission strategies of the two most common trematodes found in *C. carinata*: *M. subdolum* and *Levinseniella* sp.. Two short-term experiments were performed to compare their transmission success from *H. ulvae* to *C. carinata*. Both microphallids penetrated the isopod through the pleopods and encystment took place in the middle-region of the host's body. The two parasites showed different strategies with respect to cercarial production and dispersal ability (cercarial behaviour). *M. subdolum* produced a high quantity of swimming cercariae, whereas *Levinseniella* sp. created few crawling free larval stages. These results can be interpreted as adaptations to different crustacean hosts. The strategy exhibited by *Levinseniella* sp. is more efficient to infect *C. carinata*, a relatively quiet bottom-dwelling isopod.

The sixth chapter focus on the population dynamics of *C. carinata* at the Mondego Estuary, from 1993 to 2002, to assess the development of a restoration programme for the seagrass beds of *Z. noltii* endangered by eutrophication. The mitigation measures implemented since 1998 have contributed to a more stable *C. carinata* population density at an inner unvegetated sand flat area, where these isopods were abundant. This species was resilient to the occurrence of macroalgal blooms and floods, although both events have caused dispersion of isopod individuals to other areas of the estuary. Despite the increasing environmental quality in the estuary, *C. carinata* could not establish itself successfully in rooted-macrophyte beds, in contrast to other common estuarine species (*e.g. H. ulvae* or *Scrobicularia plana*) that prospered at all sites (bare sand or vegetated mud flats).

Finally, there is a general discussion of the role of microphallid trematodes in explaining the irregular presence of *C. carinata* at the *Z. noltii* bed, where the mud snail *H. ulvae* is a dominant species and all the elements needed to complete these parasites' life cycles are present. The infection characteristics and parasite effects in their macroinvertebrate hosts are also summarised. The possible full restoration of the intertidal areas to the original *Z. noltii* coverage is discussed, as well as the possible consequences for the *C. carinata* population. Relatively to the results obtained in this work, it is suggested that unexpected animal responses to habitat modification, such as parasite-hosts interactions, should also be thoroughly considered in environmental management.

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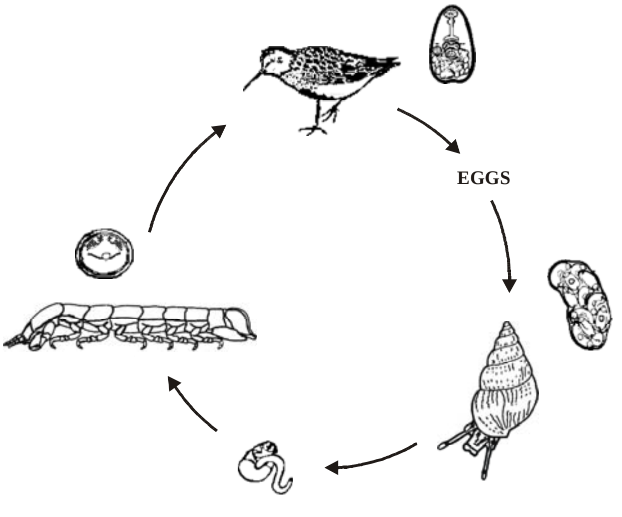
Introduction



General introduction

ESTUARIES - ECOLOGICAL IMPORTANCE AND ENVIRONMENTAL PRESSURES

Estuaries are considered one of the most productive ecological systems in the world (McLusky 1989, Valilela 1995, Clarck 1996, Crooks & Turner 1999, Kennish 2000). Since ancient times, the natural resources provided by these habitats have drawn the attention of mankind. Estuaries are critical sites for the development of many freshwater and marine wild life populations that use them for nesting, feeding, breeding and refuge, which includes many commercial species from coastal food chains (Clarck 1996, Crooks & Turner 1999, Kennish 2000). Apart from sustaining biological diversity, the estuarine systems also provide other benefits for the human society, such as protection against natural hazards (storms, floods and erosion of bordering landmasses), serving as repository of large quantities of sediment and contributing to ameliorate water quality (Clarck 1996, Crooks & Turner 1999, Kennish 2000). A large part of the human population was attracted to live on estuarine shores by the wealth of marine resources and economical opportunities. Actually, two-thirds of the world's largest cities are situated in coastal areas, being currently estimated that 37% of the human population is concentrated in these regions (Crooks & Turner 1999). The expansion of the human population has created pressure and detrimental impact that, together with an inadequate ecological and socio-economical management, cause environmental degradation and wetland loss (Clarck 1996, Crooks & Turner 1999, Kennish 2000). Activities like, a) land reclamation and drainage for urban, recreational, commercial, industrial and agricultural purposes; b) solid wastes and sewage discharges; c) over-exploitation of natural resources; d) deliberate or accidental introduction of exotic species; e) habitat modifications (*e.g.* bottom dredging, construction of fixed engineering structures, sea mining), have been conducting to the degradation of water quality (*e.g.* eutrophication, chemical contamination) and basically to the destruction of coastal ecosystems (McLusky 1989, Clarck 1996, Crooks & Turner 1999, Kennish 2000, Elliot & Jonge 2002).

Eutrophication is recognised as a major worldwide pollution threat to coastal systems. It is mostly connected to the enhanced availability of growth limiting nutrients to primary producers, such as nitrogen and phosphorus, consequent of anthropogenic activities (Jørgensen & Richardson 1996, Beukema & Cadée 1997, Valiela et al. 1997, Raffaelli et al. 1998, Cloern 2001). The processes triggered by excessive nutrient enrichment of coastal waters are complex, but usually promotes extreme growth of opportunistic algae, which can build-up dense blooms, often dominated by one or few species (Jørgensen & Richardson 1996, Norkko & Bonsdorff 1996a,b, Raffaelli et al. 1998, Martins et al. 2001, Sfriso et al 2001). Such blooms have harmful effects, sometimes extremely devastating in shallow estuaries with restricted water circulation. One of the most severe consequences occurs when an algal crash takes place, in which there is an oxygen depletion of the water column as a result of bacterial decomposition of the bloom biomass. Notwithstanding, there are other subtle processes involved in eutrophication. Algae can compete for light and nutrients with seagrasses, overgrowing and shading these rooted-macrophytes (Valiela et al. 1997, Kennish 2000, Jonge et al. 2000, Elliot & Jonge 2002, Cardoso et al. 2004). The processes involved usually result in shifts of primary producers, with loss of seagrass plants along with the functions, services and goods that they provide (Duarte 2000, 2002). These changes are ultimately reflected in the structure of food webs, lowering species richness, total biomass and secondary production (Norkko & Bonsdorff 1996a,b, Den Hartog & Phillips 2000, Livingston 2001, Elliot & Jonge 2002, Dolbeth et al. 2003, Cardoso et al. 2004).

Recently, there has been a growing concern, from people in general, regarding environmental issues, as increasing global pollution, global warming, shortage of drinkable water reserves and food stocks, etc… Thus, it has been made an effort to develop management strategies, with the purpose of achieving a sustainable utilisation of coastal resources, in order to not compromise future generations. These strategies should aim to maintain ecosystems integrity and, therefore, a holistic approach across several scientific disciplines should be undertaken, to provide required information upon which sound policy decisions are to be based (Crooks & Turner 1999). Estuaries are characterised by physical, chemical and biological conditions that turns them into highly dynamic and unique ecosystems (Hobbs & Norton 1996). To attain a sustainable management of coastal zones under the pressures of global environmental change, it will be necessary to preserve and restore degraded estuaries (Crooks & Turner 1999, Kennish 2000, Vivian-Smith 2001, Elliot & Jonge 2002). Consequently, research will always be required to a) assess the present stock of the resources available, b) evaluate the effects of socio-economical activities in the diversity, resilience and integrity of these ecosystems, c) determine the costs and benefits that management options provide and d) develop flexible policies accordingly (Crooks & Turner 1999). In this context, long-term studies are extremely important to identify and evaluate ecological phenomena, as well as to predict ecosystems responses and to test ecological concepts, upon which decision makers should rely on (Franklin 1989).

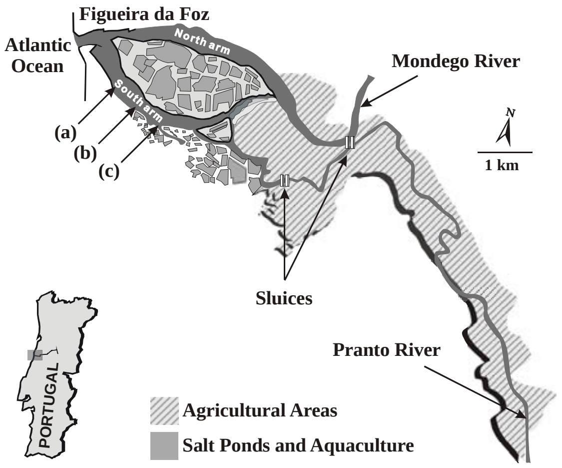


Figure 1. Schematic representation of the Mondego Estuary and its location. Indication of the of the south arm reference intertidal areas monitored since 1993: a) a *Z. noltii* bed, b) an intermediate mud flat and c) an eutrophic sand flat.

THE MONDEGO ESTUARY – A CASE STUDY

Environmental scenario

The head of the Mondego River lies in the centre of Portugal and the river drains an almost 6 670 km^2 hydrological basin, until flowing out into the Atlantic Ocean. Steep valleys of mountain landscape confine the river course until the city of Coimbra. Afterwards, the river flows through 35 km of alluvial plain in the Lower Mondego Valley. Farmers intensively use this area of approximately 150 km^2, producing mainly maize in the middle section and rice in the last 20 km of the river basin. Since the 1960's, it has been executed an intervention plan to benefit the local region, which is not yet concluded. Several channels and water dams have been constructed, the river course was confined to an artificial bed, in order to regulate the river flow, supply water and electricity to the population, prevent floods and improve irrigation efficiency in the agricultural fields. These actions contributed to the development of the region, but implied several environmental modifications, which were reflected in the river flow, water quality and even in the extent of the estuary itself (with saline influence rising upwards).

The Mondego Estuary is located in the central western coast of Portugal (Fig. 1), being influenced by a warm-temperate climate. This estuary has a great regional value, sustaining several industries, salt-works and aquacultures. It is divided into two arms with different hydrological characteristics by the alluvial Murraceira island. The north arm is under a great human pressure. The city of Figueira da Foz (up to 50 000 habitants) and its mercantile harbour (with cellulose pulp as the most important exportation product) are situated in its northern shore. Its bottoms are frequently dredged to allow the navigation of larger ships. Consequently, the north arm is a deeper channel (4-8 m deep at high tide, with a tidal range of 1-3 m), where tidal penetration happens much faster and where the daily salinity fluctuations are more pronounced. The south arm is a shallower system (2-4 m deep at high tide), with large intertidal areas, where *Spartina maritima* and *Scirpus maritimus* salt marshes and *Zostera noltii* beds are commonly found. The upstream section of this arm was silted-up until 1998, which constrained the Mondego River freshwater to flow out essentially through the north arm. This system could be considered almost as a coastal lagoon, with water circulation being dependent mostly on tides and on a small freshwater input from the tributary Pranto River. The flow of this river is in turn controlled by a sluice that is open according to the water needs of upstream rice fields in the Lower Mondego Valley (Flindt et al. 1997, Martins et al. 2001).

As many coastal systems worldwide, the Mondego Estuary has been suffering from ongoing eutrophication since the 1980's. The most common symptoms have been the occurrence of seasonal macroalgal blooms, the progressive reduction of *Z. noltii* beds and impoverishment of the associated macrobenthic assemblages (Flindt et al. 1997, Marques et al. 1997, 2003, Lillebø et al. 1999, Pardal et al. 2000, Martins et al. 2001, Cardoso et al. 2002, 2004, 2005, Dolbeth et al. 2003, Ferreira et al. 2004, Patrício et al. 2004, Verdelhos et al. 2005). Since 1993, the south arm of the Mondego Estuary has been monitored along a spatial gradient of eutrophication symptoms, for nutrient concentrations in the water, biomass and productivity of benthic primary producers, population dynamics and secondary production of macrofaunal key species, interannual variations of wading birds and impacts of macroalgal blooms (Flindt et al. 1997, Marques et al. 1997, 2003, Múrias et al. 1997, Lillebø et al. 1999, Lopes et al. 2000, Pardal et al. 2000, Martins et al. 2001, Cardoso et al. 2002, 2004, 2005, Dolbeth et al. 2003, Ferreira et al. 2004, Patrício et al. 2004, Verdelhos et al. 2005). Three sampling stations have been used as reference areas since then (Fig.1): a) a *Z. noltii* bed, located in downstream mud flats, which corresponds to a non-eutrophic situation, representing the original undisturbed state of the system (Marques et al. 2003, Pardal et al. 2004, Patrício et al. 2004); b) an intermediate area, located in the middle section, a mudflat that had *Z. noltii* coverage until approximately 1988; and c) a eutrophic area, a sandier mud flat in the inner parts of the estuary, from where *Z. noltii* disappeared without trace more than 25 years ago and where seasonal green macroalgal blooms (*Ulva* sp.) usually occur.

Several measures were implemented in 1998, to face against habitat degradation and to improve environmental quality. These measures aimed to detain and reverse the eutrophication processes in progress, by a) reducing nutrient loading into the estuary, b) improving freshwater circulation, plus c) protecting and recovering the seagrass beds. In order to achieve the first goal, the opening of the Pranto River sluice was reduced and its freshwater is now diverted to the Mondego River, by another sluice located further in an upstream section of the estuary. Farmers have also been advised to reduce the utilisation of fertilisers and also to use the already nutrient enriched water from the irrigation channels, which has also been monitored. The hydraulic regime in the southern system of the estuary was also improved, by enlarging the connection between the two arms, to allow water exchanges at high water of spring tide situations. The existing *Z. noltii* beds were protected with fences and people were informed to not disturb these areas. So, the long-term survey, performed in the Mondego Estuary for more than one decade, consents to settle a reference condition before the implementation of the mitigation measures and evaluate the rehabilitation progresses.

Cyathura carinata as a key species

Cyathura carinata (Krøyer, 1847) is an estuarine isopod (Fig. 2), very common along the Atlantic coasts of Europe (Cléret 1960, Legrand & Juchault 1963, Amanieu 1969, Bamber 1985, Köhn & Sammour 1990, Sola & Arzubialde 1993, Ferreira et al. 2004), Baltic (Muus 1967, Jażdżewski 1969, Wägele 1979, Ólafsson & Persson 1986) and Mediterranean Seas (Argano 1979). It is a euryokous species, occurring in different salinities (Burbanck & Burbanck 1979), tidal ranges and biotopes, from pebble-dominated sediments (Bamber 1985) to rooted-macrophyte beds (Amanieu 1969, Ólafsson & Persson 1986, Ferreira et al. 2004). This tube-dwelling macroinvertebrate is omnivorous. It has been reported to feed on detritus and diatoms, but they can predate other invertebrates, including dead or injured individuals of their own species (Burbanck & Burbanck 1979, Wägele 1979). In turn, they are also important links in estuarine trophic webs, as a food item to birds (Goss-Custard et al. 1991, T. Múrias & P. Pedro personal observation) and fishes (Ólafsson & Persson 1986, Martinho 2005, Leitão 2005). *C. carinata* specimens are long and slim (Fig. 2). They have a first pair of pereopods (gnathopods) extremely developed, in shape of a claw, that they use to grab and immobilize their prey. The pleopods are overlapped and the first pair is more robust than the others, acting as an operculum. Males are generally larger than females.

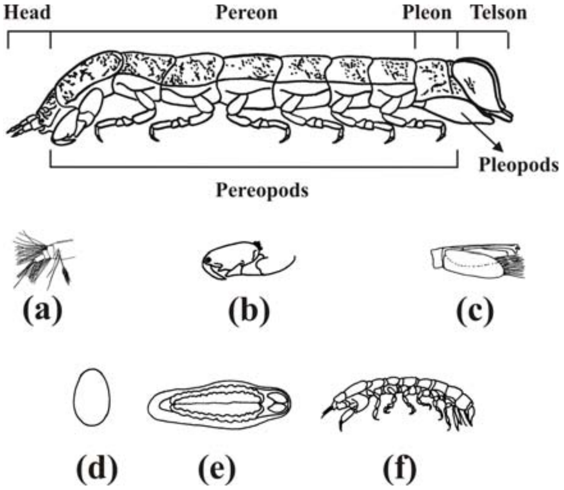


Figure 2. Schematic illustration of *C. carinata*, with indication of the male sexual characters: a) the tuft of sensory hairs in the antenna, b) a dense brush of short bristles along the exterior margin of the gnathopods and c) two male appendix under the second pair of pleopods. Also represented the 3 embryonic developmental stages: d), e) and f) (by time sequential order of maturation).

They can only be distinguished during the breeding season (Fig. 2), by the presence of a) a tuft of sensory hairs in the terminal ends of the antenna, b) a little dense brush of short bristles occurring along the exterior margin of the gnathopods and c) two male appendix under the second pair of pleopods (Jażdżewski 1969). Gravid females temporarily acquire three pairs of oostegites, respectively in the third, fourth and fifth pereon segments, during the exoskeleton moult that precedes oviposition. These females develop a ventral brood pouch that will hold the offspring. Embryos can be found in three developmental stages (Fig. 2): stage I, corresponding to oval homogeneous embryos, recently laid; stage II, referring to elongated embryos, sometimes with pereopods and antennae already identifiable; and stage III, indicating newly hatched juveniles still inside the brood pouch of the female (Jazdzewski 1969, Bamber 1985, Ólafsson & Persson 1986, Marques et al. 1994, Ferreira et al. 2004).

Previous studies presented *C. carinata* as one of the most abundant populations of the intertidal mud flats, both in terms of density and biomass (Marques et al. 1994, Martins et al. 1997, Pardal et al. 2002). Naturally, *C. carinata* has been one of the key species used to survey the eutrophication processes evolving in the south arm. The long-term data acquired along this study has allowed clarifying some doubts raised by previous works, such as life span, recruitment frequency and evidence of protogynous hermaphroditism (a process by which some females sex reverse into males). From January of 1993 to September of 1995, this isopod was found to be most abundant in the eutrophic inner area (Fig. 1), where seasonal macroalgal blooms usually occurred. Nevertheless, its density decreased towards downstream areas, where *Z. noltii* beds persisted (Ferreira et al. 2004). Concerning *C. carinata*'s biological features, this isopod revealed to: a) have a 2-year life span, b) produce a single cohort per year; c) show continuous growth (with lower rates during winter); d) evidence protogynous hermaphroditism and e) present a high growth production and a low turnover ratio (Ferreira et al. 2004). Regarding the occurrence of macroalgal blooms, *C. carinata* seemed to temporarily benefit from the presence of macroalgae, due to higher energy resources and more efficient protection against predators (Raffaelli et al. 1998, Norkko et al. 2000, Pardal et al. 2000, Cardoso et al. 2002). Nevertheless, algal blooms had negative consequences afterwards, particularly evident on the recruitment success, which had repercussions for the population abundance and for the secondary production (Ferreira et al. 2004). Thus, it is important to assess how this isopod population will evolve in a eutrophic estuary and how it will respond to the mitigation measures introduced for rehabilitating environmental quality.

Digenetic trematodes – the variable incognita

Metacercarial cysts of digenetic trematodes were observed inside *C. carinata* from the Mondego Estuary, with a higher incidence in individuals from the *Z. noltii* beds. Prior to this study, only little information existed about parasites in this isopod (Reimer 1963, Schulenburg et al. 1999).

Investigation of diseases of marine and estuarine organisms is an important pre-requisite for consolidating the knowledge about life in oceans and coastal waters (Kinne 1983). Parasitism is a fundamental and significant feature of nearly all natural systems, yet parasites are the most ignored group in respect to studies of biodiversity, community and trophic structure. Methodological and taxonomical problems in documenting parasite-host interactions constitute an obstacle to assess just how important parasitism can be for intertidal macrobenthic assemblages (Huxham et al. 1995, Mouritsen & Poulin 2002).

Small-scale studies indicate digenetic trematodes as major components of intertidal macroinvertebrate and avian parasite fauna in coastal systems (Lauckner 1980, Mouritsen & Poulin 2002, Skirnisson & Galaktionov 2002). Their complex life cycle almost always involves a mollusc. Among these, mud snails are typical first intermediate hosts to many of these parasites, as they occur in high densities in mud flats from European estuaries and coastal lagoons. From 50 digeneans known to infect *Hydrobia* spp., there are about a dozen species of xiphidiocercaires (family Microphallidae) that use crustaceans as second intermediate hosts, a transport vector to reach a final water bird host (Deblock 1980). In the Mondego Estuary, these trematodes are transmitted to *C. carinata* through *Hydrobia ulvae* (Pennant), which can reach densities of almost 300 000 ind m^{-2} (Lillebø et al. 1999, Cardoso et al. 2002, 2005). So, the life cycle of microphallid trematodes found in *C. carinata* proceeds according to the scheme presented in Fig. 3. The adult form of the parasite occurs in the intestine of a wading bird. The eggs of digeneans are released outside, through the birds' faeces, and some are accidentally ingested by *H. ulvae*. Within the gonads of the mud snail, larval trematodes undergo an asexual reproduction process, producing numerous cercariae that leave the snail host. When these parasites find *C. carinata*, they penetrate the isopod's exoskeleton, invade the host's body cavity (mesocercariae stage), where they mature and encyst (metacercariae stage). The cycle is completed when birds feed on infected isopods (Mouritsen & Jensen 1997).

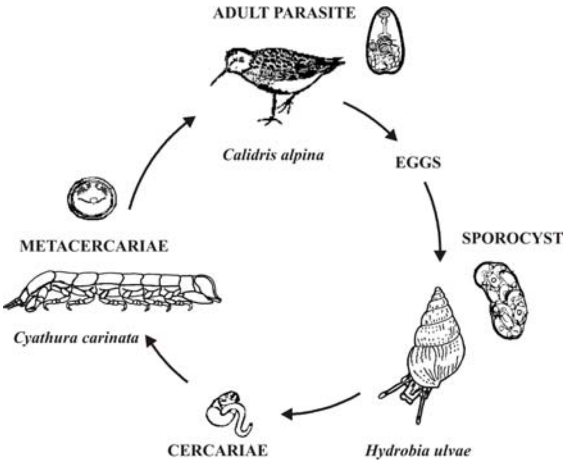


Figure 3. Schematic illustration of microphallids' life cycle infecting wading birds, *C. carinata* and *H. ulvae*. The several stages of the parasites larval development are written in capital letters.

The spatio-temporal patterns of digenean parasites in intermediate host populations are often linked to the phenology of their final hosts (Esch et al. 2001), but there are usually spatial, seasonal and interannual variations that are not yet explained. The role of some of these parasites (*e.g.* microphallids) in estuarine processes becomes more important as their distribution is widespread through coastal systems, used as wintering ground and/or resting areas by final shorebird hosts on their migratory routes (Esch et al. 2001). Recent application of molecular techniques demonstrated that there is not yet a proper knowledge of the numbers of trematode species infecting mud snails. Many of them have strong impact on the ecology of the host organisms and may be cryptical determinants of their dynamics. Therefore, digeneans may be an important factor in the dynamics of most estuarine community assemblages, which is often disregarded and neglected. In order to achieve progress in ecological research and to build a sound fundament for environmental protection, it is necessary to measure and evaluate the total amount of stress of a living system. So, the knowledge acquired about the diseases of coastal organisms is also an important issue for protecting life in coastal systems and for compensating the damage already induced by anthropogenic activities (Kinne 1983).

Outline of the major aims of this study

Given the above, the present work has two major intents, which are to follow *C. carinata* through a period of almost 10 years, in a eutrophic estuary where an environmental rehabilitation programme was implemented, and investigate the influence of trematode infections on its population dynamics. In conformity, the main objectives are summarised as the following:

a) Identify digenetic trematodes infecting *C. carinata*;

b) Describe temporal patterns of prevalence and intensity of trematodes species in the isopod population;

c) Assess infection characteristics of digenetic trematodes within *C. carinata* and also compare strategies used by the most common trematodes found within these isopods;

d) Identify key processes determining the flux of parasites;

e) Evaluate the impact of trematodes on survivorship, growth and reproduction of the isopods;

f) Since *H. ulvae* is the first intermediate host of the trematodes found within *C. carinata*, it is also relevant to identify digenetic trematodes within this mud snail, describe temporal variations of parasite prevalence and infer possible impacts;

g) Evaluate how *C. carinata* responds to the mitigation measures implemented in the south arm of the Mondego Estuary, by analysing its population dynamics variation for almost a decade and assessing the influence of episodic or rare events (*e.g.* algal blooms, floods, parasites)

h) Finally, as conservation or rehabilitation of estuaries require detailed information of the system as a whole, this work pretends to provide a better understanding of the processes functioning in the Mondego Estuary, aiming to contribute for the success of its restoration programme.

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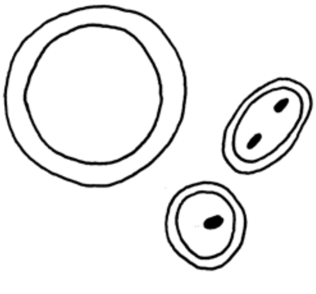
Chapter 1



Trematodes in a *Cyathura carinata* population from a temperate intertidal estuary: infection patterns and impact on host

ABSTRACT

A study was initiated to identify trematode patterns in *Cyathura* from two key sites in the Mondego Estuary: a *Zostera* bed and a sand flat. The two subpopulations of *Cyathura* differ, as the annual recruitment success is much higher and more regular on the sand flat than on the *Zostera* bed.

Counting and sizing of trematode cysts inside the body of preserved *Cyathura* specimens enabled a description of trematode patterns in space and time. Further identification of trematodes was based on DNA studies of unpreserved cysts as well as on identification of parasites in co-occurring mud snails. Two trematode species dominated in *Cyathura*: *Maritrema subdolum* (cysts around 190 μm) and a hitherto unknown *Levinseniella* species (cysts around 340 μm), the latter being the most frequent one. Generally, the prevalence of both species peaked during winter months, when migratory water birds occur in the estuary. *Cyathura* from the *Zostera* bed harboured more infections per specimen than those from the sand flat. A much higher density of mud snails ≥ 2 mm (which can be host to microphallids) and a low abundance of *Cyathura* are thought to be the main reasons for this pattern. Field data did indicate a host size dependent maximum number of cysts in *Cyathura* that could be a result of enhanced mortality at high cyst intensities. Furthermore, parasites were underrepresented among ovigerous *Cyathura* specimens, suggesting a negative impact on gametogenesis. As a consequence, microphallid trematodes may be a critical factor controlling recruitment strength in *Cyathura,* especially at the *Zostera* site.

Key words: *Cyathura carinata*, Mondego Estuary, Dynamics, Prosobranchia, Isopoda, Parasite, Portugal

INTRODUCTION

In brackish water ecosystems, such as estuaries and shallow water lagoons, where numerous fish and birds congregate, digenean trematodes are widespread among macrozoobenthic organisms that are intermediate hosts to parasites using the vertebrates as their final hosts. As such macroparasites may have considerable impact on host populations (see Mouritsen & Poulin 2002), environmental studies focusing on abundance patterns of invertebrates should include parasites as a possible determining factor. Descriptions of spatio-temporal patterns and identification of processes controlling the dynamics of digeneans are furthermore essential for improving our understanding of their importance in ecosystems. In a long-term study on distribution and dynamics of dominant benthic species, the Mondego Estuary (Portugal) has been used as a model system (Cardoso et al. 2002). Like other estuaries, this system is dominated by a few species (Marques et al. 2002). Among these are the mud snail *Hydrobia ulvae* (Pennant, 1777) and the isopod *Cyathura carinata* (Krøyer, 1847), which have been used as target species in several studies (Marques et al. 1994, Lillebø et al. 1999, Cardoso et al. 2002). The present ecosystem accommodates an abundant avifauna, with waders being a predominant group during winter (Lopes et al. 2002). Together these faunal components predetermine that parasites can be abundant in the system because mud snails are known to host several species of digenean trematodes utilizing benthic peracarid crustaceans (amphipods and isopods) as second intermediate hosts and birds as their final hosts (Deblock 1980). So far, only scattered information on parasites in *Cyathura* has been published (Reimer 1963, Schulenburg et al. 1999). The purpose of the present study is to provide a description of the temporal pattern in prevalence and intensity of trematode species in *Cyathura* from two type localities in a southern European intertidal estuary, which is a wintering area to waders. We attempt to identify key processes determining the flux of parasites through the *Cyathura* population and discuss possible consequences of parasitism to the host population.

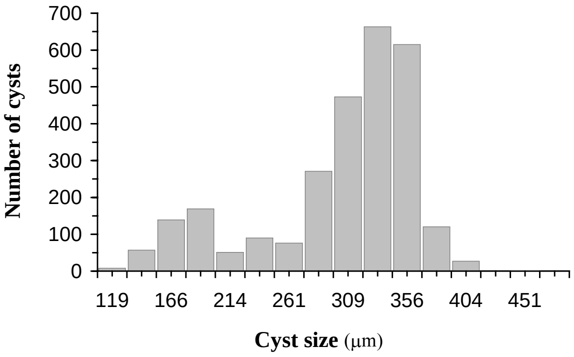


Figure 1. Size distribution (diameter) of all microphallid cysts collected from *Cyathura* from the two study sites in Mondego Estuary.

MATERIALS AND METHODS

Study site and sampling programme

The Mondego Estuary is a small temperate intertidal estuary (1 072 ha of wetland habitats) located on the Atlantic coast of Portugal (40º08‘N, 8º50W) (Fig. 1 in Cardoso et al. (2002)). During the last two decades it has been subject of a long-term environmental study. Two sites have been monitored in particular: a) a *Zostera noltii* bed and b) a sand flat (‘eutrophicated area’, Fig. 1 in Cardoso et al. (2002)). From the mid-1980’s until 1997 the extent of the *Zostera* bed declined from 15 ha to 0.02 ha. The sand flat is seasonally covered by green macroalgae, especially *Enteromorpha* sp. (Cardoso et al. 2002). The substratum at the two sites differ with respect to median grain size (*Zostera*: 74 to 105 µm *versus* sand flat: 116 to 144 µm, seasonal variation) (Lillebø 1996) and content of organic matter (*Zostera*: 6.8 ± 1.0% *versus* sand flat: 3.7 ± 1.0% (mean ± S.D.) (Cardoso et al. 2002). Higher salinity values were registered in low water pools at the *Zostera* plot (20-30) than at the sand flat plot (20-30). Water temperature in low water pools at the study sites showed an annual range from 8 to 32ºC. Sampling was performed from January 1993 to September 1995, until mid-1994 with fortnightly intervals and monthly thereafter. On each site, 6 to 10 cores (13.5 cm diameter) were sampled to a depth of 20 cm, sieved through a 0.5 mm mesh and preserved in 4% buffered formalin. Later, animals were separated and kept in 70% ethanol.

Data on the abundance of waders were provided by Múrias et al. (1997) and Lopes et al. (2002).

Laboratory procedures

Specimens of *Cyathura* were counted, measured for cephalic length and sexed. Total body length (mm) of each individual was determined from cephalic length (mm) using the equation: total body length = 0.7451 + 9.0101 × cephalic length, (r^2 = 0.97, n = 115) (Marques et al. 1994). *Cyathura* has a 2-year life span in the Mondego Estuary and it reproduces once a year. Cohorts were distinguished from each other if overlapping through a modal analysis following the method described by Marques et al. (1994).

All sampled *Cyathura* individuals were inspected for trematode cysts located inside their abdomen. These cysts were removed, counted and measured. Three different methods have been applied for identifying the trematodes in *Cyathura*: a) as mud snails typically will be first intermediate host to digeneans occurring in *Cyathura*, snails collected from the Mondego Estuary in 2000 and 2001 were screened for infections. Snails were incubated individually in a small Petri-dish under artificial light at temperatures of 20-24°C to stimulate shedding of cercariae from the infected snails. Cercariae were identified under a light microscope according to Deblock (1980); b) DNA was extracted from cysts sampled from viable

Cyathura specimens collected in the Mondego Estuary and from microphallid cercariae harvested from infected snails. PCR of the ITS-regions of rDNA was sequenced and aligned with sequences of known microphallid species to verify identifications based on morphological examinations (Warberg 2003); c) Cysts of unknown microphallids were heated to 38°C and incubated in trypsin to stimulate excystment (Rebecq 1964). Morphometric characteristics of excysted specimens were measured and compared to reported descriptions of known species (Warberg 2003).

Hydrobia snails were counted and measured. Shell height (mm) was determined by measuring maximum shell width (mm) and using the equation: maximum width = 0.4369 × total height + 0.2091 ($r^2 = 0.97$, n = 339) (Lillebø et al. 1999, Cardoso et al. 2002).

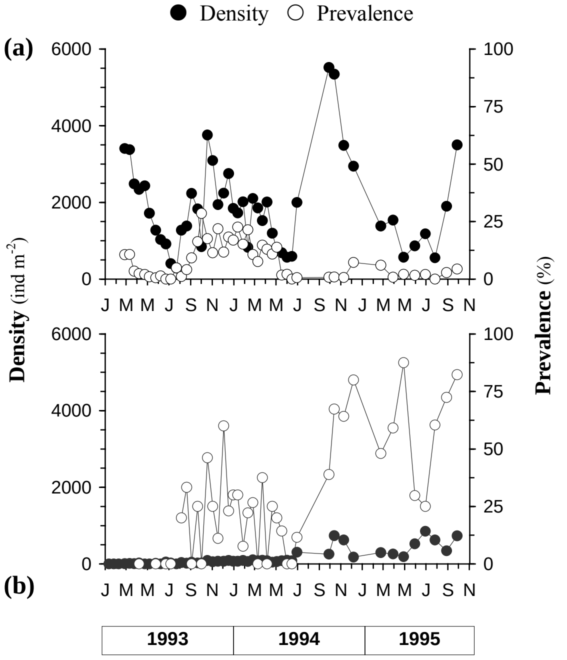


Figure 2. Temporal variation in density of *Cyathura* and prevalence of microphallid cysts in the isopod from a) the sand flat site and b) from the *Zostera* site.

Statistical analysis

Statistical analyses were performed using the MINITAB 10.2 and the Statistical Package for the Social Sciences version 10 software packages. All data were inspected for violation of required assumptions and, if so, proper transformations or non-pararametric tests were used.

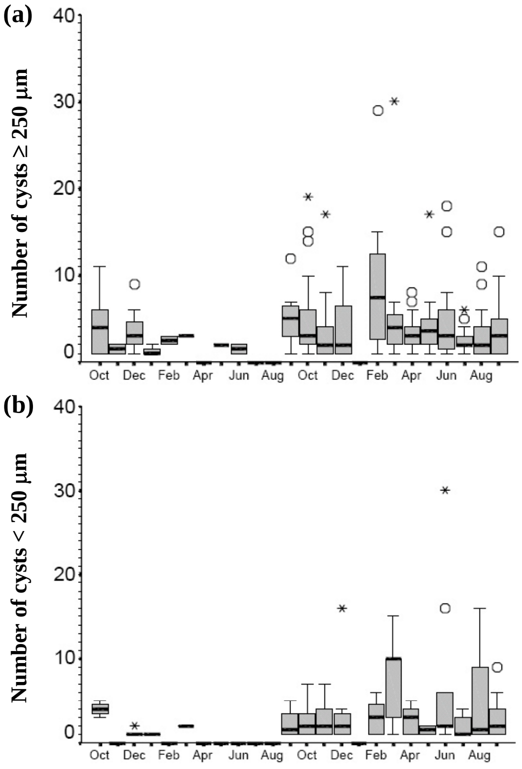


Figure 3. Boxplot of cyst numbers in *Cyathura* from the *Zostera* site from October 1993 until September 1995. a) Cysts ≥ 250 µm. b) Cysts < 250 µm. Boxplots show the median, interquartile range, outliers and extreme cases of individual variables (vertical line: is drawn from the upper quartile to the most extreme point that is within a distance of 1.5 of the upper quartile; outliers: values between 1.5 and 3 box length; extremes: values more than 3 box lengths; box length: interquartile length).

RESULTS

Trematodes

The size-frequency of microphallid cysts registered in *Cyathura* indicates that two size groups were dominant: one with a cyst diameter of 180-190 µm and another of 330-360 µm (Fig. 1). These modes represent the final sizes attained by mature cysts of some microphallid species. As they grow for up to six weeks within their host before reaching these sizes (maturation time depends on species and temperature) (Galaktionov et al. 1997, Damsgaard 2002), a high fraction of the cysts with sizes below the peaks probably represent immature cysts of the two dominant species groups. Identification of microphallid species cannot alone be based on mature cyst dimensions, but the smaller size group (180-190 µm) fits with the size of *Microphallus claviformis* (Brandes, 1888) or

Maritrema subdolum Jaegerskioeld, 1909, whereas the larger cysts belong to a hitherto unknown *Levinseniella* species (Warberg 2003). Screening of a few thousands *Hydrobia* specimens from the Mondego Estuary in 2002 resulted in 60 microphallid shedding snails. Of these, 53 snails were infected with *M. subdolum*, 2 with *M. claviformis* and 5 with the unknown *Levinseniella* sp. Therefore, it is reasonable to assume that a major fraction of the small cysts belong to *M. subdolum*. DNA-sequence analyses (ITS2-sequence data) have further verified that the small spherical cysts in *Cyathura* were identical to *M. subdolum* (unpublished data). Cercariae of the new *Levinseniella* species exhibit behaviour and morphology similar to what is reported for *Levinseniella brachyosoma* (Deblock 1980), except its lack of tail (Warberg 2003). The cercaria moves in a leech-like fashion. Both infection experiments and ITS2 sequencing have verified the identity between the large cysts found in *Cyathura* and the cercariae larvae found in *Hydrobia* (Warberg 2003). For the analysis of parasite patterns in *Cyathura*, we will for convenience distinguish between two groups of cysts: a) < 250 µm and b) ≥ 250 µm. As indicated, a majority of cysts within these groups belong to *M. subdolum* and the unknown *Levinseniella*, respectively.

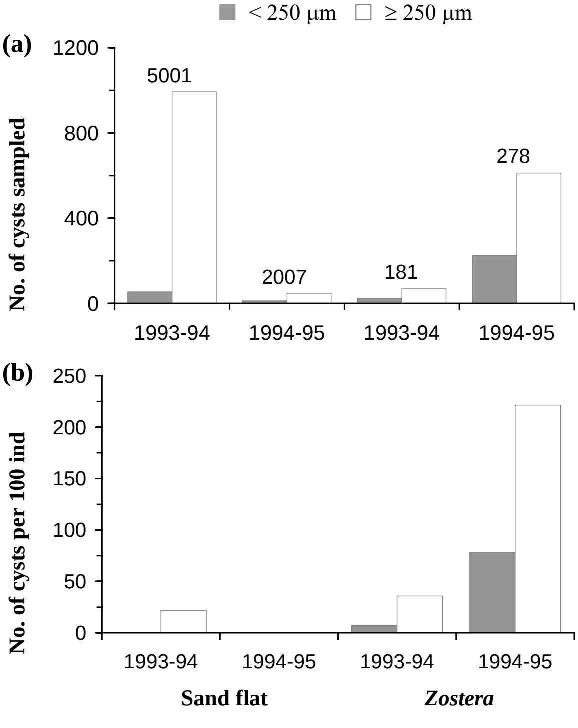


Figure 4. a) Total numbers of small cysts (< 250 µm) and large cysts (≥ 250 µm) recorded from *Cyathura* in the period June 1993 to May 1994 (1993-94) and June 1995 to May 1995 (1994-95) from the two study sites in Mondego Estuary. The figures above the columns indicate the number of *Cyathura* examined. b) Numbers of cysts per 100 specimens of *Cyathura* calculated from the data shown in Fig. 4a.

Temporal and spatial variation in density and infections of *Cyathura*

a) Sand flat site. The temporal density variation of *Cyathura* reflects the annual pattern of reproduction and recruitment (Fig. 2a). Ovigerous females had their peak occurrence mid-May to mid-June. From mid-July and onwards, there was steep increase in density due to recruitment, resulting in peak numbers in October. Afterwards, there was a more gradual decline until the following summer. Generally, infected specimens constituted a minor fraction of the whole population, during most of the study period (Fig. 2a). However, from autumn 1993 until summer 1994, up to 30% of the 1993 year-class was infected. The prevalence increased from August until October and remained at the same level until the die-off of most of the 1993 year-class. In this period, there was a positive correlation between the numbers of uninfected and infected specimens (Pearson correlation coefficient, $r = 0.815$, $p < 0.001$, $n = 23$). The development of infections in the 1994 year-class was different, as there was no increase in prevalence before November and only 7% (December 1994) of this cohort became infected.

The intensity pattern for the 1994 year-class did not show much seasonal variation. The median values were less than 2 cysts for both microphallid species, but a few specimens with more than 10 large cysts (≥ 250 µm) were found in the winter months. Overall, 66% of the specimens infected with large cysts had only 1 cyst and less than 1% of the infected had more than 10. For small cysts, infected isopods accommodated less than 5 cysts each.

b) *Zostera* site. Until late June 1994, the density of *Cyathura* was less than 100 ind m^{-2} (Fig. 2b). At this time, the 1994 year-class recruited and a peak density of 738 ind m^{-2} was reached in October that declined within a month to a few hundred individuals. Again, there was recruitment in 1995 resulting in a small density peak. Basically, the recruitment pattern of *Cyathura* at the *Zostera* bed showed similarity to the pattern at the sand flat site, but the peak density of recruits was much lower.

The prevalence of microphallid cysts in *Cyathura* from the *Zostera* stock was lowest during summer (June-July 1994 and 1995) and highest during winter months (Fig. 2b). As a reflection of the low density and thus small sample sizes of *Cyathura* until mid-1994, the prevalence of cysts fluctuated widely (between 0 and 50%). The prevalence in the 1994 year-class of *Cyathura* increased during autumn 1994, in contrast to what happened on the sand flat, and reached high levels from October 1994 until May 1995 (between 40 and 90%). A major decline took place when the 1995 year-class substituted the 1994 year-class, in summer 1995.

Concerning the development in intensity of trematode cysts in *Cyathura*, there was a weak seasonal pattern for the most abundant cysts (≥ 250 µm) in 1994-95. They had a peak in median intensity in February 1995 (Fig. 3a). At this time, 25% of the infected *Cyathura* stock harboured between 6 and 12 large cysts per specimen. It is obvious that the distribution of cysts in the host population is aggregated. Up to 30 cysts (≥ 250 µm) were observed in a single specimen in March 1995. The small and less frequent cysts did not show a pronounced pattern, though the highest median numbers were registered in March 1995. A single individual contained 32 small cysts (Fig. 3b).

Overall distribution of cysts in *Cyathura*

In total, 1995 cysts have been collected from sampled *Cyathura* specimens between June 1993 and May 1995. Of these, 52.3% came from the sand flat stock of *Cyathura*, in the period from June 1993 to May 1994, whereas 41.6% were collected from the *Zostera* stock from June 1994 to May 1995 (Fig. 4a). However, the number of *Cyathura* collected from the *Zostera* site in 1994-95 accounted only for 3.7% of all specimens sampled, whereas those from the sand flat (1993-94) accounted for 67%. To facilitate comparison in space and time across host density, the numbers of microphallid cysts per 100 specimens of *Cyathura* have been calculated (Fig.4b). From this graph, it appears that cysts ≥ 250 µm are more frequent than those < 250 µm. It is also obvious that the *Zostera* stock of *Cyathura* accommodated more cysts per host specimen in the period from June 1994 to May 1995 than in the previous year, but it harboured also more cysts per specimen than *Cyathura* from the sand flat in both years.

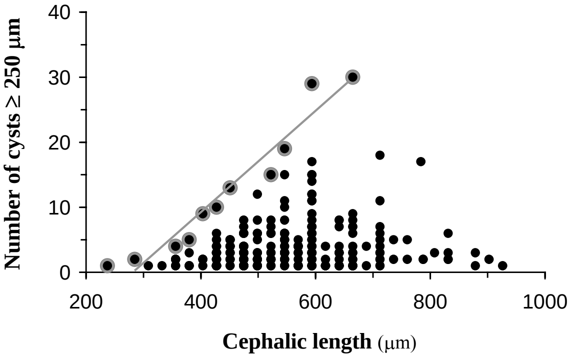


Figure 5. Numbers of cysts (≥ 250µm) as a function of cephalic length of *Cyathura* from the *Zostera* site. Maximum cyst numbers of each length group is indicated by black symbols enclosed in an open circle. (For each cephalic length a maximum cyst number was chosen if it exceeded the preceding values). A line is fitted to the data points indicating maximum cyst numbers.

Trematode infections in relation to host size and ovigerous females

The number of cysts in *Cyathura* (data from the *Zostera* site) shows a clear size-dependent maximum intensity (≥ 250 µm) until a host length of 7 mm (cephalic length 700 µm) (Fig. 5). Up to this threshold, there is a linear relationship between the maximum cyst number (MCN) for each size group and cephalic length (CeL) ($MCN = 0.076 \times CeL - 21.165$; $r^2 = 0.93$). For each CeL interval a maximum cyst number was chosen if it exceeded the preceding values. Using the slope of this formula, it appears that an increase in CeL of 200 µm (corresponding to an increase in body length of 2 mm) enhances MCN with about 15 cysts.

Cysts were very rarely observed in ovigerous females or in females with brood pouches (Fig. 6). During the three reproductive seasons (defined by the presence of females with brood pouches), 4 066 females were collected. Of these, 15% (609 individuals) had brood pouches with or without eggs. Among the females without brood pouches, 8.5% (295 individuals) were infected, whereas only 0.5% (2 individuals) of those with brood pouches had infections. This difference was highly significant ($\chi^2_1 = 51.5$, p = 0.000).

Temporal patterns of first intermediate host and final host

To understand spatial and temporal patterns of cysts in *Cyathura*, the phenology of the first intermediate and final hosts is important. The numbers of waders (final host) were highest during the winter months (November to February), due to the importance of the Mondego Estuary as a wintering ground for many migratory wader species, in particular the Dunlin *Calidris alpina* and the Avocet *Recurvirostra avosetta* (Fig. 7). Smaller peaks occurred during spring (April to May) and autumn migration (August to September).

Hydrobia (first intermediate host) densities were different between the two study sites. At the *Zostera* site, *Hydrobia* reached densities of nearly 300 000 ind m^{-2}, but this was mainly due to the occurrence of high numbers of small recruits (Cardoso et al. 2002). Generally, the density of snails ≥ 2 mm (Fig. 8) fluctuated around 63 000 ind m^{-2} at this site, whereas the adult density (≥ 2 mm) on the sand flat rarely exceeded 7 000 ind m^{-2}. Nevertheless, the population reached nearly 135 000 ind m^{-2} in March 1993, concomitantly with a bloom of green macroalgae.

DISCUSSION

Trematodes in *Cyathura*

The present study shows the dominance of cysts from at least two microphallid species in *Cyathura*: *M. subdolum* and *Levinseniella* sp.. The presence of these two species has been verified from DNA studies of cysts taken from *Cyathura* in 2001 (Warberg 2003). In addition, we found *M. claviformis* in *Hydrobia* specimens collected in 2001 in the Mondego Estuary. This species can also be expected to account for a fraction of the cysts recovered from *Cyathura*, as recent experiments have indicated that all the mentioned microphallids are able to infect *Cyathura* (SM Ferreira personal observation). Previously, Reimer (1963) registered *M. subdolum* in *Cyathura* from the Baltic Sea. Schulenburg et al. (1999) have reported the presence of unidentified microphallid species in *Cyathura*, from different European localities, using DNA techniques.

The present paper provides the first data of the unknown trematode *Levinseniella* sp.. It is remarkable that *Levinseniella* is quantitatively dominant to *M. subdolum* in *Cyathura* because, in 2001, more *Hydrobia* specimens from the estuary were infected with either *M. subdolum* or *M. claviformis* than with *Levinseniella* (a factor of 11). Furthermore, snails infected with *M. subdolum* have a much higher shedding rate of cercariae than snails infected with *Levinseniella* (SM Ferreira personal observation). In addition, the latter has a longer development time in *Cyathura* (3 weeks *versus* 5-6 weeks) (Galaktionov et al. 1997, Damsgaard 2002, KT Jensen personal observation). New recordings of infections in *Cyathura* from the Mondego Estuary, in 2001, have confirmed the dominance of *Levinseniella* over *M. subdolum* cysts.

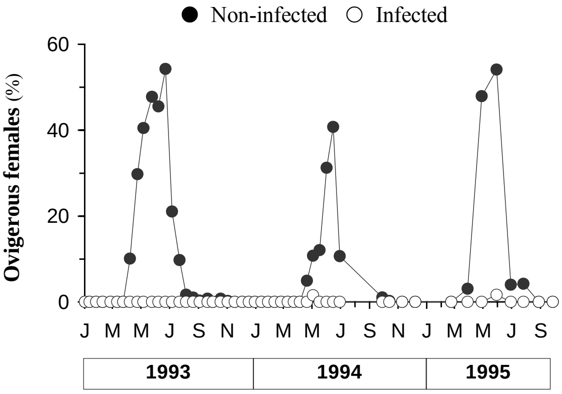


Figure 6. Temporal variation in the percentage of non-infected and infected reproductive females (females with brood pouches with or without eggs) relative to all adult females of *Cyathura* from the sand flat site in Mondego Estuary.

To generate the prevalence pattern of the two species, some selective mechanisms may be involved. Of course, it is possible that other amphipods or isopods from the estuary have another infection profile, with *M. subdolum* being dominant, as this would contribute to an understanding of the patterns within snails. Other potential crustacean hosts do occur in the estuary (Marques et. al. 2002). However, this will not explain the pattern in

Cyathura, as we can expect specimens to be exposed to more infective propagules of *M. subdolum* than of *Levinseniella*. Therefore, it suggests that *Levinseniella* exhibit specific adaptations to infect *Cyathura*. *Levinseniella* cercariae are tailless and do not swim. They stick to the bottom, where they move slowly around in a leech-like manner. In contrast, *M. subdolum* larvae swim in the water layer, close to the bottom. Therefore, we propose that *Levinseniella* is better adapted behaviourally than *M. subdolum* to infect a sluggish mud-dwelling isopod, such as *Cyathura.* But functional differences in penetration capability of cercariae of the two species, such as shape and size of stylets, could also be important in explaining host specificity.

Prevalence and intensity patterns

Although there are interannual and inter-site differences, the microphallid infections in *Cyathura* generally peak in autumn and winter months. This temporal pattern is associated with the phenology of waders that primarily are using the estuary as a wintering area. As *Cyathura* is prey to dunlins, and other waders (Kentish, ringed and grey plovers) (Múrias personal observation), their presence will promote dispersal of trematode eggs. As a consequence, the development of infections in the *Hydrobia* population will be closely coupled with the increase in bird numbers. At present, we do not have data showing the phenology of infections in *Hydrobia*, but we expect a peak in the prevalence pattern of snails a few weeks after the peak occurrence of waders. Generally, an increase in shedding rates and a decline in maturation time can be expected, following the temperature rise from March-April to mid-summer (Lillebø et al. 1999). Until November-December, where the temperature is still around 14-16°C, there will be a potential for cercariae production, if the temperature-dependent shedding rate of the unknown *Levinseniella* follows the pattern observed for other microphallids (Mouritsen & Jensen 1997). The number of infected snails will limit the total production of cercariae. The prevalence of infections in snails will not be expected to increase until the arrival of migratory birds from October onwards. Given a development time of a few weeks, from ingested parasite eggs to cercariae, there will be a relatively short period in October-December, with rising bird numbers and an appropriate temperature, where supplies of cercariae can increase again. Several factors may contribute to the patterns of microphallid cysts in *Cyathura*. The pattern is a result of the balance between supplies of larval propagules shed from snails and the removal of infected *Cyathura* specimens from the population through different mortality agents.

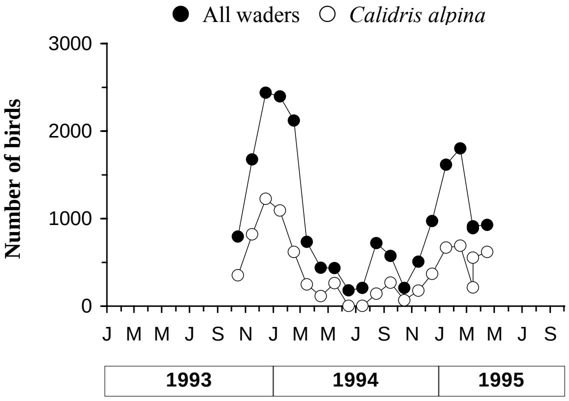


Figure 7. Monthly variation in abundance of all waders and Dunlins (*Calidris alpina*) in Mondego Estuary (October 1993 to May 1995). Data reproduced with permission from Múrias et al. (1997).

To explain the difference in prevalence in *Cyathura* between sites in the Mondego Estuary, the density of infected *Hydrobia* specimens, in particular, is supposed to be important. The density of snails is generally high on the studied sites, but the stock from the sand flat is strongly dominated by juveniles, in contrast to those from the *Zostera* site. As snails smaller than 2 mm (shell height) do not host microphallids (Probst & Kube 1999), it is obvious that the *Zostera* site potentially could accommodate more infected snails than the sand flat site. Except for a short period in 1993, the abundance of snails on the sand flat being larger than 2 mm is less than 7 000 ind m^{-2} (Cardoso et al. 2002). Apart from snails, there could also be environmental factors that may reduce transmission rates between snails and isopods.

Microphallids have been shown to cause mortality among heavily infected specimens of *Corophium volutator* (Pallas) (Muus, 1967 Mouritsen & Jensen 1997). It has also been demonstrated that surface acitivity in *Corophium* is promoted by parasites, probably because heavily infected specimens may have difficulty swimming and making burrows (Damsgaard 2002). Such surface active specimens are exposed to mortality agents, such as predators, and unfavourable abiotic factors. Actually, the observed decline in maximum cyst intensity for *Cyathura*, with a body length above 7 mm, could be a result of such mortality. The presence of an upper limit for the cyst number, as indicated by the formula for MCN, could also be a result of parasite induced host mortality at high cyst intensities. Because of space limitation inside the body of *Cyathura*, there is an upper limit to the number of large cysts that a specimen can accommodate. Along a 2 mm *Cyathura* body, there can be 6 mature cysts (each 340 µm) in a row. So, to approach the maximum numbers (15 cysts for a length increase of 2 mm), there should be two parallel rows. Undoubtedly, such a number of cysts will damage host tissue and will probably interfere negatively with host functioning (respiration, locomotion, behaviour).

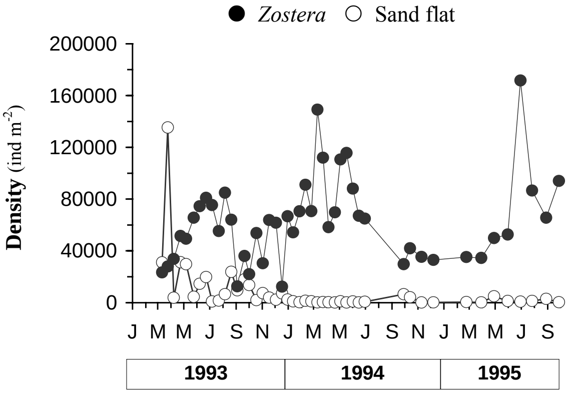


Figure 8. Temporal variation in density (ind m^{-2}) of *Hydrobia* (shell length ≥ 2 mm) at the sand flat site and at the *Zostera* site in Mondego Estuary from March 1993 until October 1995 (data provided by Lillebø et al. (1999) and Cardoso et al. (2002).

Our data suggest that microphallids could have negative impact on offspring production, as we found microphallid cysts significantly underrepresented among ovigerous females of *Cyathura*. Decreased reproduction of crustaceans, following microphallid infections, has previously been reported (Muus 1967, Bick 1994, Thomas et al. 1995). Perhaps, the most likely explanation of ovigerous females being underrepresented among the infected individuals is that infections interfere negatively with ecdysis and gametogenesis (Muus 1967, Thomas et al. 1995). Only those able to resist infective propagules may reproduce. Naturally, this could have significant impact on population development in infected areas. Our results suggest that microphallid cysts are responsible for the low recruitment on the *Zostera* site as only few ovigerous females were registered at this site. Experimental results with *Cyathura* have furthermore shown that even few microphallid infections may have strong negative impact on reproduction of *Cyathura* (SM Ferreira personal observation). Therefore, we hypothesize that microphallids contribute to the low offspring production and weak recruitment of *Cyathura* at the *Zostera* bed. As *Cyathura* is a mud-dwelling isopod with direct development, recruitment success depends on local offspring production. Consequently, recruitment failure can have long lasting consequences, as recolonisation of a site must await immigrants from elsewhere. Thus, population development may be controlled by microphallids influencing the reproductive capability of their hosts and they may further induce population fragmentation. Hidden as they are inside their host, such trematodes are often ignored in field studies examining the impact of environmental factors on target species. The present results suggest that trematodes in *Cyathura* potentially may be cryptic determinants of its population dynamics. It is therefore recommended that parasites are included in population studies of estuarine crustaceans known to host microphallid trematodes.

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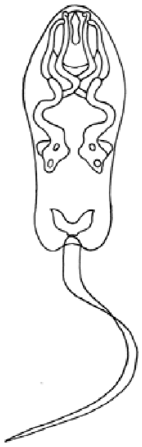
Chapter 2



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

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 25ºC, *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.

Key words: Parasitism, *Maritrema*, *Cyathura*, *Hydrobia*, Sediment

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, Mouritsen & Jensen 1997, Mouritsen et al. 1997, 1998, Meissner & Bick 1997, 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 24ºC and salinity of 20, 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 15ºC.

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 15ºC and a salinity of 20.

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 versus 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 (salinity of ≈ 22). Brown substratum was used in half of the containers and yellow substratum in the other half. The aquaria were kept in a 25ºC 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 5 aquaria containing *M. subdolum* infected *H. ulvae* (treatments) and 5 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 1 304 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 surface 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 15ºC. The aquaria were emptied and live *C. carinata* were counted. Afterwards, they were placed at 6ºC 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 25ºC room, after having recovered for one week at 15ºC. 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 ± 8% *versus* brown substratum: 22 ± 5%, 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 ± 5 parasites *versus* brown substratum: 7 ± 3 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 hours 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* = 0.33, $p > 0.05$, n = 10).

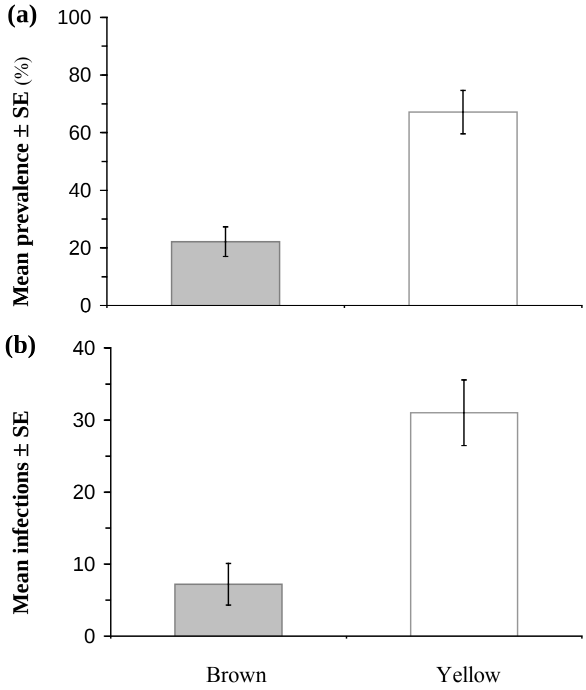


Figure 1. a) Prevalence of *M. subdolum* in *C. carinata* and b) intensity (mean ± SE) of the total number of infections found in each replicate of the brown and yellow substrata aquaria.

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

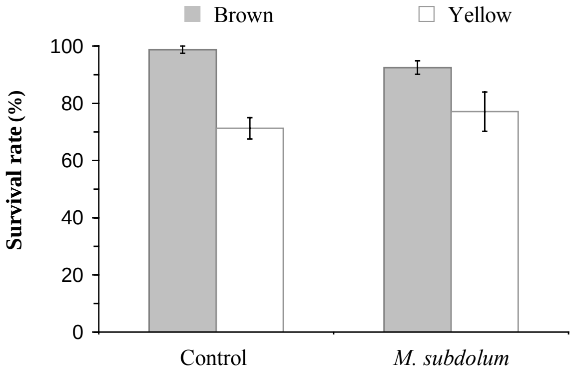


Figure 2. Survival rate (mean ± SE) of *C. carinata* as a function of substratum and parasite treatment (control: isopods incubated with *Cryptocotyle* infected snails).

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 versus* 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 ($r_{58} = 0.079$, $p > 0.05$).

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 (SM Ferreira personal 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 (SM Ferreira personal 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.

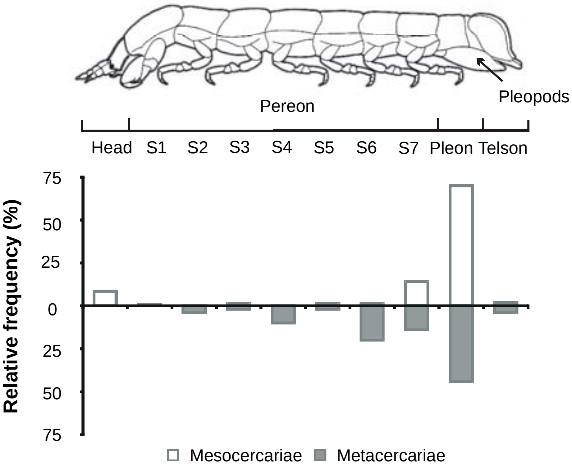


Figure 3. Distribution of meso (n = 140) and metacercariae (n = 50) of *M. subdolum* in different body parts of infected *C. carinata* (n = 60). S1 to S7 are the seven segments of the pereon region of the isopod's body.

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.

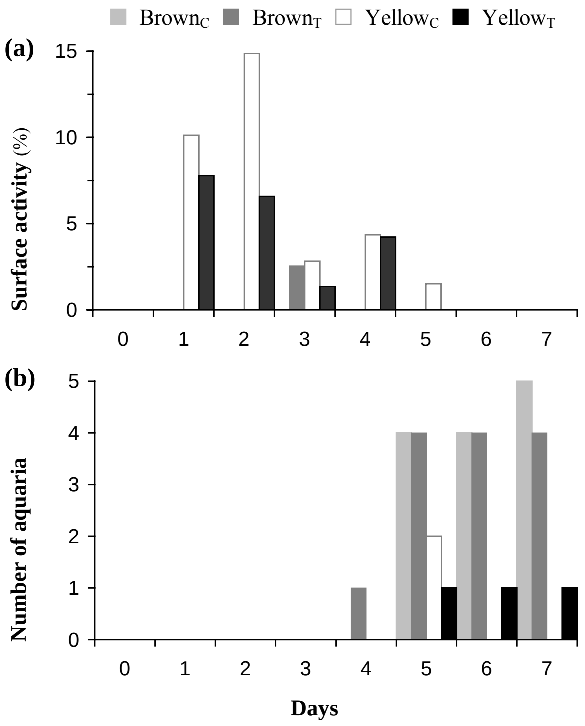


Figure 4. Surface activity of *C. carinata* in the brown and yellow substrata aquaria. a) Relative frequency of isopods swimming, crawling on the surface or extruding the burrow, during light hours. b) Number of aquaria with isopod tracks on the sediment's surface, made during darkness. (**C**: control,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

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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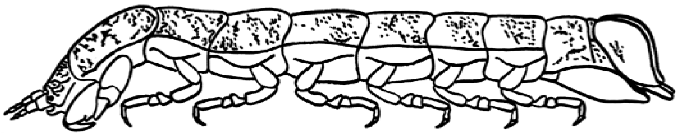
Chapter 3



Impact of microphallid trematodes on the survivorship, growth and reproduction of an isopod (*Cyathura carinata*)

ABSTRACT

Crustaceans are second intermediate hosts to several microphallid species (Trematoda). Some of these parasites are potentially pathogenic or manipulative. A laboratory experiment was performed to assess the impact of microphallids on the survival, growth and fecundity of *Cyathura carinata*, a protogynous hermaphroditic isopod, widespread within European estuaries. For nearly 12 weeks, experimental populations of infected and non-infected isopods were kept at 25ºC. *C. carinata* carrying microphallid cysts showed higher mortality rates than non-infected specimens and were not able to produce embryos. The reduced fecundity of infected isopods could be caused by parasite-induced castration and/or by mating failure due to behavioural modifications in one of the sexes. It might also be associated with lower growth rates and lower moulting frequencies, since infected *C. carinata* were significantly smaller than the non-infected after 9 weeks. This may imply a setback for the isopods to achieve sexual maturity (which may also affect the population sex ratio) and for females to lay their eggs in the marsupia. Regardless of the mechanisms involved, microphallids may have severe consequences for their host populations, through negative effects on survival, growth and fecundity. For species with direct development, such as *C. carinata*, parasite induced reproduction failure may contribute to temporal fluctuations of abundance. Based on the present results, it is recommended to include parasites as an important factor influencing host populations from shallow-water ecosystems.

Key words: Isopod, Microphallid, Parasite, Fecundity, Growth, Mortality

INTRODUCTION

Amphipods and isopods from shallow water ecosystems are hosts to a variety of trematode species. These parasites are potentially pathogenic or manipulative and thus may influence the population dynamics of their hosts (Helluy 1984, Thomas et al. 1995, Mouritsen & Jensen 1997, Meissner & Bick 1999). At least 10 species of microphallid trematodes using mud snails *Hydrobia* spp. (first intermediate hosts) are represented in benthic peracarid crustaceans from the northwestern European estuaries and coastal lagoons (Deblock 1980). By using crustaceans as their second intermediate hosts, the parasites are capitalising on existing prey-predator relationships to reach water birds, their final hosts. Reported effects of microphallids on crustaceans include enhanced host mortality (Mouritsen & Jensen 1997, Jensen et al. 1998, Meissner & Bick 1999), behavioural control to promote transmission to the final host (Helluy 1984, Combes 2001), reduced offspring production and prolonged intermoult stages (Muus, 1967; Thomas et al. 1995, 1996a,b). Microphallid species exhibit a variety of life histories and each of them may have different effects in different host species, reflecting the individual parasite-host evolution. This makes it rather unpredictable whether microphallids are harmless, manipulative or pathogenic.

Recognizing the possible effects of microphallid trematodes in crustaceans, it is necessary to consider these in population studies of host organisms. The mud snail *Hydrobia ulvae* and the isopod *Cyathura carinata* are two widely distributed macrozoobenthic species in European brackish water ecosystems. These two macroinvertebrates co-occur in the Mondego Estuary (western Portugal) and both are infected with different larval stages of the same microphallid species (Jensen et al. 2004). Up to 90% of *Cyathura carinata* from a *Zostera noltii* meadow were infected by these trematodes, with a maximum mean intensity of 12 cysts (Jensen et al. 2004).

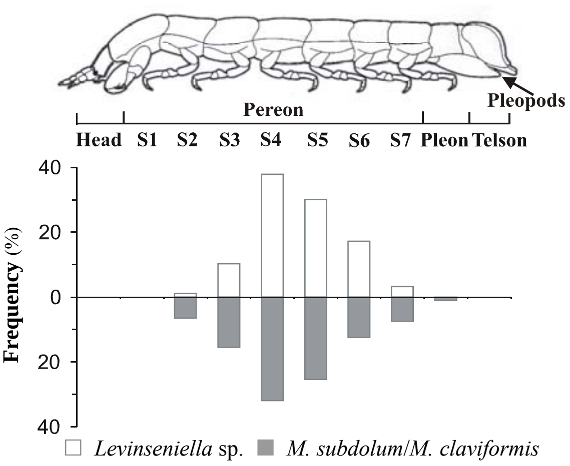


Figure 1. Location of *Levinseniella* sp. (n = 309) and *M. subdolum*/*M. claviformis* (n = 236) cysts within the body of *C. carinata* (n = 131, specimens alive after the 37th day, when the larger cysts were mature and in a fixed position).

Descriptive data suggest that microphallids control the reproductive success of the isopod, as ovigerous females were considerably underrepresented among infected individuals (Jensen et al. 2004). At the Mondego Estuary, *C. carinata* has a life span of 2 years, shows continuous growth rates and generates a single cohort per year (Ferreira et al. 2004). To understand the importance of microphallid parasites for the population dynamics of this isopod, a laboratory experiment was conducted to study the survivorship, growth and fecundity of non-infected and infected specimens. As *C. carinata* is a protogynous hermaphrodite (1 year-old females may endure sex reversal and reproduce as males in the second year), microphallids could also potentially influence sex ratio and male differentiation of the isopod. Possible mechanisms explaining the complex of effects caused by microphallid infections are discussed, as well as their consequences for the population dynamics of *C. carinata*.

MATERIALS AND METHODS

Collection and treatment of experimental animals, sediment and water

C. carinata specimens were collected from two different sites of the Mondego Estuary (40º08'N, 8º50'W), at the end of January of 2003. One of the sites is a sand flat, located in an inner area of the estuary, where the isopods are abundant and almost non-infected (Jensen et al. 2004). The other site is a *Z. noltii* meadow, situated 600 m downstream of the previous area, where *C. carinata* are scarce and usually infected by microphallids (Jensen et al. 2004). All individuals used in the experiment belonged to the same population, since the isopods (especially juveniles) generally colonise the *Z. noltii* meadow, after the reproduction season (Ferreira et al. 2004). Sediment containing burrowed *C. carinata* was collected with a hand corer and sieved in estuarine water, through a 0.5 mm mesh. The isopods retained were sorted *in situ*, until achieving 500 specimens from each site. The captured organisms were transported to laboratory and kept at 25ºC. Each *C. carinata* was inspected for the presence of cysts under a stereomicroscope. Infected specimens were separated from the others. Most of them had only 1 cyst, which was much lower than the intensity observed in the natural population, in previous years (Jensen et al. 2004). To enhance the parasite load, the infected isopods were immediately incubated in a container for 24 h, together with infected *H. ulvae*. *Maritrema subdolum*, *Microphallus claviformis* and one until now undescribed *Levinseniella* sp. were the microphallid species used, being the only ones so far identified in the Mondego Estuary (Jensen et al. 2004). The cysts from the first 2 species have more or less the same size (≈ 170 μm diameter) (Deblock 1980) and cannot be distinguished from each other when inside living hosts. Still, both are very different from the third one, whose cysts are very large (≈ 340 µm diameter) (Jensen et al. 2004). The maturation of these cysts may last between 4 weeks for *M. subdolum*/*M. claviformis* and 6 weeks for *Levinseniella* sp. (Galaktionov et al. 1997).

For the experiment, sediment was sieved through a 0.5 mm mesh size and bubbled with nitrogen for 24 h to remove all macroinvertebrates. Clean seawater (salinity of ≈ 35) was collected at the mouth of the estuary, diluted with demineralised water (until a salinity of 20) and stored at 10ºC.

Experimental design

Forty glass jars of 1.5 l were used as aquaria. Each contained a sediment layer of 3 cm depth and 0.65 l of brackish water (salinity of 20). To avoid evaporation, a lid was loosely attached to the jars. They were established in a room at 25ºC, with a 12 h photoperiod. Aeration was provided during 30 min at each 4/2 h of light/darkness, to avoid oxygen depletion and disturbing isopods' normal activity. To assure stable environmental conditions, the jars were left undisturbed for 48 h. Then, *C. carinata* specimens were added, 2 weeks after being collected and acclimated to the experimental conditions.

Twenty jars with experimental animals were established. They consisted of 2 sets of 10 replicates, respectively for non-infected and infected isopods. Since only 197 infected *C. carinata* were found, these determined the abundance to be used. Corresponding to a density of 2 680 to 2 822 individuals per m^{-2}, comparable to the natural population in the estuary during the same period of the year (Ferreira et al. 2004), 7 jars of each set contained 20 specimens and 3 were left with 19. To avoid injuring the isopods and ensure equal size distribution within the infected and non-infected populations, isopods were matched pairwise according to their body length. The initial size of the two coexisting cohorts in both experimental populations was determined through size-frequency distribution analysis of *C. carinata* collected from the Mondego Estuary, in the same period of the year (Ferreira et al. 2004). This analysis was performed by ANAMOD software, which is based on the probability paper method (Cassie 1954). After 45 days, the cephalic lengths of all living *C. carinata* were measured, using photographs taken with a digital video camera mounted on a stereomicroscope. The size of the isopods after 45 days was also analysed in accordance with the natural population structure (Ferreira et al. 2004).

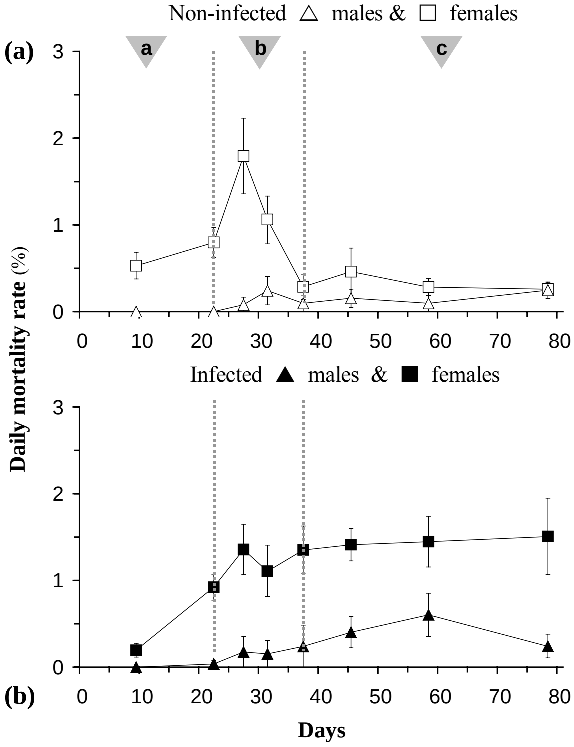


Figure 2. Comparison of daily mortality rates between: a) males and females within the non-infected isopods and b) males and females within the infected *C. carinata*. The results express the proportion (%) of individuals that died per day, between two consecutive examination dates, represented by the mean (± SE) of all the replicates.

During the experiment, *C. carinata* specimens were regularly fed with frozen *Hediste diversicolor*. Periodically, the aquaria were disassembled to track the population development (counting and recognising males, non-reproductive and ovigerous females by their relative size, plus the cysts within infected specimens). It was done only 8 times in total to minimise disturbances. The 20 spare jars were used to replace the disassembled ones, which were left undisturbed until the next inspection, under the same temperature and light conditions.

All ovigerous females found were isolated in 25 cm^2 cell culture flasks. Environmental conditions were exactly the same as in the experiment, except for the sediment. A layer of fine sand (SDS sand Fontainebleau, ref. 1960027, 99.8% SiO_2, granulometry 150-300 ppm) was used instead of mud. Generally, cyathurans are not very specific about the sediment in which they live (Burbanck & Burbanck 1979). This procedure was necessary for counting and following the embryonic development of offspring. Embryos were classified in 3 developmental stages, according to literature (Jazdzewski 1969, Bamber 1985, Ferreira et al. 2004).

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

Infected *C. carinata* hosted 1 to 19 microphallid cysts (4.2 ± 0.2, mean ± SE). Among these, *Levinseniella* sp. was more abundant (2.4 ± 0.2 cysts, mean ± SE) than *M. subdolum*/*M. claviformis* (1.8 ± 0.2 cysts, mean ± SE). Infected females (2.9 ± 0.4, mean ± SE) accommodated significantly more cysts of *Levinseniella* sp (Wilcoxon test: $W = 1\,965$, $p < 0.05$) than males (1.8 ± 0.2, mean ± SE). The cysts were located within the pereon region of the isopods, most of them (> 50%) being concentrated in the 4th and 5th segments (Fig. 1).

The experiment was divided in 3 periods: a) before, b) during and c) after the appearance of ovigerous females, in accordance with their removal to individual culture flasks as they came along. The pattern of the mean daily mortality rates differed between these periods (Fig. 2). The first period can be considered as a reference value for the background mortality. For both non-infected and infected isopods, mortality increased gradually until half of the second period. High rates were observed between the 22nd and 27th days, when ovigerous females were found more frequently. Then, the mortality of the non-infected *C. carinata* declined and remained at a lower level during the third period, close to the initial reference value (Fig. 2a). Contrarily, the infected isopods maintained high rates until the end of the experiment (Fig. 2b). Overall, significantly more infected (73%) than non-infected (44%) specimens died during the whole experimental period (*t*-test: $t_{18} = -5.33$, $p < 0.001$). Females exhibited higher mortality rates than males, both within the non-infected (Wilcoxon test: $W = 145$, $p < 0.001$ Fig. 2a) and infected *C. carinata* (*t*-test: $t_{18} = 11.94$, $p < 0.001$, Fig. 2b). After the appearance of the last ovigerous female (third period), the half-life time estimated for the non-infected females (74 days) was higher than for the infected ones (20 days). Both were much lower than the half-life of non-infected (280 days) and infected (161 days) males.

Microphallid's influence on the mortality rates of parasitised *C. carinata* was analysed from the second period onwards, when all cysts were already visible. No differences were found between the initial and final numbers of isopods infected with both groups of trematode species, or just with *Levinseniella* sp. or *M. subdolum*/*M. claviformis* (Chi-square test: $\chi^2_2 = 0.325$, $p > 0.05$). Neither was there any selective mortality in relation to isopods infected with 1, 2 and 3 or more cysts (Chi-square test: $\chi^2_3 = 1.576$, $p > 0.05$ for *Levinseniella* sp.; $\chi^2_3 = 1.015$, $p > 0.05$ for *M. subdolum*/*M. claviformis*).

In the non-infected population, almost all males became distinct from females between the 9th and the 22nd days (Fig. 3a), giving a sex ratio of approximately 1:2 (Fig. 3b). In the infected population, this ratio was initially lower (1:8, Fig. 3b) and new males kept on differentiating from females throughout the whole experimental period (Fig. 3a). Afterwards, both sex ratios increased gradually (Fig. 3b), mostly due to the higher mortality rates of females.

A similar size composition was given at the beginning of the experiment, as infected and non-infected isopods were matched pairwise according to their body length. However, after 45 days, the infected isopods of the 1 year-old cohort were significantly smaller than the non-infected, both for males and females (Table 1). From the 2-years old cohort, there were no infected males and very few infected females. Either infected *C. carinata* grew significantly less than the non-infected or there was a differential size-selective mortality between the two groups. No significant correlations existed between *C. carinata*'s size (males and females) and the number of cysts, considering all microphallid groups (r_{41} = 0.203 and r_{63} = -0.008, $p > 0.05$), or just *Levinseniella* sp. (r_{41} = -0.031 and r_{60} = -0.067, $p > 0.05$), or females infected with *M. subdolum*/*M. claviformis* (r_{24} = 0.109, $p > 0.05$). There was a significant correlation between males' size and the number of *M. subdolum*/*M. claviformis* cysts ($r_{21} = 0.583$, $p < 0.05$).

Ovigerous females appeared during 2 weeks, starting from the 22nd day. A higher frequency was observed until the 27th day (Fig. 4a). From all females within the control population, 29% produced embryos, 3% of which died (Fig. 4b). This was higher than the 2% of ovigerous females registered among the infected isopods (Chi-square test: $\chi^2_1 = 28.124$, $p < 0.001$), half of which died as well. The digenean intensity within these females was rather low and varied between 1 and 2 cysts. The only one with 7 *M. subdolum*/*M. claviformis* cysts died. The embryonic development period lasted 28 ± 2 days (mean ± SD). Each of the 3 considered developmental stages endured similar time intervals, between 8 to 10 days. The last one was more variable because it depended on the reliability of juveniles to live the females' brood pouches. A linear regression was established between the females' cephalic length and their offsprings (number of embryos = 81.2 × female's cephalic length - 29.9, r^2 = 39%, n = 46). The few infected ovigerous females that did survive contained a number of embryos within the confidence limits (5%) of the estimated equation.

DISCUSSION

Initially, the mortality rates of both infected and non-infected *C. carinata* were low. Previous experiments have also shown that *C. carinata* is not affected by low infection levels on a short-term time scale (Ferreira et al. 2005). Mortality rates increased during the second period, when males and ovigerous females were found among both infected and non-infected *C. carinata*. This suggests that some individuals may be less fit to cope with the demanding physiological processes taking place during reproduction (*e.g.* sex reversal and egg production). An idea reinforced by the fact that the non-infected females returned to the background mortality rates afterwards. Moreover, the low rates observed among males suggest that these are the most robust specimens. The isolation of ovigerous females in individual containers cannot explain the mortality differences between the infected and non-infected isopods. In fact, during the third period, the mortality rates of the ovigerous females were similar to those of the non-infected females remaining in the experimental jars.

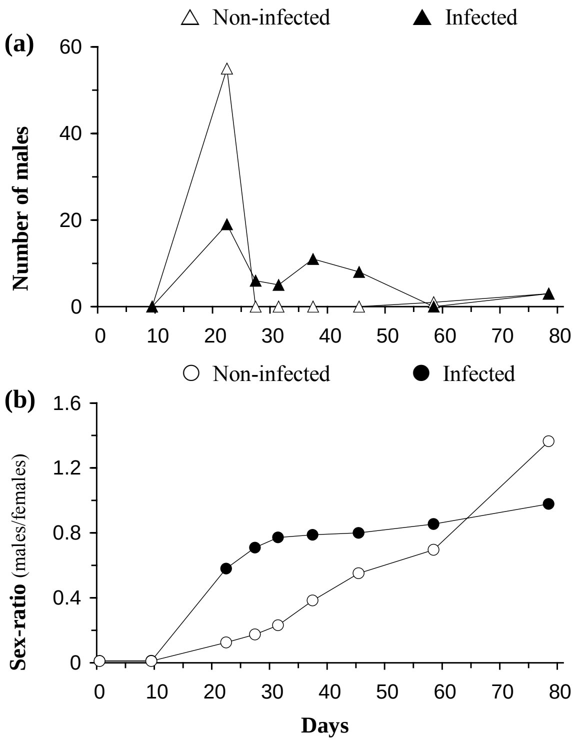


Figure 3. a) Number of new males that differentiated from females within the non-infected and infected *C. carinata* populations. Body length plus the number and type of cysts allowed recognising and tracking these individuals during the experiment. b) Sex ratio within both non-infected and infected populations.

C. carinata's growth was also affected by the presence of microphallid cysts, as reported before in amphipods (Muus 1967, Thomas et al. 1995, 1996a,b). After 45 days under experimental conditions, infected *C. carinata* males and females were significantly smaller than the non-infected. Size-selective mortality is an alternative explanation to the growth differences. However, there was no obvious sign that it happened. All specimens were counted, sexed and identified by their relative size each time they were inspected. Concerning the infected isopods, it was easy to follow each individual by the number and location of cysts. A higher mortality among larger infected individuals accommodating more cysts was not observed.

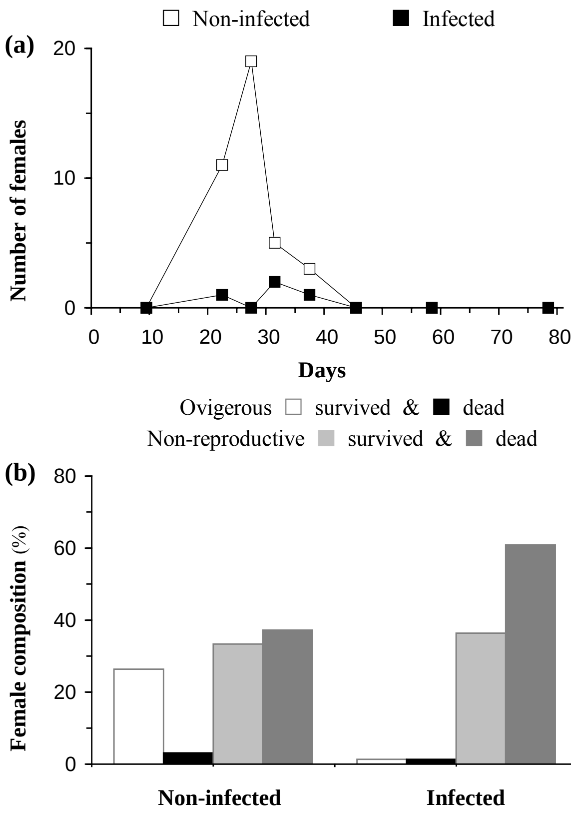


Figure 4. a) Number of new ovigerous females within the non-infected and infected *C. carinata* throughout the experimental period. b) Female composition within the non-infected and infected isopods, classified as surviving or dead ovigerous females and surviving or dead non-reproductive females.

C. carinata males can develop by two different ways: a) sexual maturity of the 1 year-old individuals, or b) sex reversal of the 2 year-old females (protogynous hermaphroditism). Both processes occurred during the same time interval in the control population. Almost all non-infected males were identified at the 22nd day, resulting in a sex ratio of nearly 0.5 (or 1:2). Among the infected isopods, new males continued to appear until the end of the experiment, although some were able to differentiate at the same time as the non-infected ones. The initial sex ratio of the infected isopods was significantly lower (0.125 or 1:8). This may be explained by the longer time required for the 1 year-old individuals to achieve sexual maturity, as a consequence of lower growth rates caused by microphallid cysts. The timing of appearance of these males can perhaps be related to the background of their infection, namely for how long they had been infected and the intensity of their infection. No 2 year-old infected males were observed, so there was no sex reversal of infected females.

C. carinata were able to breed under the present experimental conditions. They produced offspring 1½ month earlier than under natural conditions (Ferreira et al. 2004), probably stimulated by the high temperature and photoperiod used during the experiment (Jazdzewski, 1969, Bamber 1985). The embryonic development was also shorter than 45 days estimated from field observations (Ferreira et al. 2004). Microphallids had a negative influence on *C. carinata*'s reproduction. Almost no infected females were able to produce embryos. The few specimens that did manage to accomplish it and survive (1% of the total population) contained a low abundance of cysts. The fact that females were slightly more infected with *Levinseniella* sp. than males could potentially contribute to their higher mortality rates. Still, they showed the same pattern as the non-infected females. The mortality rates increase during the second period, probably also as a consequence of a reproduction effort. A possible interpretation could be that these females do try to reproduce, but somehow microphallids have a negative impact on their capability to produce offspring. That attempt could also be related with the age of their infection, since parasite trematodes affect the growth rates of crustaceans, according to the results of this experiment and of other similar works (Muus 1967, Thomas et al, 1995, 1996a,b). For instance, those that acquired microphallid cysts close to reproduction would be able to breed at the same time as the non-infected females. However, those that had been infected for longer time would achieve a later sexual maturity and develop reproduction mechanisms at different moments in time, during and after the second period. This might explain why they kept higher mean daily mortality rates during the third period, while the non-infected returned to the initial values. All surviving specimens were kept under surveillance, after the experiment ended. No more ovigerous females were

Table 1. Comparison of mean cephalic lengths (± SD) of experimental non-infected and infected *C. carinata* males and females (n - sample size). The initial length of all isopods is indicated, as it was not possible to differentiate between males and females at the beginning of the experiment. Two cohorts composed the population structure: the 1 (0+) and 2 (1+) year-old cohorts.

Cephalic length (mm)		**Initial**		**After 45 days**				
Cohort	**Sex**	**1. All**	**n**	**2. Non-infected**	**n**	**3. Infected**	**n**	***t* - test**
0+	**Males**	0.41 ± 0.07	447	0.81 ± 0.10	46	0.73 ± 0.11	43	t_{87} = 3.32, p < 0.01; μ1 ≠ μ2
	Females			0.71 ± 0.07	51	0.65 ± 0.08	62	t_{111} = 4.95, p < 0.01; μ1 ≠ μ2
1+	**Males**	0.76 ± 0.12	26	1.13 ± 0.10	16	-	0	-
	Females			0.87 ± 0.04	10	0.89 ± 0.06	3	-

found until the death of the last individual, 6 months after the experimental set-up. Notably, 43.5% of all the ovigerous females died within 1-month after releasing their offspring, which sustains that reproduction is a physically demanding process. These results do also corroborate the strong post-reproduction mortality observed in southern European populations, where only 10 to 20% of the 1year-old individuals are able to survive, after the reproduction peak, and thus accomplish 2 years of life span (Amanieu 1969, Ferreira et al. 2004).

Fecundity and growth reduction in invertebrates hosting macroparasites has previously been reported among crustaceans, insects and others (Muus 1967, Ward 1986, Thomas et al. 1995, 1996a,b, Hurd & Webb 1997, Bollache et al. 2002). In the present case, few cysts were responsible for the effects observed. Therefore, nutrient competition between host and parasite does not seem to be the most plausible explanation, although it may be an important one. The uptake of nutrients (Ginetsinskaya 1988, Galaktionov et al. 1996) by microphallids may interfere with the host's allocation of resources to growth and reproduction. However, the way by which microphallids interfered with *C. carinata*'s reproduction remains unclear, even though several possibilities can be presented. a) Since moulting is a prerequisite for egg fertilization and oviposition, ecdysial problems (Muus 1967) and reduced moulting frequency caused by parasites (Thomas et al. 1996a,b) may result in infertility. b) Mechanical damages inflicted by the presence of parasites in the host's body may also have a negative impact on reproduction. *C. carinata* eggs are deposited in a marsupium, made up of overlapping oostegites on the 3rd, 4th, and 5th thoracic segments (Burbanck & Burbanck 1979). The ovaries are located in the same region, with the oviducts emerging in the internal side of the 5th pair of pereopods. The incidence of microphallid cysts in this area may interfere, either mechanically or chemically, with gametogenesis and egg development. In particular, the larger *Levinseniella* sp. cysts may be more harmful, as they occupy almost all the body cavity of the host. c) If males or females normal behaviour is altered, then their mating success may be reduced. d) Parasites may also inhibit oocyte maturation or cause other gametogenesis disorders (Hurd & Webb 1997) through their impact on the host's hormonal system. Studies across several host-parasite complexes (all with insects as hosts) have shown that parasite induced fecundity reduction is associated with a lower vitellin protein content (yolk) of the host ovaries (Hurd & Webb 1997). Furthermore, it has been revealed that vitellogenin synthesis and secretion in fat bodies, plus the ability of the ovaries to uptake it from hemolymph (ovarian patency) are markedly reduced, as well as the binding capacity of juvenile hormone, responsible for the regulation of these processes (Hurd & Webb 1997). Considering that crustaceans share similarities with insects, it is likely that digenean cysts may also mediate their effects through an impact on the crustacean host endocrine system. However, further studies are needed to understand the ways by which trematodes interfere with the metabolism of crustaceans.

Irrespective of the mechanisms involved, the present work shows that microphallid cysts may have severe impact on *C. carinata* populations. It is demonstrated that parasites might be a significant mortality agent. Yet, the negative impact on reproduction may be even more severe to such a species with direct development, limiting its dispersal ability. Although, *C. carinata* is one of the most important species of the Mondego Estuary intertidal mudflats (Ferreira et al. 2004), it exhibits fluctuating densities in areas where adult mud snails potentially hosting microphallids are abundant

(Jensen et al. 2004). Based on the results obtained, this work supports the idea that microphallids can be one of the factors contributing to host fluctuations, causing mortality and recruitment failure. The persistence of local *C. carinata* populations, co-occurring with dense stocks of mud snails, may depend on the continued appearance of dispersing specimens from elsewhere. As prevalence of microphallids also exhibits interannual fluctuations, perhaps related to the phenology of waders, their impact on the isopods will also vary accordingly. Generally, parasites may contribute to population fragmentation and limitation of the efficient population size. Therefore, knowledge of the parasite fauna is required to understand spatial distribution and dynamics of *C. carinata* and should be considered in future studies.

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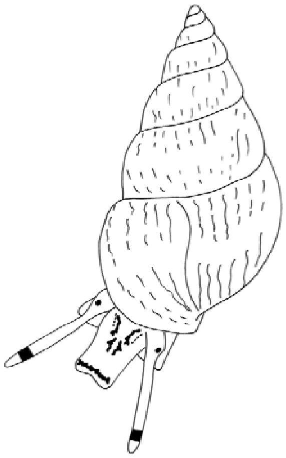
Chapter 4



Digenetic trematodes in a southern European population of the estuarine gastropod *Hydrobia ulvae*

ABSTRACT

Digenetic trematodes Haploporidae, Plagiorchiidae, Echinostomatidae and Microphallidae were observed in a southern European population of the mud snail *Hydrobia ulvae* (Mondego Estuary, Portugal). Higher prevalence of these parasites occurred in autumn/winter (4.3%) and again in spring (3%), decreasing until 1% in summer. The relatively low prevalences, however, corresponded to 800 - 5 440 infected mud snails per m^2. Digenetic trematodes were found in mud snails from size classes between 2.0 and 5.9 shell height and those > 5.9 mm were non-infected. Parasitised *H. ulvae* had generally thin shells, very easy to crush, with large and round whorls, fouled by epibionts.

Key words: *Hydrobia*, Trematode, Population structure, Prevalence, Digenean

INTRODUCTION

Mud snails are some of the most important deposit feeding invertebrates in European estuaries. They serve as intermediate hosts to several digenetic trematode species. Infections by digenetic trematodes have been reported to cause host debilitation (Jensen et al. 1996), castration (Mouritsen & Jensen 1994, Huxham et al. 1995), changes in mobility (Mouritsen & Jensen 1994) and enhanced growth or "gigantism" (Mouritsen & Jensen 1994, Gorbushin 1997). As *H. ulvae* is an important food source for birds (Lopes et al. 2000) and fishes (Aarnio & Matilla 2000), digeneans may interfere with food web dynamics (Huxham et al. 1993). In the Mondego Estuary (Portugal), *H. ulvae* is the most abundant invertebrate in the intertidal areas, especially at *Zostera noltii* beds, where it can reach more than 300 000 ind m^2 (Lillebø et al. 1999, Cardoso et al. 2005). Therefore, it is intended to: a) identify digenean species infecting *H. ulvae*, b) describe temporal variations of parasite prevalence and c) infer possible repercussions for the snail population structure and dynamics.

MATERIALS AND METHODS

Study site

The Mondego Estuary is located in the central western coast of Portugal (40º08'N; 8º50'W), where a *Zostera noltii* bed has been monitored since 1993. The period from September of 1994 to September of 1995 will be in focus, as high levels of trematode infections were reported for a second intermediate isopod host, *Cyathura carinata* (Jensen et al. 2004). Monthly macrobenthic samples consisted of 6 random cores taken to a depth of 15 cm. Every core was washed in a 0.5 mm mesh sieve and the remaining material was preserved in 4% buffered formalin. Later in laboratory, all animals were sorted and kept in 70% ethanol. On all occasions, low water pools temperature and salinity were measured *in situ*. Water temperature reflected the typical annual pattern of a temperate climate, being low in winter and high in summer, varying from 9 to 31ºC (Cardoso et al. 2005). Salinity remained generally above 21, except in rainy years, when it could decline to values lower than 5 (Cardoso et al. 2005).

Table 1. Digenetic trematodes found within *H. ulvae* from the Mondego Estuary (n), with indication of their mean annual prevalence (± SE), mean annual density (± SE) and groups of final and second intermediate hosts. The symbol "…?" stands for the fact that there are still too many uncertainties regarding the identification of species from families Haploporidae and Plagiorchiidae, for which they will be treated as a whole group. A reference is made to the species positively recognized, but there can be more that were not identified. A hithertho unknown *Levinseniella* species has also been observed in living snails from the same site in 2001-04, but not in this particular study.

Digenean family	Species	n	Prevalence (%)	Density (ind m^{-2})	Second int. hosts	Final hosts
Haploporidae	Gymnophallid sp. no. 3 Gymnophallid sp. no. 4 ... ?	40	0.85 ± 0.17	539 ± 69	not existent or unknown	fishes? birds?
Plagiorchiidae	*Cercaria tetralophocerca* Cercaria sp. no. 20 ...?	22	0.47 ± 0.15	239 ± 85	unknown	
Echinostomatidae	*Himasthla continua* *Himasthla interrupta*	2	0.04 ± 0.03	24 ± 16	bivalves	birds
Microphallidae	*Maritrema subdolum*	33	0.68 ± 0.17	579 ± 210	crustaceans	birds
	Microphallus claviformis	22	0.58 ± 0.18	410 ± 194		
	Levinseniella brachysoma	3	0.07 ± 0.04	43 ± 23		
	Levinseniella sp.	-	-	-		
(Not identifiable)		3	0.06 ± 0.04	37 ± 20	?	?

Laboratory work an data analysis

In a first phase, *H. ulvae* specimens were counted and measured for maximum shell width. Shell height (mm) and biomass (g AFDW) were estimated according to the conversion equations: shell maximum width = 0.4369 × shell height + 0.2091 (n = 339, $r^2 = 0.97$) and biomass = 0.0564 × shell height$^{2.2381}$ (n = 191, $r^2 = 0.98$), (Lillebø et al. 1999). Afterwards, the mud snail population structure was determined by tracking recognisable cohorts from successive dates (Lillebø et al. 1999, Cardoso et al. 2005). In a second phase, *H. ulvae* juveniles with shell height < 1.8 mm (Bachelet & Yacine-Kassab 1987) were removed, as they would not have mature gonads to allow the larval development of digeneans (Probst & Kube 1999). The remaining mud snails were sub-sampled to achieve 300 to 700 specimens of all adult individuals for each sampling date. These *H. ulvae* were dissected, sexed and inspected for the presence of parasites. The trematodes found within them were identified using the Deblock's (1980) identification key. Special attention was paid to the shape of the shell, presence of epibionts, corrosion marks, contours of the whorls and softness when crushed. Statistical analyses were performed using the MINITAB 10.2 software. All data were inspected for violation of required assumptions and, if so, proper transformations or non-pararametric tests were used.

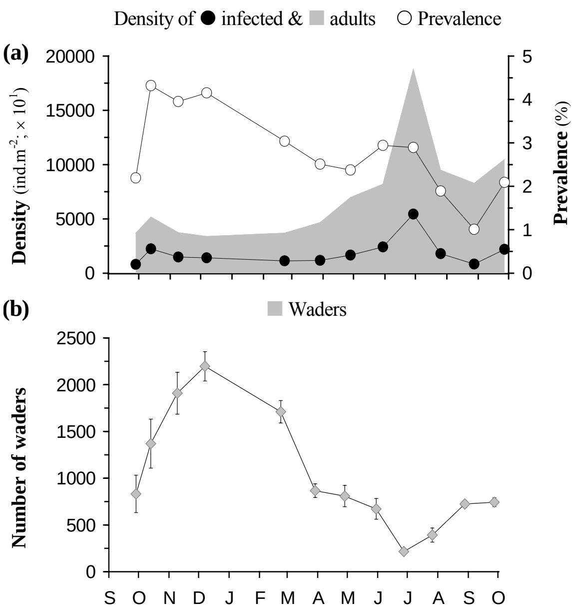


Figure 1. Density of adult *H. ulvae* (× 10^1 ind m^{-2}) and of infected mud snails (ind m^{-2}), plus trematode prevalence at the Mondego Estuary, from September of 1994 to September of 1995. b) Abundance of wading birds (mean ± SE) from censuses carried out from October of 1993 to January of 2000 (Lopes et al. 2000).

RESULTS

Four families of digenetic trematodes were found within *H. ulvae* from the Mondego Estuary (Table 1): Haploporidae (32%), Plagiorchiidae (17%), Echinostomatidae (1%) and Microphallidae (47%). The remaining 2% represented unidentifiable immature larval stages. The proportion of mud snails infected with trematodes (Fig. 1a) varied between 4.3% in October of 1994 and 1% in July of 1995. As the density of *H. ulvae* with shell height ≥ 1.8 mm was usually higher than 30 000 ind m^{-2} (reaching a maximum of 187 900 ind m^{-2} in the summer of 1995), infected gastropods varied between a minimum of 800 to a maximum of 5 440 individuals per m^2 (Fig. 1a). The total density of infected mud snails was well correlated with the density of the adult population ($r_{10} = 0.87$, $p < 0.05$) and showed no relation with the presence of birds in the estuary ($r_{10} = -0.44$, $p > 0.05$). These birds arrived from north in the beginning of autumn (Fig. 1b) and many remained in the estuary during winter (almost 2 200 waders). By the end of spring, a majority departed again towards north. A population of 200 - 300 birds remained in the estuary during summer, composed mostly of *Charadrius alexandrinus*, *Himantopus himantopus*, *Calidris alpina*, *Tringa totanus* and *Numenius phaeopus*. The density of *H. ulvae* infected with Haploporidae (Fig. 2a) and Plagiorchiidae (Fig. 2b) varied between a maximum of 940 and 570 ind m^{-2}. Mud snails infected with *Himasthla* sp. (Fig. 2c) and *L. brachysoma* (Fig. 2d) were scarce. The occurrence of *M. subdolum* and *M. claviformis* infected gastropods varied seasonally (Fig. 2e & 2f), from less than 600 ind m^{-2} in autumn-winter, up to nearly 2 400 ind m^{-2} in the summer.

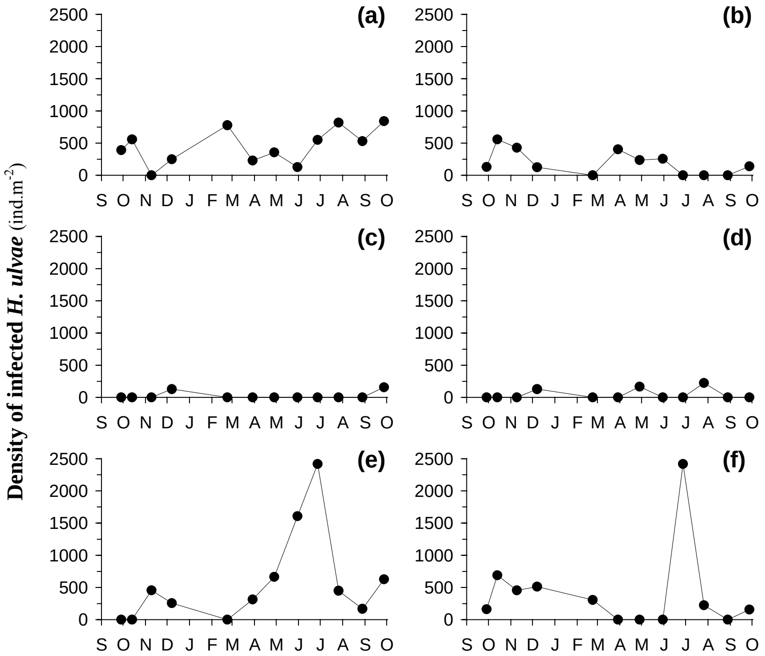


Figure 2. Density of adult *H. ulvae* (shell width ≥ 1.0 mm) infected with a) Haploporidae, b) Plagiorchiidae, c) *Himasthla* spp., d) *L. brachysoma*, e) *M. subdolum* and f) *M. claviformis* trematodes, at the Mondego Estuary, from September of 1994 to September of 1995.

Previous works showed that *H. ulvae* exhibits a well-structured population at the *Z. noltii* bed (Lillebø et al. 1999, Cardoso et al. 2005), having all size/age groups represented (shell height classes from 1.6 mm to 7.5 mm). Digenetic trematodes were only found in *H. ulvae* individuals between 2.0 and 5.9 mm shell height. Infected mud snails showed different shell shape more often than the non-infected ones (Wilcoxon test: $W = 89.0$, $p < 0.001$, Fig. 3a). Infected individuals had typically long and thin shells, very easy to crush, with large pronounced whorls, showing corrosion marks and also germination of green algal spores (Fig. 3b). The frequency of these shells showed a somewhat irregular pattern, not clearly related to the prevalence of parasites ($r_{10} = 0.431$, $p > 0.05$). No significant differences were found between the abundance of mud snails with irregular shells infected with rediae or sporocysts (*t*-test: $t_{22} = 0.45$, $p > 0.05$).

DISCUSSION

The prevalence of digenetic trematodes in *H. ulvae* from the Mondego Estuary was lower than in other northern European estuaries (Jensen & Mouritsen 1992, Huxham et al.1995, Field & Irwin 1999, Montaudouin et al. 2003). Still, the prevalence observed in the *Z. noltii* bed corresponded to several thousands of infected mud snails per m^2, which is a similar to the densities reported in the previous studies. Co-occurrence of all the hosts involved in a digenean life cycle is an important feature for the successful transmission of these parasites (Field & Irwin 1999, Esch et al. 2001). Accordingly, the high numbers of mud snails infected with Microphallidae in the summer, may have resulted from a combination of dense stocks of first (*H. ulvae*) and second (crustaceans, 965 to 2 518 ind m^{-2} – IMAR’s database) intermediate hosts, together with crustacean eating birds present in the estuary.

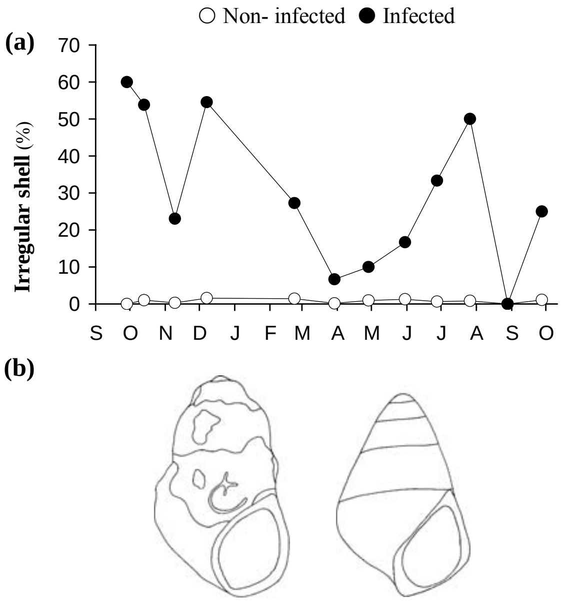


Figure 3. a) Proportion of individuals with irregular shell shape in non-infected and infected *H. ulvae*, at the Mondego Estuary, from September of 1994 to September of 1995. b) Shells of two *H. ulvae*: one was infected with *M. subdolum* (left) and the other was not infected (right). Both had the same width (1.2 mm), but the infected mud snail had a shell height of 2.5 mm, whilst the non-infected measured 2.3 mm.

Digeneans have been reported to induce population structure changes and shorten life span of *H. ulvae* (Huxham et al. 1995, Montaudouin et al. 2003) and *Littorina littorea* (Huxham et al. 1993). Positive correlations were observed between mud snails’ shell height and trematode infections (Mouritsen & Jensen 1994, Gorbushin 1997, Field& Irwin 1999, Probst & Kube 1999, Montaudouin et al. 2003), which have been attributed to factors such as “gigantism” induced by parasites and hosts’ age (older/larger individuals were exposed to infection for longer time). Nevertheless, in the present study, none of the examined *H. ulvae* with shell height > 5.9 mm were parasitised. Most of the infected individuals had a different shell shape, which might have resulted from induced modifications in the growth processes. If digenetic trematodes in fact allocate host energy, previously destined for reproduction, to enhance shell length (Mouritsen & Jensen 1994, Gorbushin 1997, Probst & Kube 1999), this may have consequences for the shell structure (*e.g.* by nutrient limitation for shell formation). Previous findings have also suggested an interaction between epibioses and infection by trematode parasites (Mouritsen & Bay 2000). Epibionts may promote dislodgment, reduced growth rates, shell destruction, reduced fecundity and death of gastropods (Laukner 1980, Wahl 1996). Yet, infected mud snails may not live long enough to become “gigant” gastropods, due to the typical warm-temperate climate from southern Europe. Increasing temperatures are reported to enhance cercarial release (Mouritsen & Jensen 1997, Mouritsen 2002), which causes tissue damages and thus host debilitation. So, a higher mortality of infected *H. ulvae* should be expected in summer, considering the lower thermal tolerance of snail hosts (Lauckner 1980, Huxham et al. 1993, Jensen et al. 1996). Cercarial emergence from *H. ulvae* is greater in water pools during low tide, when solar radiation increases water temperature (Mouritsen 2002), which is usually above 20ºC in the Mondego Estuary, frequently reaching more than 30ºC in the summer (Lillebø et al. 1999). Under these circumstances, larger infected mud snails may shed high quantities of cercariae, which is more deleterious for those infected with rediae (Haploporidae, Plagiorchiidae and Echinostomatidae) than for those infected with sporocysts (Microphallidae) (Huxham et al. 1993, Gorbushin 1997, Montaudouin et al. 2003). This might account for the fact that all infected *H. ulvae* were smaller than 4.3 mm shell height in summer.

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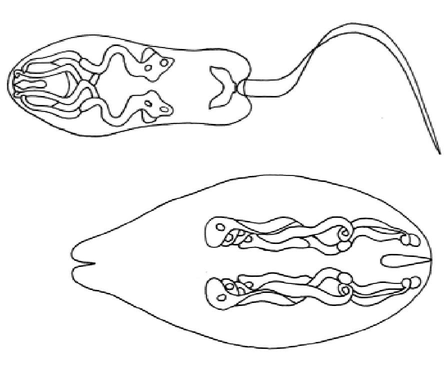
Chapter 5



Infection characteristics and transmission strategies of digenetic trematodes (*Maritrema subdolum* and *Levinseniella* sp.) infecting the isopod *Cyathura carinata*

ABSTRACT

Maritrema subdolum and a *Levinseniella* species not yet described are two microphallid parasites that use the estuarine isopod *Cyathura carinata* as a second intermediate host. Two short-term experiments were performed to compare their transmission success from *Hydrobia ulvae* to *C. carinata*. Mud snails infected with *Levinseniella* sp. shed less cercariae than those infected with *M. subdolum*, but *Levinseniella* sp. was more successful than *M. subdolum* to infect *C. carinata*. Both microphallids penetrated the isopod through the pleopods and encystment took place in the middle-region of the host's body. The two parasites showed different strategies with respect to cercarial production and dispersal ability (cercarial behaviour). *M. subdolum* produced a high quantity of swimming cercariae, whereas *Levinseniella* sp. originated few crawling free larval stages. These results can be interpreted as adaptations to different crustacean hosts. The strategy exhibited by *Levinseniella* sp. appears to be very appropriate for infecting *C. carinata*, a relatively quiet bottom-dwelling isopod.

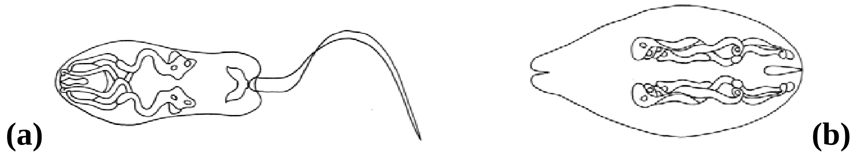
Key words: Parasite, Digenea, *Maritrema*, *Levinseniella*, *Cyathura*, *Hydrobia*

INTRODUCTION

The prosobranch mud snails *Hydrobia* spp. are used as first intermediate hosts by at least 50 digenetic trematodes. Among these, there are about a dozen microphallid species represented in benthic peracarids (second intermediate hosts) from northwestern European shallow water systems (Deblock 1980). Recently, prevalence and intensity patterns of microphallid cysts were described for a *Cyathura carinata* population from the Atlantic coast of Portugal (Jensen et al. 2004). *C. carinata* is a tube-dwelling isopod, common in many European estuaries. It preys on other small invertebrates, using an ambush technique of sitting and waiting quietly inside its burrow. Two microphallid trematodes infecting this isopod were identified: *Maritrema subdolum* and a *Levinseniella* species hitherto not described. The two parasites have slightly different cercarial morphology and behaviour. The most conspicuous feature is that *M. subdolum* cercariae can swim by using a long tail, whereas the *Levinseniella* sp. are tailless and crawl on the surface of the sediment. Cysts of these two microphallids, together with those from *Microphallus claviformis*, have recently been demonstrated to cause reduced survivorship, growth and reproduction failure in *C. carinata* (Ferreira et al 2005a). Two similar laboratory experiments were performed, one with *M. subdolum* and the other with *Levinseniella* sp., in order to compare their infection patterns in this isopod. *C. carinata* were incubated together with infected *H. ulvae* to examine how these parasites, with different strategies of their free-living larval stages, can reach the same burrowed host. *C. carinata*'s behaviour,

Table 1. Schematic representation and morphometric characteristics (in µm) of cercariae and metacercariae from a) *Maritrema subdolum* and b) *Levinseniella* sp.

		Maritrema subdolum	*Levinseniella* sp.
cercariae	body	135 x 45	197 x 60
	stylet	13 - 18	30
	tail	140 x 14	unexistent
metacercariae	shape	round	round
	diameter	170	330
reference		Deblock 1980	Warberg 2003



infection characteristics and parasite distribution in the host's body were also analysed.

MATERIALS AND METHODS

Specimen's collection and storage

C. carinata specimens were collected in the Mondego Estuary (Portugal, 40º08'N; 8º50'W), at a sand flat site where they are abundant and almost all non-parasitised by trematodes (Ferreira et al. 2004, Jensen et al. 2004). These isopods were kept in laboratory at 15ºC, until further procedures. *H. ulvae* were gathered at a *Zostera noltii* bed from the same estuary, where their population is abundant and mature (Cardoso et al. 2005). In laboratory, these mud snails were individually inspected for cercarial emission, under light conditions, at 24ºC and salinity of 20. Shed cercariae were identified to species level, according to Deblock (1980). *H. ulvae* infected with *Cryptocotyle* sp., *M. subdolum* and *Levinseniella* sp. were stored separately in three small aquaria at 15ºC. The characteristics of the two microphallid species are described in Table 1.

M. subdolum experiment

Ten plastic containers were used as experimental aquaria. They were covered with a lid to avoid intensive evaporation. All contained 1 l of brackish water (salinity of ≈ 20) and a 4 cm layer of sediment on the bottom. Sediment was previously sieved through a 0.5 mm mesh to remove macroinvertebrates and was characterised by a median grain size of ≈ 210 µm. These aquaria were placed randomly in a 25ºC room, with a 12 h light/darkness cycle, in order to avoid gradient effects. Air was supplied for 15 min each 4 h of light and each 2 h of darkness, with a glass pipette inserted through the lid, to prevent oxygen depletion. These systems were put to rest for 24 h, before inserting the experimental animals. Then, a net cylinder (1 mm gauge mesh, 35 mm in diameter) was placed in the centre of each aquarium, to enclose 5 *H. ulvae* and prevent their escape from the sediment-water system. Five aquaria contained mud snails infected with *M. subdolum* (treatments), while the other five contained *H. ulvae* infected with *Cryptocotyle* sp. (controls). *Cryptocotyle* sp. use fishes as second intermediate hosts and thus do not infect crustaceans. Mud snails with this type of infection were used rather than non-parasitised ones, as these last specimens can be infected by immature parasites or, for some other reasons, fail to shed cercariae (Curtis & Hubbard 1990). Afterwards, 16 *C. carinata* were introduced in each aquarium. It corresponded to a density of 1 304 ind m^{-2}, similar to the natural density (Ferreira et al. 2004). Both *H. ulvae* and *C. carinata* were carefully chosen to secure identical sizes in every aquarium. Furthermore, the net container did not prevent the isopods to get in contact with the mud snails, during the 7 days (168 h) while this experiment lasted. Temperature, salinity and animal behaviours were daily checked, as well as the presence of tracks on the sediment surface of all aquaria and the number of isopods swimming, crawling or extruding the burrows. At the end, each group of *H. ulvae* was removed to individual small aquaria and stored at 15ºC. Then, the experimental containers were emptied and living *C. carinata* were counted. Afterwards, they were stored at 6ºC, until being dissected. All isopods were sexed, measured for cephalic length (Ferreira et al. 2004) and inspected for the presence, abundance and location of parasites (as mesocercariae, a tailless form of recently infecting cercariae crawling in the host's tissues, or as metacercariae, the encysted form of the parasite).

Levinseniella **sp. experiment**

It was used almost the same procedure as in the previous experiment. Nevertheless, it was necessary to reduce the number of mud snails in each container, as well as the number of aquaria, because *H. ulvae* individuals infected with *Levinseniella* sp. were very rare. So, only 8 aquaria were used: 4 of them with 2 *Levinseniella* sp. infected mud snails in each (treatment) and 4 other with the same amount of *Cryptocotyle* sp. infected *H. ulvae* (control). This experiment ran for 8½ days (204 h).

Cercarial emission

After recovering for one week at 15ºC, the *H. ulvae* infected with *M. subdolum* and *Levinseniella* sp. were incubated again in the 25ºC room for 48 h, under the same environmental conditions as those during the experiments. Afterwards, the mud snails were transferred to clean Petri dishes with only brackish water (≈ 20). After 1 h, they were re-established in their original aquaria. The cercariae released in the Petri dishes were stained with neutral lugol and counted afterwards. The same procedure was repeated in the 2 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

After being incubated together with infected *H. ulvae*, 22 ± 5% (mean ± SE) of *C. carinata* specimens became infected with *M. subdolum*, whereas 23 ± 9% (mean ± SE) of the isopods were found carrying *Levinseniella* sp. larvae (Fig. 1a).

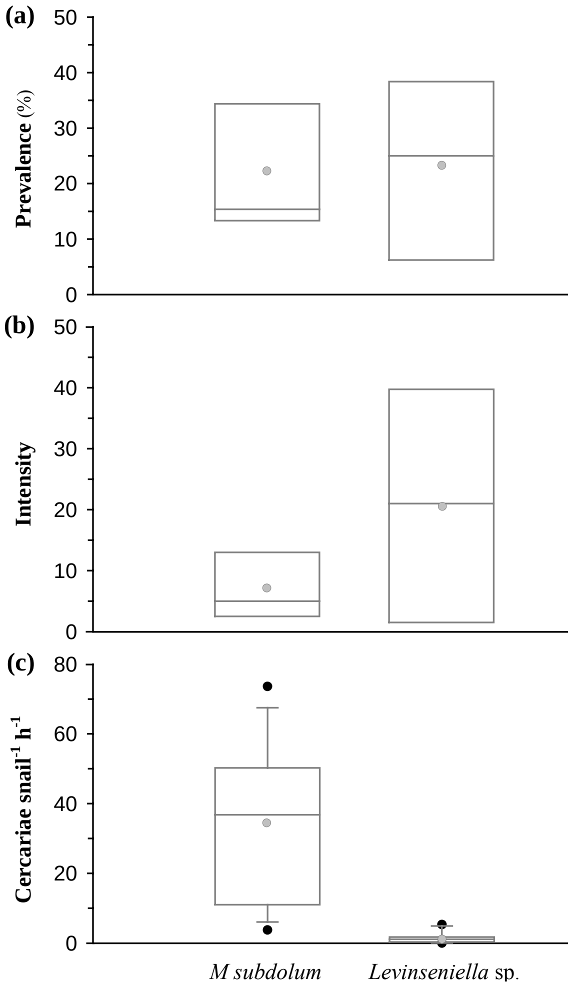


Figure 1. Box plots of (a) the parasites prevalence within *C. carinata*, (b) total number of infections (intensity) found within each replicate aquarium and c) cercarial emission from *M. subdolum* and *Levinseniella* sp. infected *H. ulvae*, estimated per hour and for each individual snail, in the two experimental procedures. 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. Black circles represent outliers and the grey circles indicate the mean.

There were no significant differences between the prevalence rates obtained by the two microphallids (*t*-test: $t_7 = -0.11$, $p > 0.05$). No trematode infections were observed in isopods from aquaria containing *Cryptocotyle* sp. infected mud snails. Infected *C. carinata* hosted from 1 to 6 *M. subdolum* parasites and from 1 to 33 *Levinseniella* sp. larvae. There were no statistical differences between both microphallid species, regarding the number of infections obtained per aquaria (Fig. 1b, Wilcoxon test: $W = 22.0$, $p > 0.05$). Nevertheless, the number of infections ranged from 0 to 41 larvae in the *Levinseniella* sp. experimental aquaria (21 ± 10, mean ± SE) and between 2 and 18 in the replicates with *M. subdolum* (7 ± 3, mean ± SE).

No differences were detected between cercarial emissions (Fig. 1c) from the experimental aquaria with *H. ulvae* infected by *M. subdolum* (one-way ANOVA: $F_{4,10} = 1.50$, $p > 0.05$), nor in those with mud snails infected by *Levinseniella* sp (one-way ANOVA: $F_{3,8} = 0.88$, $p > 0.05$). The average shedding rate of *H. ulvae* infected with *M. subdolum* (35.4 ± 6.5 cercariae snail^{-1} h^{-1}, mean ± SE) was significantly higher (Wilcoxon test: $W = 298.0$, $p < 0.001$) than the shedding rate of *Levinseniella* sp. infected mud snails (1.6 ± 0.4 cercariae snail^{-1} h^{-1}, mean ± SE). There were no significant correlations between the shedding rate of *M. subdolum* infected *H. ulvae* and the prevalence ($r_3 = 0.713$, $p > 0.05$) or intensity ($r_3 = 0.815$, $p > 0.05$) of this parasite in *C. carinata.* Neither were there any significant correlations between the shedding rate of *Levinsiniella* sp. infected mud snails and the prevalence ($r_2 = -0.784$, $p > 0.05$) or intensity ($r_2 = -0.287$, $p > 0.05$) of this trematode within the isopods. *H. ulvae* infected with *Levinseniella* sp. produced on average 22 times fewer cercariae than those parasitised by *M. subdolum*, but *Levinseniella* sp. was 54 times more efficient to infect *C. carinata* than that later parasite. There was no significant difference in the survival of *Cyathura carinata* (≈ 90%), or in the intensity of microphallid larvae within males, gravid and non-reproductive females from both experimental procedures ($p > 0.05$). Moreover, there was no relationship between *C. carinata*'s cephalic length and the infection intensity of either *M. subdolum* ($r_{15} = 0.246$, $p > 0.05$) or *Levinseniella* sp. ($r_{11} = 0.001$, $p > 0.05$).

M. subdolum mesocercariae (Fig. 2a) were all concentrated in the posterior region of *C. carinata*'s body (60% in the pleon and 20% in each of the two last pereon segments), while immature metacercariae (with just a thin primary wall) were scattered along the isopods' body cavity (52% were accommodated in the pleon). *Levinseniella* sp. mesocercariae (Fig. 2b) were also abundant in the pleon (49%), as well as in an anterior segment of the pereon (14% in S2), being scarce along the rest of the middle region of the hosts' body. Contrarily, *Levinseniella* sp. immature metacercariae (Fig. 2b) were mostly located in the central segments of the pereon region (47.5 % in S4 and S5). No trematodes were found inside the daily-examined dead isopods. The absence of metacercariae from dead individuals could be a result of a high decomposition rate of dead host tissues. Therefore, the total number of infections may have been underestimated.

DISCUSSION

Levinseniella sp. was more successful than *M. subdolum* in infecting *C. carinata*. The results from the present experiments corroborate field observations, in which *Levinseniella* sp. cysts dominated within *C. carinata* from the Mondego Estuary (Jensen et al. 2004), despite the fact that this microphallid is a very rare species among *H. ulvae* (authors' unpublished data). The two strategies used by these parasites (many swimming *versus* few crawling cercariae) are not equally appropriate for infecting *C. carinata*. Cercaria's ability to disperse can be expected to represent an adaptation to reach a second intermediate host (Combes et al. 1994). *M. subdolum* cercariae have long tails that allows them to swim in the water layer near the bottom, enabling them to reach crustaceans living in this zone (Mouritsen 2002). The tailless *Levinseniella* sp. cercariae crawl on the sediment surface to get in touch with bottom-dwelling hosts. Parasite distribution within *C. carinata*'s body indicated that both microphallids infected this isopod in a similar way. Pleopods were the main infection path, as shown by the high abundance of mesocercariae found inside the hosts' pleon region. These structures are used for ventilation, which create subtle water currents that may draw cercariae. So, *Levinseniella* sp. seems to have higher probabilities to reach *C. carinata* than *M. subdolum*. Crawling cercariae can actively search for a burrowed host, while swimming cercariae have to be pulled inside the isopods' burrows. Although *M. subdolum* occurs naturally within *C. carinata* (Reimer 1963, Schulenburg et al. 1999, Jensen et al. 2004), the infection by this parasite might be casual (Ferreira et al. 2005b). For this same reason, *M. claviformis* cercariae, which also have a long tail and swim close to the bottom, have not yet been observed within *C. carinata*. Preliminary laboratory experiments confirmed that *M. claviformis* is able to infect this isopod, but only when both parasite and host are incubated together in small containers (authors' unpublished data). *C. carinata*'s head may be another susceptible region, as it often extrudes the burrow. This activity may increase the exposure to microphallid cercariae, as observed before with *M. subdolum* (Ferreira et al. 2005b), and may account for the *Levinseniella* sp. mesocercariae found within the 2nd segment of the isopods' pereon. When in contact with the isopods, cercariae may penetrate the host's cuticula and then search for an encystment place. At this stage, the shape and length of the stylet may be important. *Levinseniella* sp. has a more robust and longer stylet than *M. subdolum*, being probably more successful in penetrating the isopod's exoskeleton. *Levinseniella* sp. showed a preference for encysting within the 4th and 5th segments of *C. carinata*'s pereon region, while *M. subdolum* metacercariae were spread within the body of the host. These observations were consistent with those from a previous long-term experiment, carried out to assess the effects of mature cysts on *C. carinata* (Ferreira et al. 2005a). No survival differences were observed between infected and non-infected isopods from the present short-term experiments. Still, cysts of *Levinseniella* sp. attain a larger size than those of *M. subdolum*, developing thicker layers in the cysts walls. Thus, they require more time to mature (Galaktionov et al. 1997) and may cause more physical damage within the hosts' body (Ferreira et al. 2005a). Generally, digenean cercariae are able to locate their hosts by typically exhibiting a behaviour that directs them towards the appropriate host-space (Combes et al. 1994). *M. subdolum* is found within most European estuaries, reaching a wide spectrum of second intermediate crustacean hosts, such as amphipods (*Gammarus* sp., *Corophium* sp., *Melita* sp.), isopods (*Idotea* sp., *Sphaeroma* sp.) and decapods (*Carcinus* sp.). (Reimer 1963, Deblock 1980). So far, *Levinseniella* sp. has only been reported in *C. carinata* from the Mondego Estuary and seems to fit the behaviour of this isopod very well. The adaptation to narrow spectra of host species may have been an important segregating factor promoting diversification of digenetic trematodes in shallow-water ecosystems.

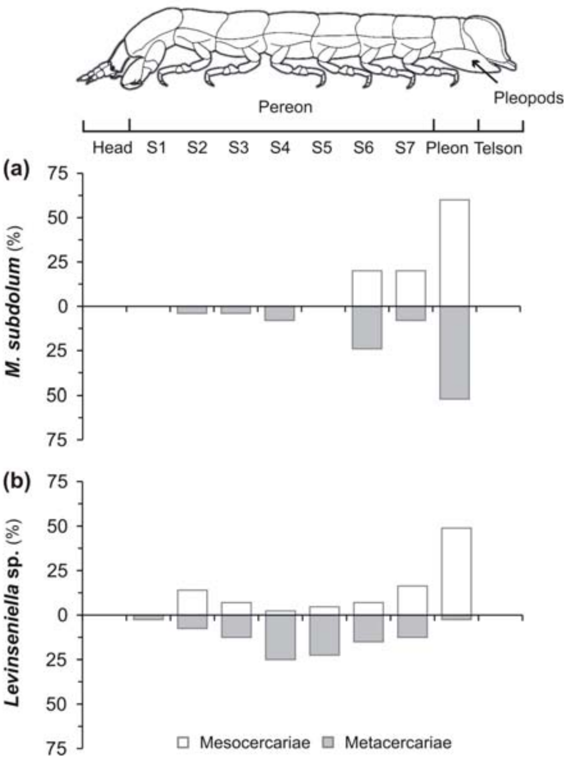


Figure 2. Distribution of (a) meso (n = 10) and metacercariae (n = 25) of *M. subdolum*, plus of (b) meso (n = 43) and metacercariae (n = 40) of *Levinseniella* sp. in different body parts of infected *C. carinata* (respectively, n = 15 and n = 13). S1-S7 are the seven segments of the pereon region of the isopod's body.

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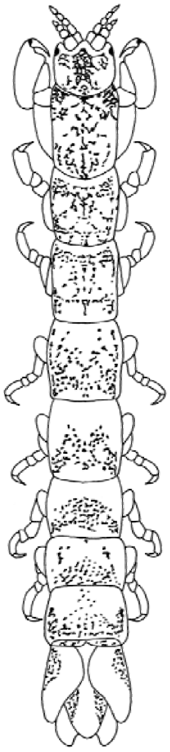
Chapter 6



Effects of restoration management on the estuarine isopod *Cyathura carinata*: mediation by trematodes and habitat change

ABSTRACT

In the Mondego Estuary (Portugal), a restoration programme aimed to recover seagrass beds of *Zostera noltii* endangered by eutrophication. A long-term survey of 10 years was used to assess the development of the processes involved, focusing on one of the key species (*Cyathura carinata*, Isopoda). The mitigation measures implemented since 1998 (nutrient loading reduction, freshwater circulation improvement and seagrass bed protection) enhanced water quality and seagrass recovery, by preventing favourable conditions for the development of macroalgal blooms. These changes have contributed to a more stable *C. carinata* population density at an inner unvegetated sand flat area, where these isopods were abundant. This species was resilient to the occurrence of macroalgal blooms and floods, although both events have caused dispersion of isopod individuals to other areas of the estuary. *C. carinata* could not establish successfully in rooted-macrophyte beds, in contrast to other common estuarine species (*e.g. Hydrobia ulvae* or *Scrobicularia plana*) that prospered in all sites (bare sand or vegetated mud flats). Microphallid trematode parasites might be responsible for discontinuity of the isopod population at the *Z. noltii* bed, where the mud snail *H. ulvae* is a dominant species and all the elements to develop their life cycle were present. If the intertidal areas become fully restored to the original seagrass coverage, microphallids might exclude *C. carinata*. Some mud flats where *H. ulvae* is not a dominant species should be preserved to safeguard this key isopod species, as its stability will be dependent on the parasitic pressure and on its capability to disseminate within the estuary. Therefore, unexpected animal responses to habitat modifications, such as parasite-host interactions, should also be carefully considered in environmental management.

Key words: Eutrophication, Ecological restoration, Estuarine management, *Zostera*, Isopod, Population dynamics, Parasitism

INTRODUCTION

Coastal zones are recognised as some of the world's most productive ecosystems (Clark 1996), in which eutrophication is one of the major threats (Kennish 1997, Livingston 2001, Dolbeth et al. 2003). The processes triggered by excessive nutrient enrichment of coastal waters are complex, but usually cause shifts in primary producers and ultimately in the structure of food webs, lowering species richness, total biomass and secondary production (Hartog & Phillips 2000, Norkko et al. 2000, Livingston 2001, Dolbeth et al. 2003, Cardoso et al. 2004, 2005, Verdelhos et al. 2005). In fact, one of the most common signs of eutrophication is the decline of seagrass assemblages, due to proliferation of opportunistic algae (Valiela et al. 1997, Raffaelli et al. 1998, Cloern 2001, Cardoso et al. 2005, Verdelhos et al. 2005). Nowadays, frameworks for tidal wetlands restoration have been developed and the recovery of seagrass beds and of their associated faunal

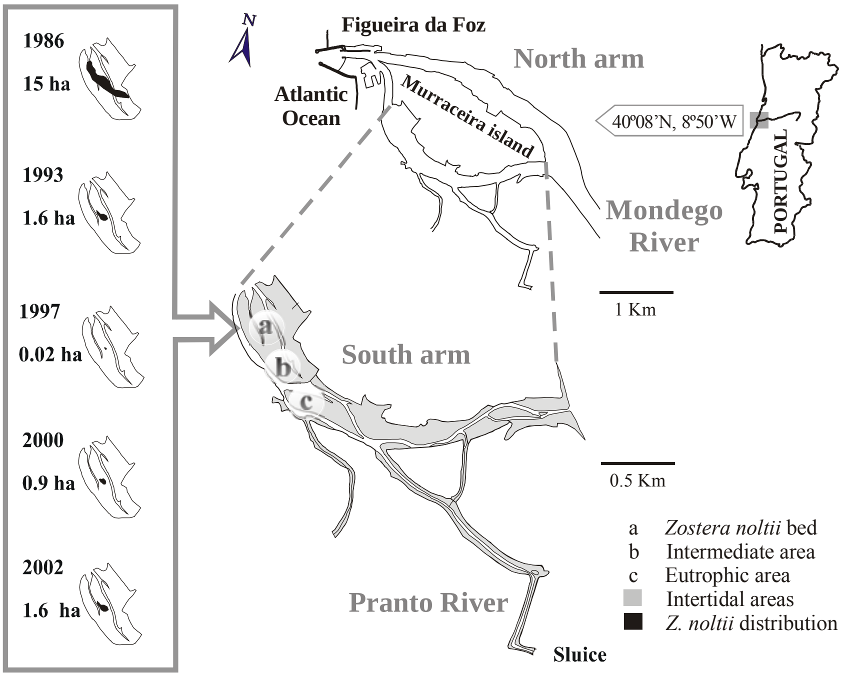


Figure 1. Location of the sampling stations at the south arm of the Mondego Estuary and variation of the *Z. noltii* beds' area of occupation from 1986 to 2002. Mapping was based on field observations, aerial photographs and GIS methodology (ArcView GIS 8.2).

communities has been addressed (Jonge et al. 2000, Katwijk & Hermus 2000, Reise 2002, Cardoso et al., 2005).

The Mondego River (Fig. 1) forms a small temperate intertidal estuary (1 600 ha) located in the south of Europe. This coastal system supports several industries and receives an agricultural run-off from 15 000 ha of rice and corn fields cultivated in upstream areas. This well-documented estuary has shown increasing symptoms of eutrophication since the 1980's (Marques et al. 2003). Seasonal proliferations of green macroalgae *Ulva* spp. occurred along with a drastic reduction of *Zostera noltii* beds and impoverishment of macrobenthic communities (Marques et al. 2003, Pardal et al. 2000, Cardoso et al. 2004, 2005, Dolbeth et al., 2003, Ferreira et al. 2004, Verdelhos et al. 2005). The implementation of a recovery programme was necessary to avoid the total loss of the *Z. noltii* beds and to improve their surrounding environment. Since 1998, several mitigation measures were introduced with the aim of protecting the existing beds, by improving freshwater circulation and reducing nutrient loading into the south arm system. Data from a long-term survey (10 years) will be used to assess the effectiveness of the restoration programme (Hobbs & Norton 1996). As resilience of invertebrates is not yet fully understood, emphasis will be placed on the population dynamics of *Cyathura carinata* before and after estuarine management. This invertebrate is a tube-dwelling predatory isopod, which is an important member of macrobenthic assemblages from many European estuaries, with a significant role in estuarine trophic webs (Muus 1967, Amanieu 1969, Jażdżewski 1969, Wägele 1979, Bamber 1985, Ólafsson & Persson 1986, Köhn & Sammour 1990, Goss-Custard et al. 1991, Sola & Arzubialde 1993, Ferreira et al. 2004). *C. carinata* has the particularity of being a protogynous hermaphrodite, in which females may sex reverse into males. This isopod is also a second intermediate host to microphallid trematodes (Jensen et al. 2004) and these parasites have an important role on its population dynamics (Ferreira et al. 2005). Long-term studies are not very common in Ecology, but they are important to ensure that gathered evidence is logically integrated (Taylor 1989), namely in the cases of rare and stochastic episodes or slow events with years of duration. The long-term approach allows the prediction of ecosystem responses and tests ecological concepts (Franklin 1989). Therefore, it is also intended to evaluate the influence of episodic or rare events (*e.g.* algal

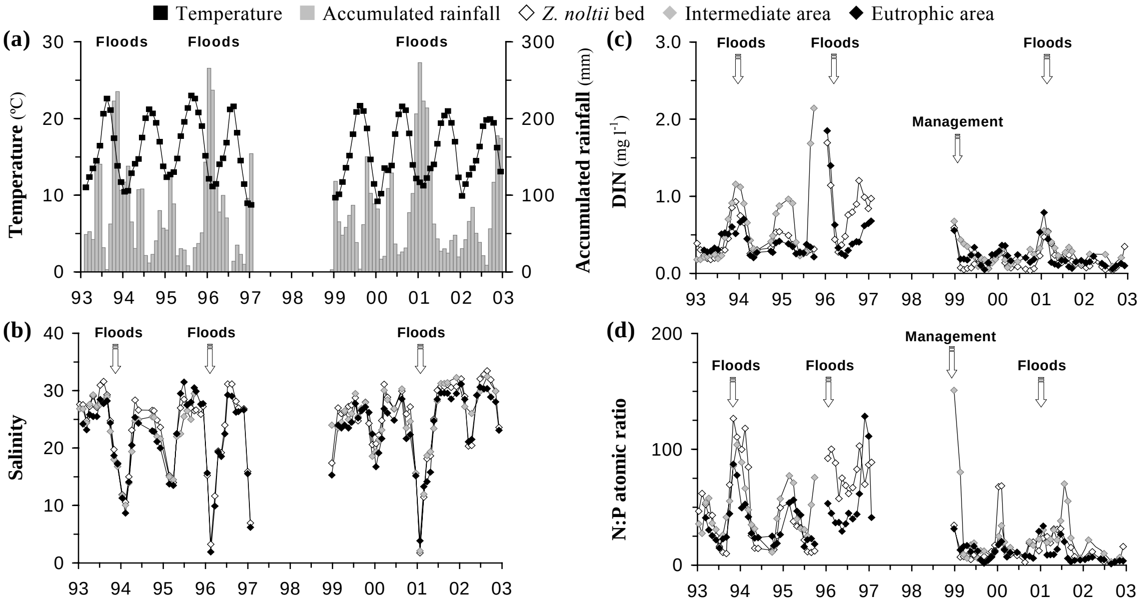


Figure 2. Variation of a) air temperature and precipitation at the Mondego Estuary region, b) water salinity, c) dissolved inorganic nitrogen and d) N:P atomic ratio at the 3 sampling stations of the Mondego Estuary, from January 1993 to December 2002.

blooms, floods and trematode parasites) in slow ecological processes (*e.g.* population dynamics of *C. carinata*) over a period of 10 years.

MATERIALS AND METHODS

Study site

The Mondego Estuary is located in the central western coast of Portugal (Fig. 1). A small island divides it into 2 arms with different hydrological characteristics. The north arm is a navigation channel, being frequently dredged (4-8 m deep at high tide, with a tidal range of 1-3 m). The south arm is shallow (2-4 m deep at high tide) and has extensive intertidal areas. Before 1998, this arm was silted-up in the upstream sections, which obstructed the Mondego River outflow. So, water circulation in this system depended mostly on tides and on a small freshwater input from Pranto River. This tributary is controlled by a sluice, which used to be open according to rice fields drainage. After 1998, the opening of the sluice was reduced to lower nutrient loading into the south arm. Pranto freshwater is now discharged by another sluice located further upstream in the Mondego River and the connection between the 2 arms was enlarged, in order to improve the hydraulic regime in the southern system of the estuary.

More than 3 decades ago, *Z. noltii* beds covered most of the south arm intertidal areas. In 1986, they were reduced to 15 ha (Fig. 1). In 1993, 3 sampling stations were established along a spatial gradient of different eutrophication symptoms (Fig. 1), already described in Pardal et al. (2000), Dolbeth et al. (2003), Marques et al. (2003), Ferreira et al. (2004) and Verdelhos et al. (2005). a) A *Z. noltii* bed located in downstream mud flats, which represents a non-eutrophic situation, more or less corresponding to the original state of the system (Marques et al. 2003, Patrício et al. 2004). After 1998, this site was protected with fences to avoid direct human disturbance. b) An intermediate area located in the middle section, a mud flat which was covered by *Z. noltii* until recently before 1993. c) A eutrophic area, which is a sand flat in the inner parts of the estuary, from where *Z. noltii* disappeared more than 25 years ago and where seasonal green macroalgal blooms (*Ulva* sp.) occurred before the restoration measures.

Field programme

Sampling was carried out during mornings, from January 1993 to December 2002, fortnightly in the first 18 months and monthly thereafter. To facilitate seagrass recovery, no samples were collected between September 1995 and December 1998 at the intermediate area and *Z. noltii* bed, whereas at the eutrophic area the sampling interruption started in January 1997. Samples consisted of 6-10 cores (13.5 cm diameter) taken to a depth of 15 cm. Each core was washed on a 0.5 mm mesh sieve and the remaining material was preserved in 4% buffered formalin. Later in the laboratory, all animals and plant material were sorted out and the animals were kept in 70% ethanol. On all occasions, salinity was measured *in situ*. Likewise, water samples were analysed for dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP), according to standard methods (Strickland & Parsons 1972, Limnologisk Metodik 1992). The nearby Coimbra forecast station of the Portuguese Institute of Meteorology provided the monthly precipitation and atmospheric temperature data.

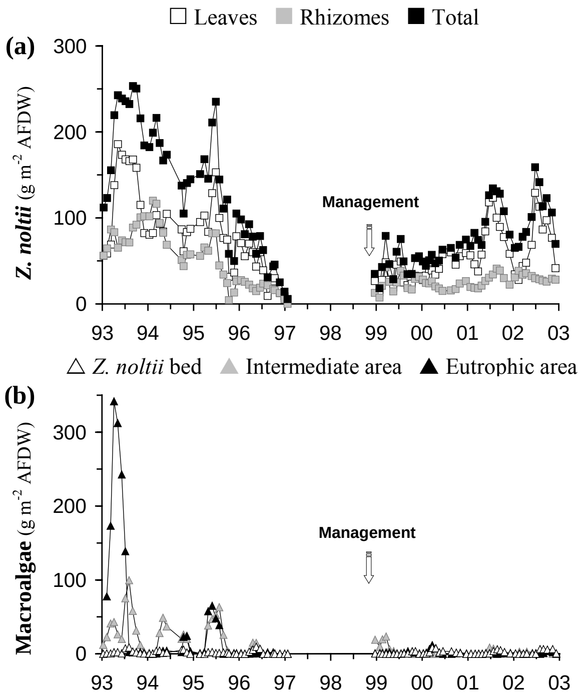


Figure 3. Variation of a) seagrass leaves, rhizomes and total biomass at the *Z. noltii* bed and b) green macroalgal biomass at the 3 sampling stations of the Mondego Estuary, from January 1993 to December 2002.

Seagrass, macroalgae and *Cyathura carinata*

After being sorted, green macroalgae and *Z. noltii* (leaves and rhizomes) were dried for 48h at 60ºC and their ash free dry weight (AFDW) was determined by loss on ignition (8h at 450ºC).

C. carinata specimens were counted, sexed and measured for cephalic length. Total body length (mm) and biomass (g AFDW) were determined by the regression models used already by Ferreira et al. (2004): total body length = 0.745133 + 9.01010 × cephalic length, r^2 = 0.97, n = 115 and biomass = 0.0190549 × cephalic length$^{2.71815}$, r^2 = 0.96, n = 800. All specimens were also inspected for the presence of microphallid trematode cysts inside their abdomen, which were removed and counted (Jensen et al. 2004).

Data analyses

Statistical analyses were performed using the MINITAB 10.2 and SigmaStat 2.0 software. If required assumptions were invalidated, proper transformations or non-pararametric tests were used.

C. carinata population structure was defined by tracking recognisable cohorts in size-frequency distributions from successive dates, using ANAMOD software. Field growth rates were expressed by the equation of Gaschütz et al. (1980), $L_t = L_\infty\ (1\text{-e}^{-[kD(t\text{-}to) + C\ (kD/2\pi)\ \sin 2\pi\ (t\text{-}ts)]})\ 1/D$, where L_t is the length of the organism at a given time t; L_∞ the maximum possible length of the organism; t_0 the instant when the organism would have a length equal to zero; t_s the time interval between the start of growth (when t = 0) and the first growth oscillation, being growth expressed by a sine curve with a one year period; *K* the growth constant; *C* the parameter in which the values can range from 0 to 1, depending on the species; and *D* the parameter that expresses metabolic deviations from the von Bertalanffy's $^2/_3$ rule.

Secondary production was based on the increment summation method. The values of growth production (P) were expressed as:

$$P = \sum_{n=1}^{N} P_{cn}\ ,$$

where P_{cn} is the growth production of a cohort *n*. The mean population biomass $\overline{B}$ was calculated as:

$$\overline{B} = (1/T)\sum_{n=1}^{N} \overline{B}_n t\ ,$$

where *T* is the period of study; *N* the number of cohorts in the period *T*; $\overline{B}_n$ the mean biomass of the cohort *n* and *t* the duration of that same cohort.

RESULTS

Environmental characteristics

The annual patterns of temperature and precipitation varied according to the warm temperate climate of the Mondego region. The winters of 1993-94, 1995-96 and 2000-01 presented higher rainfall than the others (Fig.2a). During those periods, an extensive opening of the Pranto River sluice caused strong salinity declines (Fig. 2b), sometimes to values lower than 5. After the introduction of management measures, DIN concentrations (Fig. 2c) were significantly reduced in all areas (Wilcoxon tests: W = 1 816 for the *Z. noltii* bed; W = 1 484 for the intermediate area; W = 1 705.5 for the eutrophic area, $p < 0.001$), as well as the brackish water N:P atomic ratios (Fig. 2d; Wilcoxon tests: W = 1 374 for the *Z. noltii* bed; W = 1 455.5 for the intermediate area; W = 1 538.5 for the eutrophic area, $p < 0.01$). Between 1993 and 1997, the *Z. noltii* bed suffered an area reduction from 1.6 to 0.02 ha (Fig. 1), as well as a decline of the seagrass biomass (Fig. 3a) from 253 to 5 g m^{-2} AFDW. The scenario changed when management measures were implemented (Wilcoxon test: W = 2 689, $p < 0.001$), with a recovery of the seagrass biomass (maximum of 159 g m^{-2} AFDW) and area of occupation (up to 1.6 ha in 2002). Green macroalgae (Fig. 3b) were scarce at the *Z. noltii* bed (1.90 ± 0.26 g m^{-2} AFDW, mean ± SE), both before and after management. In the intermediate and eutrophic areas, algal blooms were frequent before 1998 (19.80 ± 3.60 and 35.10 ± 12.00 g m^{-2} AFDW, mean ± SE, respectively), but afterwards they ceased to occur (Wilcoxon tests: W = 2 573 for the intermediate area, W = 2 416 for the eutrophic area, $p < 0.001$).

Cyathura carinata

The *C. carinata* population exhibited a characteristic annual pattern of variation. Density and biomass (Fig. 4a & 4b) increased during summer, achieving maximum values in autumn/winter and then declined until late spring. *C. carinata*'s density was higher at the eutrophic area than at the other sampling sites. The maximum densities achieved in the rainy autumn/winters of 1993-94 (which was also preceded by a spring algal bloom) and 2000-01 were significantly lower than the corresponding ones of 1994-95 (one-way ANOVA: $F_{7,35} = 2.96$, $p < 0.05$). These values became more stable since 1998, showing some tendency to increase. *C. carinata* had a very unstable population at the intermediate area (Fig. 4a). There were only few isopods in this site prior to 1998, but afterwards they became more abundant, existing 2 000 ind m^{-2} in January of 1999 and again in the end of 2000. In the beginning of 1993, *C. carinata* was absent from the *Z. noltii* bed (Fig. 4a),

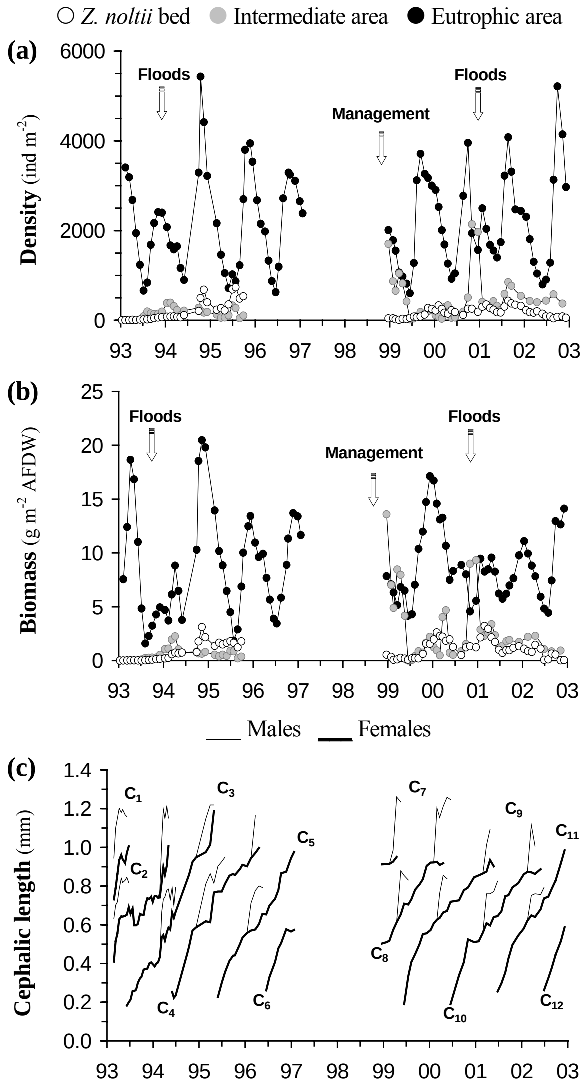


Figure 4. Variation of *C. carinata*'s a) density and b) biomass at the 3 sampling stations of the Mondego Estuary, from January 1993 to December 2002. c) Linear growth of males and females from each cohort (C_x) identified at the eutrophic area from January 1993 to December 2002 (the methodology used could not assess the population structure at the other areas, due to the low abundance of isopods).

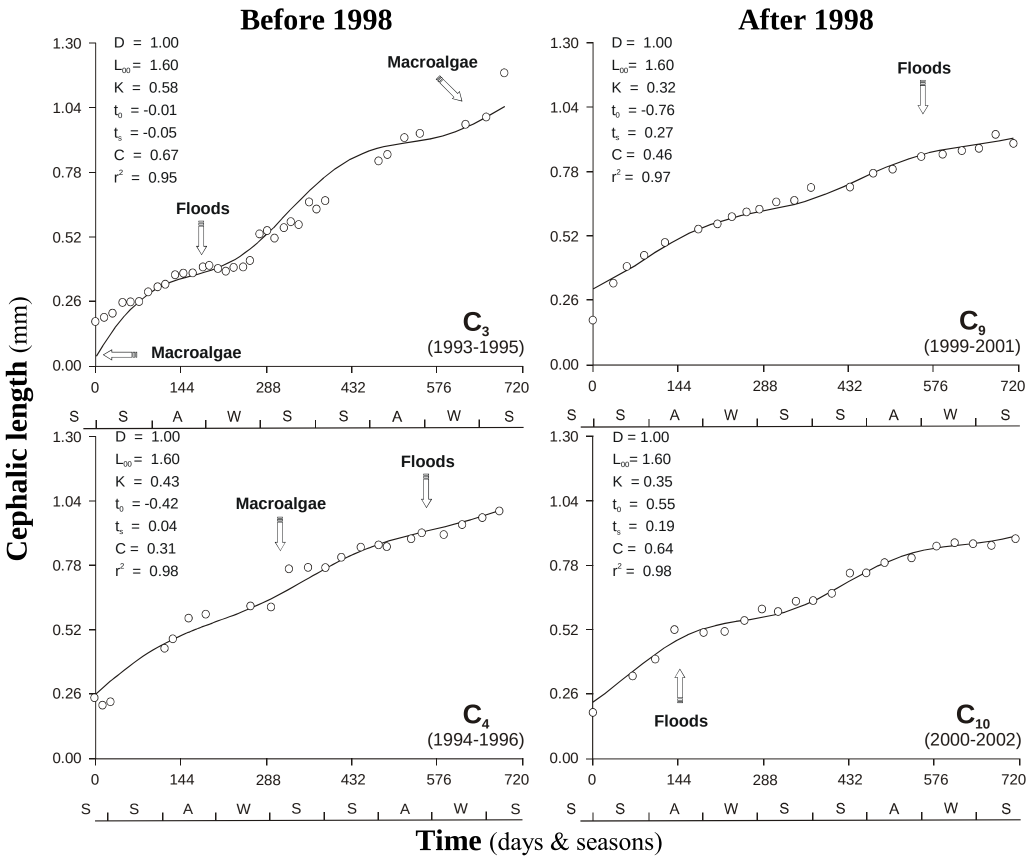


Figure 5. *C. carinata* growth models (Gaschütz et al. 1980), calibrated with field growth data of females from cohorts C_3, C_4, C_9 and C_{10}, with indication of the respective parameters. Males were disregarded, as there are few observations.

however its population sprouted until the end of 1995, reaching 1 000 ind m^{-2}. After 1998, the population recovered from a non-existing situation, similar to the one of 1993-95. There were approximately 550 isopods per m^{-2} from autumn 1999 until spring of 2002. Then, the population started to decline, being on the verge of disappearing from this site by the end of the year.

It was possible to recognise and follow 12 cohorts along the 10 years of sampling (Fig. 4c). Each cohort was estimated to last 23 to 24 months. Every year, two cohorts coexisted. A new cohort urged at the end of spring, while the oldest one (2 year-old) disappeared at the same time. So, the population recruited once per year, for an extended period of time, producing a single annual cohort. The 1 year-old cohorts were also able to reproduce, with males differentiating from females at the 8th month of life (by direct development). Like the 2 year-old males (which resulted from sex reversal of females - protogynous hermaphroditism), they showed higher mean cephalic lengths than females and disappeared after reproduction. When a cohort recruited, the newborn isopods accounted for 80 to 90% of the population. At the time that they reproduced for the first time, these 1 year-old isopods suffered an abrupt decline and represented only 10 to 20 % of the population until the end of their life span. Growth was continuous throughout the isopods' entire life, varying with age, sex and seasonality, being higher in the early life stages, in males and during spring and summer (Fig. 4c & Fig. 5). The growth constants (*K*) of females from cohorts C_3 and C_4 were higher than those from C_9 and C_{10} (Fig. 5). The cohorts prior to 1998 reflected seasonality more markedly than those subsequent to the restoration programme, growing slightly larger during the reproduction seasons.

A lower fraction of the isopods was infected with microphallid trematodes in the eutrophic area than at the other sites (Fig. 6a, Kruskal-Wallis: $H = 36.8$, $p < 0.001$), and the mean number of microphallid cysts per infected host was lower as well (Fig. 6b, Kruskal-Wallis: $H = 11.7$, $p < 0.01$).

Throughout the entire study period, both prevalence and mean intensity of microphallids followed the same annual pattern as the isopods' density, at all sampling stations. It was most clear at the eutrophic area, where infected isopods represented 6.6 ± 0.7% of the population and carried 1.3 ± 0.1 microphallid cysts (mean ± SE). In the *Z. noltii* bed, microphallid infections increased gradually along with *C. carinata* density. When prevalence approached 100%, like in the summers of 1999 and 2002, density declined and the population crashed afterwards, as might have happened before 1993 and after 1995. In those periods, the mean intensity of trematodes increased as well, reaching approximately 10 cysts per infected host. Prevalence and intensity of microphallid trematodes followed a similar pattern at the intermediate area, although in a more erratic manner and not so drastically.

Growth production (P, Table 1) showed a seasonal tendency to be lower in spring and higher in autumn. It seemed to be higher in periods when green macroalgae developed and lower when precipitation was more abundant. Production estimates from the seasons following both types of phenomena were also negatively affected. The lower annual estimates were obtained in 1995, 2000 and 2001, when lower secondary productions occurred in rainy autumns and/or in winters, and also in seasons subsequent to algal growth. The annual estimate from 1993 was balanced between an extraordinary high growth production during the major spring algal bloom and a low production in the rainy fall. The highest annual value was in 1994, when secondary production increased during a small autumn algal growth. The mean population biomass ($\overline{B}$, Table 1) was usually higher in autumn and winter. It followed the same tendency as growth production, regarding the occurrence of algal development and intensive rainfall. Annual P/$\overline{B}$ ratios tended to decrease until 1998 and increase afterwards, being the highest estimates obtained in spring and summer.

DISCUSSION

The mitigation measures implemented in 1998 are improving water quality in the south arm of the Mondego Estuary. The enlargement of the connection between the two arms reduced the residence time of water in the estuary (from 5-7 days to 1 day) and favoured nutrient dilution, by improving freshwater circulation (Verdelhos et al. 2005). Additionally, a rational management of fertilizer usage and sluice discharge reduced the input of nutrients, which all together prevent favourable conditions for the development of macroalgal blooms. Therefore, it seems that eutrophication stressors are abating and that conditions have been established for seagrass recovery. In fact, the *Z. noltii* has been recovering, both in terms of biomass and area of occupation. Nevertheless, physical engineering may be required to restore the entire south arm to its original state (Cardoso et al. 2004). Facing this scenario, what consequences may it have for *Cyathura carinata* or other similar macroinvertebrates?

Cyathura are known euryokous species, occurring in different salinities (Burbanck & Burbanck 1979), tidal ranges and biotopes, from pebble dominated sediments (Bamber 1985) to rooted-macrophyte beds (Amanieu 1969, Ólafsson

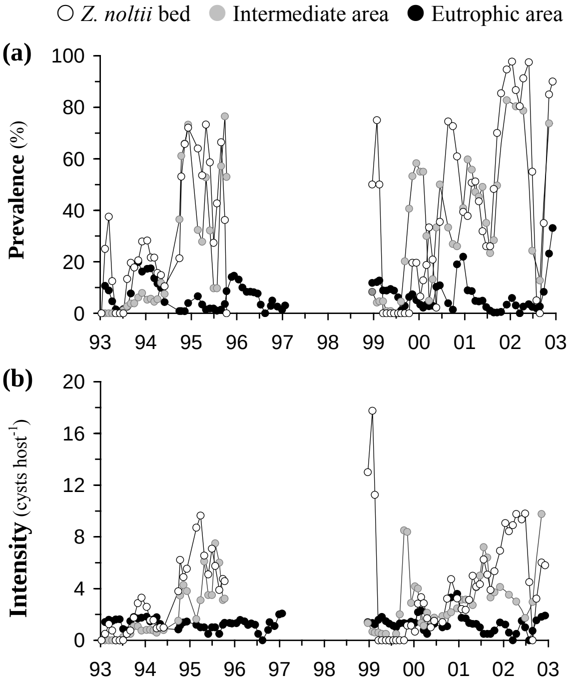


Figure 6. Variation of a) the proportion of isopods infected with microphallid trematodes (prevalence) and b) the mean number of microphallid cysts per infected isopod host (mean intensity), at the 3 sampling stations of the Mondego Estuary, from January 1993 to December 2002.

Table 1. Growth production, mean population biomass and P/$\overline{B}$ ratio of *C. carinata* at the eutrophic area, estimated annual and seasonally, from January 1993 to December 2002. Size-frequency polymodal analysis was not possible to be performed for the other two sampling stations, thus secondary production could not be assessed. The symbol (*) indicates the periods when green macroalgae occurred and (´´) refers to the ones when rainfall was more abundant. The bigger the symbol, the higher the intensity of the phenomena. The winter of 1993 (values in grey) corresponds only from the period between February and March.

	P					**$\overline{B}$**					**P / $\overline{B}$**				
	winter	**spring**	**summer**	**fall**	**all year**	**winter**	**spring**	**summer**	**fall**	**all year**	**winter**	**spring**	**summer**	**fall**	**all year**
	g.m^{-2} 3 months^{-1}				g.m^{-2} year^{-1}	g.m^{-2} (3 months)				g.m^{-2} (year)					
1993	5.83*	10.72*	1.10	2.14''	19.79	11.94*	13.64*	2.16	4.25''	6.85	0.49*	0.79*	0.51	0.50''	2.89
1994	6.09	3.41	8.10	11.59*	29.19	4.95	4.91	9.53	18.55*	8.55	1.23	0.69	0.85	0.62*	3.41
1995	2.22	3.99*	2.57	5.45"	14.8	11.80	5.55*	4.69	11.72"	8.77	0.19	0.72*	0.55	0.46"	1.69
1996	2.26''	1.66	6.04	6.32	16.41	9.96''	5.27	6.13	11.79	8.51	0.23''	0.32	0.99	0.54	1.93
1997															
1998															
1999	1.37	3.65	5.23	7.25	17.5	5.66	5.21	7.4	14.38	11.16	0.24	0.70	0.71	0.50	1.57
2000	3.84	3.05*	4.68	3.18''	14.75	13.70	9.40*	8.65	6.69''	9.91	0.28	0.32*	0.54	0.48''	1.49
2001	3.39"	1.99	5.90	4.44	15.72	8.72"	7.42	6.42	8.85	7.87	0.39"	0.27	0.92	0.50	2.0
2002	4.15	1.54	6.67	9.28	21.64	9.39	5.76	5.47	14.53	7.87	0.44	0.27	1.22	0.64	2.75

& Persson 1986, Ferreira et al. 2004). Therefore, *C. carinata* is probably not a very sensitive bioindicator of ecosystems health, however, this isopod is a key species in many European estuaries (Muus 1967, Amanieu 1969, Jażdżewski 1969, Wägele 1979, Bamber 1985, Ólafsson & Persson 1986, Köhn & Sammour 1990, Sola & Arzubialde 1993), for which it is necessary to assess and predict its responses to habitat changes. The *C. carinata* population was abundant and well age-structured at the eutrophic area, contrarily to other key species, such as the mud snail *Hydrobia ulvae* or the bivalve *Scrobicularia plana* (Cardoso et al. 2005, Verdelhos et al. 2005). This isopod contributed to a considerable fraction (25 to 61%) of the annual secondary macrobenthic production in this site (Dolbeth et al. 2003), where the eutrophication processes led to a progressive impoverishment of the benthic fauna (Dolbeth et al. 2003, Cardoso et al. 2004). Consequently, any stress factor to its population may contribute to destabilise even more an already disturbed macrobenthic assemblage. In the pre-restoration period, *C. carinata*'s individuals grew larger during the reproduction seasons. Thus, they theoretically produced more offspring, as the number of embryos in a brood pouch is related with the size of the female (Ferreira et al. 2005). It is not clear what exactly causes the growth differences. Still, these higher growth rates may have resulted also from a compensation for the occurrence of multiple algal blooms and floods, which have disrupted the normal development of the population. The isopods temporally benefited from the increasing habitat complexity, food resources and protection against predators, provided by the spring algal blooms, which was reflected in the seasonal growth productions and mean population biomass (Raffaelli et al. 1998, Norkko et al. 2000, Pardal et al. 2000, Cardoso et al. 2002). Nevertheless, algae affected negatively the recruitment of juveniles, promoting dispersion to adjacent areas and/or differential mortality (Ferreira et al. 2004). On the other hand, floods and consequent extensive sluice openings were responsible for abrupt salinity declines and stronger water currents. These events had negative repercussions for *C. carinata* (*e.g.* density, growth production and mean population biomass), once that the unvegetated sand flat offers little protection against erosion, compared to the *Z. noltii* bed or even the intermediate area (where some seagrass rhizomes still can be found). From the lowest annual abundance peak observed at the eutrophic area in 1993, it was possible to infer a synergistic action from the autumn floods and the prior harmful spring macroalgal bloom. Nonetheless, *C. carinata* was resilient to these disturbances, being able to recover in the following years. With the implementation of the mitigation measures, *C. carinata* started to present similar maximum annual density values, with a slight tendency to increase. Following always the same annual density pattern as in the eutrophic site, *C. carinata* abundance usually increased after summer in the *Z. noltii* bed and at the intermediate area, mostly due to juvenile dispersion. Although being an organism of limited mobility (Burbanck & Burbanck 1979), *C. carinata* dispersion to downstream sections of the estuary may be a general trend, as maximum densities were always achieved in autumn, after reproduction had ended. After the introduction of the restoration programme, the density of this isopod slightly increased at the frequently disturbed intermediate area, where fishermen usually revolve the sediment to search for bait. Nevertheless, *C. carinata* was unable to prosper in seagrass beds, in contrast to other common estuarine species (*e.g. H. ulvae* or *S. plana*) (Cardoso et al. 2005, Verdelhos et al. 2005). So, what prevents *C. carinata* immigrants from establishing in these areas? Many unknown reasons may be invoked, such as specific habitat selection. For instance, both macroalgae and seagrasses constitute a physical barrier that might interfere with ambush-like predatory feeding mechanisms of this isopod. Still, *C. carinata* has been observed in other rooted-macrophyte beds, namely in *Scirpus maritimus* salt marshes from the same estuary (SM Ferreira personal observation). But, if so, why does *C. carinata* develop cycles of recovery and collapse at the *Z. noltii* bed? The high prevalence and intensity of microphallid trematodes may be another explanation for the temporal fluctuations of *C. carinata* abundance (Jensen et al. 2004). The influence of these parasites in *C. carinata* was previously assessed to induce lower survivorship, growth rates and reproduction failure (Ferreira et al. 2005). The infection levels observed at the *Z. noltii* bed and at the intermediate site, in 1995, in the beginning of 1999 and in 2002 were twice those used experimentally. So, as these digenetic trematodes interfere with the population dynamics of *C. carinata*, they are a liability for the success of this isopod, once that: a) it is a species with direct development, b) reproduces only once per year, c) only 10 to 20% of the 1 year-old individuals are able to survive and achieve the 2 years estimated life span and d) males are a minority in the population (if infected, males may be unable to compete for females or mate with them) (Ferreira et al. 2004, 2005). Microphallids encyst within *C. carinata* (second intermediate host), but are transmitted by mud snails *H. ulvae* (first intermediate host), which in turn become infected by ingesting microphallid eggs released in water birds' faeces (final hosts). The low occurrence of parasites at the eutrophic area may be due to the fact that there are almost no adult mud snails (Cardoso et al. 2005), which cannot be parasitised by microphallids. On the other hand, juvenile *C. carinata* that disperse to downstream areas have a high probability of getting infected, as *H. ulvae* is a dominant species at the *Z. noltii* bed (and also at the intermediate area), reaching densities up to 300 000 ind m^{-2}, where all age classes are represented (Cardoso et al. 2005).

In conclusion, tidal wetland restoration programmes require a full understanding of the interactions between all populations and multiple stressors, in order to become successful (Vivian-Smith 2001). Management changes in ecosystems may create unexpected animal responses to habitat modifications, such as parasite-host interactions. If the intertidal areas of the south arm of the Mondego Estuary are restored to its original seagrass coverage, where *H. ulvae* is particularly abundant and a dominant species, then microphallids might exclude *C. carinata*. The success of this isopod will depend on the parasitic pressure and on the capability to disperse to the recovered sites and other intertidal mud flats. Due to the important role of *C. carinata* in trophic webs, as a food item to fish and wading birds (Burbanck & Burbanck 1979, Ólafsson & Persson 1986, Goss-Custard et al. 1991, Ferreira et al. 2004), it is vital to maintain its high abundances. To accomplish this goal, it may be necessary to preserve some intertidal flats, where adult *H. ulvae* individuals are not abundant, in order to reduce parasitic pressure on *C. carinata*.

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Discussion

General discussion

THE MONDEGO ESTUARY - A TEMPERATE EUTROPHIC SYSTEM UNDER RESTORATION

The Mondego Estuary has a relevant regional socio-economical importance, as it supports several industries, salt-works, aquacultures and mercantile harbour activities. This small temperate intertidal system (1 600 ha) receives also an agricultural run-off of 15 000 ha of rice and corn fields from upstream areas. Like most coastal systems all over the world, it has been under severe environmental stress, namely expressed as an ongoing eutrophication process developed in the south arm. During the last two decades, seasonal proliferations of opportunistic green macroalgal species (*Ulva* sp.), have been occurring along with a drastic decline of *Zostera noltii* beds (Flindt et al. 1997, Marques et al. 1997, 2003, Lillebø et al. 1999, Pardal et al. 2000, Martins et al. 2001, Cardoso et al. 2002, 2004a, 2005, Dolbeth et al. 2003, Ferreira et al. 2004, Patrício et al. 2004, Verdelhos et al. 2005). From 1986 to 1993, the area of occupation of the seagrass bed in the south arm was reduced from 15 to 1.6 ha and only 0.02 ha were remaining in 1997. The *Z. noltii* biomass declined as well from 253 g m^{-2} in 1993 to 6 g m^{-2} in 1997. These changes were followed by a progressive impoverishment in species diversity and major changes in the trophic structure (Dolbeth et al. 2003, Cardoso et al. 2004a, Patrício et al. 2004). Therefore, a rehabilitation programme was implemented in 1998 to avoid the total loss of the seagrass beds, one of the richest ecological habitats in the estuary (Marques et al. 1997, Martins et al. 2001, Dolbeth et al. 2003, Cardoso et al. 2004a), and to improve their surrounding environment. A more rational management of sluice discharges from the Pranto River, the enlargement of the connection between the two arms of the estuary, the physical protection of the existing seagrass beds and the information given to the local population have been contributing to improve water quality, by reducing nutrient loading, residence time and turbidity (Neto 2004), which all together prevent favourable conditions for the development of macroalgal blooms. Thus, it seems that eutrophication stressors are being abated and conditions are established for seagrass recovery. In fact, macroalgal blooms ceased, the area of occupation of the *Z. noltii* bed has been increasing (1.6 ha in 2002), as well as the seagrass biomass, and perspectives for the future are that the *Z. noltii* bed may cover 2 ha of the intertidal areas by 2005 (Neto 2004). Nevertheless, the biological community in the inner eutrophic sand flat did not return to its original state (Cardoso et al. 2005). Restoring this area to its earliest *Z. noltii* coverage may be dependent of more active rehabilitation initiatives, such as coastal engineering and transplantation of seagrasses (Jonge et al. 2000, Katwijk & Hermus 2000), rather than just relying on natural re-colonisation and reduction of nutrient loadings to the estuary (Cardoso et al 2004a, 2005). In order to integrate the problem in a broader geographical scale, the rehabilitation of the Mondego Estuary should also embrace its surrounding areas. All entities, benefiting from exploration of the natural resources of the estuary, should contribute to lower the impacts caused by their activities. Other solutions should be addressed, like the construction of artificial wetlands at the final end of the Pranto River. This would help to improve the estuarine water quality, to preserve the ecosystems health and to attain a sustainable management of the coastal region (Flindt et al. 1997, Neto 2004).

Cyathura carinata

Cyathura sp. are known euryokous species, occurring in different salinities (Burbanck & Burbanck 1979), tidal ranges and biotopes, from pebble-dominated sediments (Bamber 1985) to rooted-macrophyte beds (Amanieu 1969, Ólafsson & Persson 1986, Ferreira et al. 2004). Due to its capacity to live in different ecological niches, *C.*

carinata may not be a most suitable bioindicator of ecosystems health, but it is one important member of the macrobenthic assemblages of many European estuaries, with a significant role in estuarine trophic webs (Burbanck & Burbanck 1979, Ólafsson & Persson 1986, Ferreira et al. 2004). This isopod was found to be most abundant at the eutrophic sand flat monitored in the south arm of the Mondego Estuary (Ferreira et al. 2004), where the eutrophication processes led to a progressive impoverishment of the macroinvertebrate benthic assemblages (Cardoso et al. 2004a, Patrício et al. 2004). In this inner disturbed area, *C. carinata* exhibited a well age-structured and rather stable population, contrarily to other key species in the intertidal areas of the south arm, such as the mud snail *Hydrobia ulvae* or the bivalve *Scrobicularia plana* (Cardoso et al. 2005, Verdelhos et al. 2005). Alone, this isopod contributed with a considerable fraction (25 to 61%) to the annual secondary production of the macrobenthic community in the sand flat (Dolbeth et al. 2003). Therefore, any stress factor acting upon this isopod population may contribute to destabilise even more, an already disturbed benthic community, with possible major consequences for the structure of the trophic web in the Mondego Estuary.

In 10 years of survey, *C. carinata* showed always the same annual density pattern, reproducing only once per year. The highest densities were always recorded in autumn and declined progressively until late spring, when breeding occurred. During this last season, the 2-year old cohorts disappeared to give place to a new one. The physiological processes occurring during reproduction (*e.g.* sex reversal and egg production) may be too demanding that only the fittest specimens can endure it (Ferreira et al. 2005a). Accordingly, 80 to 90% of the 1-year old individuals were also not able to survive this season (Ferreira et al. 2004). Furthermore, it seems that a fraction of the isopods was originated by dispersion of individuals from upstream sections of the estuary, as the maximum annual densities were always achieved after breeding had ended. *C. carinata* is an animal of limited mobility (Burbanck & Burbanck 1979), but this dispersion to downstream areas seemed to be a general trend. *C. carinata* population became less abundant towards the *Z. noltii* bed. *C. carinata* had higher abundance fluctuations at the intermediate area, the sampling site most disturbed by fishermen revolving the sediment to search for commercial species, such as bivalves (*e.g. Cerastoderma edule*) and bait (*e.g. Hediste diversicolor*). At the *Z. noltii* bed, *C. carinata* developed cycles of population collapse and recovery. The low densities recorded in this area may be related to a specific habitat selection by these isopods. For instance, seagrasses and macroalgae may act as a physical barrier that might interfere negatively with *C. carinata* feeding mechanisms at the sediment-water interface (Hull 1987, Raffaelli 1999, 2000, Cardoso et al. 2004b). On the other hand, the high prevalence and intensity of microphallid trematodes, observed within the isopods collected at the downstream sampling sites, may also account for the temporal instability of *C. carinata* abundance in these areas, as they interfere with the isopods survivorship, growth and fecundity (Jensen et al. 2004, Ferreira et al. 2005a).

In a general way, *C. carinata* was resilient to episodic disturbances, such as macroalgal blooms and flood incidents. In intertidal areas, macroalgae can form dense mats that will interfere with the normal activities of the macrobenthic invertebrates lying beneath them. During short periods of time and in low biomasses, macroalgae increase habitat complexity, which may benefit those organisms, by providing more food resources and shelter (Everett 1994, Norkko & Bonsdorff 1996a,b, Raffaelli et al. 1998, Pardal et al. 2000, Cardoso et al 2002, 2004b). Nevertheless, massive blooms have negative impacts that not only affect the macrobenthic assemblages, but also the pelagic communities (Norkko & Bonsdorff 1996a,b, Raffaelli et al. 1998, Norkko et al. 2000). In the present case, *C. carinata* temporally benefited from the presence of macroalgae. The secondary production of this isopod species was enhanced during the spring blooms. Cyathurans are generally tolerant to hypoxia conditions (Burbanck & Burbanck 1979, SM Ferreira personal observation), what may explain the fact that *C. carinata* was comparatively less affected by the algal crash in the summer of 1993 than other macroinvertebrates at the Mondego Estuary (Pardal et al. 2000, Cardoso et al. 2002, Dolbeth et al. 2003, Ferreira et al. 2004). Still, growth production and mean population biomass of *C. carinata* showed to be detrimentally affected in the seasons following the algal blooms. Macroalgae interfered with the recruitment of juveniles, which had repercussions for the population abundance afterwards (Ferreira et al. 2004). The presence of macroalgae might have promoted juvenile dispersion to adjacent areas and/or induce differential mortality, or even embryo loss by gravid females subjected to environmental stress (*e.g.* hypoxia, entrapment on the algae matrix, etc.) (Ferreira et al. 2004). In intensive rainy years, floods occurred in the Lower Mondego Valley. The need to drain the excessive flow of the Pranto River conducted to an extensive opening of the sluices, which in turn lowered salinity and increased water currents in the south arm of the estuary. These events revealed to be unfavourable for *C. carinata* at the inner eutrophic area, causing a decline of the isopods density, growth production and mean population biomass, as this unvegetated sand flat offers little protection against sediment erosion, and consequent hauling of benthic fauna, comparatively to the *Z. noltii* bed. Therefore, both macroalgal blooms and floods contributed to population dispersion of *C. carinata*, affecting its normal development. These events may act synergistically to reduce the resilience of this species, as observed for the mud snail *H. ulvae* (Cardoso et al. 2005). In a decade time, *C. carinata* was able to surpass these episodes, being able to recover in the following seasons. Regarding the restoration of the seagrass beds in the south arm of the Mondego Estuary, this isopod was apparently not much influenced by the mitigation measures. Its population continued on exhibiting a stable annual density pattern at the inner area of the estuary, although the maximum annual densities presented similar values after 1998, with a slight tendency to increase. Contrarily to other key macrobenthic species of the Mondego Estuary (*e.g. H. ulva*e and S. *plana*), which have prospered in all the sampling sites of the south arm (Cardoso et al. 2005, Verdelhos et al. 2005), *C. carinata* was not able to establish successfully in the *Z. noltii* bed. Microphallid parasites may have an important role in these processes (Ferreira et al. 2005a).

DIGENETIC TREMATODES

C. carinata was predominantly infected by two digenetic trematodes of the family Microphallidae: *Maritrema subdolum* and a *Levinseniella* species yet to be described. These two parasites were identified by morphological and DNA analyses (ITS2-sequence data) of metacercarial cysts extracted from infected isopods and cercariae shed by infected mud snails from the *Z. noltii* bed (Deblock 1980, Warberg 2003). Two other microphallid trematodes also infected *H. ulvae*: *Microphallus claviformis* and *Levinseniella brachysoma*. The presence of these last two species within *C. carinata* was not confirmed by DNA analyses, but preliminary experiments confirmed that *M. claviformis* was also able to infect this isopod. *L. brachyosoma* was not found within living *H. ulvae* from the Mondego estuary. It was only observed within preserved mud snails from 1994-95 and therefore it was not possible to assess experimentally if it can infect *C. carinata*. Still, some of the larger cysts (400-500 μm) observed within the isopods fitted the size reported previously for this parasite (Deblock 1980, Bick 1994).

The pleopod structures of *C. carinata*'s body seem to be the main infection path for all these trematodes (Ferreira et al. 2005b). The weak water currents created by the ventilation movements of these structures may draw the microphallid cercariae inside the isopods burrow, where they can get in contact with their target hosts. *C. carinata*'s head is another vulnerable region, especially for swimming cercariae, once that is often exposed above the surface, whenever the isopod senses a possible prey in the surroundings (Ferreira et al. 2005b). Increased surface activity of the isopods, such as extruding the head out of the burrow, swimming or crawling, implies a grater exposure to infecting cercariae (Ferreira et al. 2005b). When these parasites manage to penetrate *C. carinata*'s exoskeleton, they search for a place to fix and mature, which occurs preferably within the 4th and 5th segments of the isopods body cavity (Ferreira et al. 2005a,b).

Cysts of *Levinseniella* sp. were more frequent within *C. carinata* than those of *M. subdolum*, even though the former parasite was seldom found within *H. ulvae*, contrarily to this last trematode that was the most observed species (Jensen et al. 2004). In fact, *Levinseniella* sp. seems to be very effective in infecting *C. carinata*, being probably the most damaging species, as it produces larger cysts that might interfere with the functioning of the host (Jensen et al. 2004, Warberg 2003). This efficiency may be related to selective mechanisms and different strategies of the parasites, as cercarial dispersion patterns are generally adapted to the ecology of the target hosts (Combes et al. 1994). Low water pools on the intertidal mud flats represent optimal conditions for the transmission of microphallid parasites (Mouritsen 2002). These small environments may receive high amounts of solar radiation during daytime, which increases water temperature and stimulates cercarial release from the snail hosts (Mouritsen 2002). Moreover, water pools have reduced currents that diminish the possibility of cercarial loss by dispersion and thus increase the parasites' probability of finding a benthic host. *C. carinata* remain mostly hidden inside their burrows, a behaviour that lessens the likelihood of swimming cercariae to get in contact with them. Both *M. subdolum* and *M. claviformis* cercariae swim close to the bottom to reach crustaceans that live in the superficial strata, but *M. subdolum* has more possibilities of being drawn inside the isopod's burrow by subtle ventilation currents, since it swims less actively and closer to the sediment than *M. claviformis* (KT Jensen personal observation). *L. brachysoma* cercariae are mostly inactive, remaining usually in the same place where they were shed by the mud snails. They stand attached to the bottom, stretching up and down only when they sense any movement, in an attempt to attach to a crustacean passing near them (KT Jensen personal observation). Therefore, it is also unlikely that this parasite might find and infect *C. carinata*. The hitherto unknown *Levinseniella* sp. searches actively for a host, moving in a caterpillar fashion way on the surface of sediment, which is probably the most effective method to find a bottom-dwelling isopod. On the other hand, this parasite may also be more capable to succeed in infecting *C. carinata* than *M. subdolum*, due to functional differences in the capability of penetrating the isopods exoskeleton, such as the shape and size of the stylet (Warberg 2003, Jensen et al. 2004).

Prevalence and intensity of microphallid parasites showed spatial and interannual differences within *C. carinata*. Microphallids infected proportionally more isopods at the *Z. noltii* bed and at the intermediate area than at the inner sand flat. This spatial pattern was surely related to the abundance of the parasites' first intermediate host, the mud snail *H. ulvae*. This gastropod had a distribution pattern of abundance opposite to the one of *C. carinata*. There were several thousands of *H. ulvae* per m^2 in all the sampling sites of the Mondego Estuary, but solely juveniles were represented at the eutrophic sand flat area (Cardoso et al. 2002, 2005). These individuals could not host microphallids because they lack mature gonads for the larval development of these parasites (Probst & Kube 1999). The mud snail population was only stable and well-structured at the *Z. noltii* bed, where individuals of all age classes were present (Cardoso et al. 2002, 2005). Therefore, this site potentially accommodated more infected gastropods than the eutrophic sand flat. Although the prevalence of digenetic trematodes was generally low in *H. ulvae* from the *Z. noltii* bed, there were thousands of infected gastropods per m^2, due to the high density of mature mud snails at this sampling site (Cardoso et al. 2002, 2005).

Trematode prevalence increased during autumn and winter of every year, both within *C. carinata* (Jensen et al. 2004) and *H. ulvae*'s populations. These temporal patterns were related with the phenology of wading birds that use the Mondego Estuary as resting and wintering area in the course of their annual migrations (Múrias et al. 1996, Lopes et al. 2000). Yet, these time trends resulted from the balance established between the supply of trematode eggs to the mud snails, along with consequent transmission of larval stages to *C. carinata*, and the elimination of infected specimens through different mortality agents (Jensen et al. 2004). At the eutrophic sand flat, prevalence and intensity of microphallids within *C. carinata* were low, but followed the annual density pattern of the isopod's population (Jensen et al. 2004). As microphallids enhance the mortality of their hosts

(Mouritsen & Jensen 1997, Jensen et al. 1998, Meissner & Bick 1999, Ferreira et al. 2005a), infected isopods may not survive the physiologically demanding breeding season (Ferreira et al. 2005a), which would explain the null prevalence and intensity of microphallids observed during summer at the inner sampling site (Jensen et al. 2004). Still, it was noticed that when spring blooms of green macroalgae occurred on this sand flat area, prevalence increased after reproduction. This could be related to the increasing density of adult *H. ulvae* at this site, during these particular periods, due to the combined effect of stronger benthic recruitments and dispersion of individuals proceeding from the *Z. noltii* bed, to seize the advantages offered by the increasing habitat complexity offered by the algal mats (Cardoso et al. 2002, 2005). Still, if there are no adult mud snails, how can there be infected *C. carinata* at the eutrophic site? There are at least three hypotheses: a) the few infected isopods may be proceeding from upstream sections of the estuary, where adult *H. ulvae* are abundant (*e.g. Scirpus maritimus* salt marshes); b) wading birds prefer to use unvegetated intertidal areas, where macrophytes do not interfere with their feeding mechanisms (Cabral et al. 1999). Therefore, it is possible that there are more trematode eggs per m^2 at the sand flat site and that prevalence among the few existing adult *H. ulvae* may be higher. As there are thousands of isopods per m^2, it can be easier for a microphallid cercariae to infect this second intermediate host; c) on the other hand, the environmental conditions at the eutrophic site might be suitable for a more efficient microphallid transmission, such as substratum properties and associated features (Ferreira et al. 2005b).

C. carinata were absent from the *Z. noltii* bed in the beginning of 1993 and again in 1999. At the same time as the isopod population became more abundant at this sampling site, the prevalence and intensity of microphallid cysts increased as well, and generally the population tended to collapse afterwards (Jensen et al. 2004). The presence of *C. carinata* at the *Z. noltii* bed depended mostly on spatial dispersion of juvenile individuals from upstream areas of the estuary, as the eutrophic sand flat. In spite of the low prevalence rates observed among *H. ulvae*, there were several thousands of trematode infected mud snails per m^2. The settlement of the isopods in an intertidal area, where these mud snails are a dominant species, represents an increased risk of being infected by microphallids. As these parasites interfere negatively with the population dynamics of *C. carinata*, they may cause host oscillations of abundance, by enhancing host mortality and recruitment failure (Ferreira et al. 2005a). As *C. carinata* is a tube-dwelling isopod with direct development, recruitment success depends on local offspring production. Hence, recruitment failure induced by microphallids may limit the efficient population size of *C. carinata* and cause population fragmentation (Jensen et al. 2004, Ferreira et al. 2005a).

FINAL REMARKS AND ISSUES FOR FURTHER INVESTIGATION

C. carinata was not evidently affected by the mitigation measures introduced in the Mondego Estuary for restoring the intertidal mud flats to their original seagrass coverage of *Z. noltii*. Nevertheless, the occurrence of spring macroalgal blooms has ceased since the implementation of the restoration programme. This has contributed to a more stable population density of the isopods at the eutrophic area, where *C. carinata* was most abundant. The *Z. noltii* beds have been recovering, both in terms of biomass and area of occupation. Even so, *C. carinata* has not been able to prosper as other key macrobenthic species, such as *S. plana* and *H. ulvae*. This isopod has shown cycles of population recolonisation and collapse at this site. Apart from many other factors inherent to the species physiological and ecological features, microphallids may have an important role in the distribution of this isopod. They might exclude *C. carinata* from rooted macrophyte beds and salt marshes where the mud snail *H. ulvae* usually prospers, as they induce recruitment failure and consequent population fragmentation. If the south arm intertidal areas are restored to their original seagrass coverage, these parasite-host interactions should be carefully considered and evaluated. As *C. carinata* plays an important role in the estuarine trophic web, it is imperative to maintain a stable and abundant population of this isopod. To fulfil this purpose, some mud flats where *H. ulvae* is not a dominant species should be preserved to safeguard this key isopod species, as its stability will be dependent on the parasitic pressure and on its capability to disseminate within the estuary.

H. ulvae was found infected by several digenetic trematodes, which use a panoply of other hosts rather than crustaceans, such as bivalves, fishes and birds. The prevalence of these parasites within the mud snails was low, which made it difficult to detect seasonal patterns. Several thousands of *H. ulvae* from 1994-95 were dissected, but only a few were found carrying larval stages of trematodes. The most puzzling question concerns the unknown *Levinseniella* species. How can this parasite be so abundant within *C. carinata* and not be observed within *H. ulvae* from that same period of time? The influence of digenetic trematodes on the *H. ulvae* population from the Mondego Estuary should be subject a further investigation. The preliminary results obtained with the present work provided some hints, regarding possible enhanced mortality in these gastropod hosts, related with environmental stress induced mostly by elevated temperatures during summer. As *H. ulvae* is the foremost abundant mollusc from the intertidal areas, the study of digenetic trematodes within these gastropods is surely relevant, not only by the importance that this species assumes in estuarine food webs, but also by the fact that these parasites may be cryptic determinants of the population dynamics of other estuarine organisms (*e.g. C. carinata*), inclusively of commercial interesting species.

Generally, host organisms have been adapted to co-occurring parasites and have a life-history that takes the presence of these “enemies” into account. It is often true that a high diversity of parasites may indicate a healthy state of an ecosystem. Nevertheless, the increasing hazards that threaten coastal environments, mostly derived from anthropogenic impacts, may disrupt and interfere with the natural balance between parasites and their hosts. In this scenario, the knowledge acquired about the role of parasites becomes a significant issue for the management of life processes in coastal systems, which is often disregarded. The present work contributes to the growing recognition of the importance of parasites for understanding the dynamics and distribution of populations from shallow water ecosystems.

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