

# Parasite transmission between soft-bottom invertebrates: temperature mediated infection rates and mortality in *Corophium volutator*

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**ABSTRACT:** Circumstantial evidence has suggested that marine microphallid trematodes using gastropods *Hydrobia* spp. as first intermediate hosts, amphipods *Corophium* spp. as second intermediate hosts, and various waterbirds as final hosts, may cause temperature-dependent epizootics and eventually local extinction of intermediate host populations. Therefore, we examined experimentally the impact of the microphallid trematode *Maritrema subdolum* on *Corophium volutator* with special reference to the influence of temperature, during the parasites' transmission from snail to amphipod. Trematode infected snails and amphipods were established together in experimental aquaria at temperatures of 15, 22 and 24°C for 9 d. Amphipod surface activity, survival rate, parasite prevalence, infection rate, and parasite distribution both within and between amphipod individuals were recorded during, or at the end of, the experiment. The snails' shedding rate of larval trematodes as a function of temperature was also examined. Increasing temperature resulted in higher infection levels and parasite-induced mortality in the amphipods, most likely governed by a similar temperature-dependent emergence of larval trematodes from the snails. No transmission occurred at 15°C, whereas at 24°C the parasite prevalence approached 100% and the parasite-induced mortality exceeded 50% in relation to controls. As assessed by the transparency of the amphipods' gills, infestation inflicted anaemia was the likely mechanism behind the increased surface activity observed among infected specimens. This parasite-induced behavioural change may facilitate transmission of infective stages to shorebird hosts feeding on *C. volutator*. The results demonstrate that microphallid trematodes are able to induce a significant additive mortality in *C. volutator* populations. The temperature mediated mortality emphasizes the potential significance of a density-independent process in controlling the impact of parasites on host organisms and thereby host population dynamics.

**KEY WORDS:** *Corophium volutator* · Parasitism · Mortality · Temperature · Population dynamics  
Behavioural change · Microphallids · Trematodes · Tidal flat · *Hydrobia ulvae*

## INTRODUCTION

Parasites have increasingly been recognized as agents that interfere with essential aspects of the life of an animal host, including physiology, behaviour, reproduction, competitive ability and survival (e.g. Anderson 1979, May 1983, Sousa 1983, Hamilton et al. 1990, Lively et al. 1990, Møller 1990, Minchella & Scott 1991, Lively 1992). Nevertheless, parasitism, unlike

the more commonly studied processes of predation and competition, has received relatively little empirical attention as a factor affecting the dynamics of animal populations or the structure of animal communities (Dobson & Hudson 1986, Anderson 1991, Minchella & Scott 1991, Holt 1993). This lack of attention is particularly true for populations of marine soft-bottom invertebrates (Sousa 1991). Most studies on these organisms have merely reported on epizootic outbreaks among introduced or cultured species (see Kinne 1980, 1983, Rohde 1982, and references therein). Few attempts have been made to clarify how parasites invoke signif-

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icant mortality in natural host populations, and experiments clarifying whether parasites induce depensatory, additive or compensatory mortality (the latter having no population dynamic consequences) are lacking in general (see Holmes 1982).

It seems likely that the impact of parasites in soft-bottom intertidal habitats has been overlooked rather than that parasites are noninfluential. Invertebrate populations of intertidal areas often are characterized by apparently erratic fluctuations in densities, due in part to the harsh environmental conditions, but also to periodically strong predation pressures from fish, waterbirds, and predatory invertebrates that may exploit these habitats, especially during certain periods of their life cycles (Reise 1978, 1985, Schneider 1978, Commiato 1982, Kent & Day 1983, Ambrose 1984, 1991, Quammen 1984, Murdoch et al. 1986, Peer et al. 1986, Wilson 1991). However, parasites commonly use the predator-prey systems for transmission (e.g. Lafferty 1992), which may cause the influence of the parasites to remain largely undetected. 'We see predators kill, but rarely parasites' (Price 1980).

The small and often abundant amphipod *Corophium volutator* inhabiting U-shaped burrows in the substrate of near-shore soft-sediment environments is regularly subjected to large and often unexplained fluctuations in population densities (see Watkin 1941, Reise 1978, 1985, Fish & Mills 1979, Möller & Rosenberg 1982, Murdoch et al. 1986, Olafsson & Persson 1986, Wilson 1989, Matthews et al. 1992). Parasites have previously been suggested to curtail *Corophium* survival (Muus 1967, Lauckner 1987), and more recently Jensen & Mouritsen (1992) ascribed the local extinction of an intertidal population of *C. volutator* to an epizootic caused by the microphallid trematodes *Maritrema subdolum* Jaegerskioeld, 1909 and *Microphallus claviformis* (Brandes, 1888). These digenean parasites are engaged in complex life cycles using various waterbirds as final hosts, mud snails *Hydrobia* spp. as first intermediate hosts, and *Corophium* spp. as second intermediate hosts (e.g. Lauckner 1987). The adult trematodes inhabit the final host's intestine. Their eggs are expelled to the exterior with the birds' faeces and some are eventually consumed by *Hydrobia* spp. snails. Within the gonads of the snails, the larval trematodes multiply asexually into numerous cercariae that leave the snail host in order to penetrate and encyst as metacercariae within the body cavity of *Corophium* spp. The infective metacercariae are transmitted to the bird when it feeds on these amphipods.

Jensen & Mouritsen (1992) hypothesized that the occurrence of unusually high temperatures and an accompanying high prevalence of microphallids in the coexisting population of *Hydrobia ulvae* caused a mass development of cercariae that upon transmission in-

flicted severe pathogenic damage to the amphipods, eventually causing extinction of the local *Corophium volutator* population.

The present study presents data on 3 crucial characteristics of this host-parasite association: (1) an experimental test of the potential of microphallid trematodes as agents of additive mortality in *Corophium volutator*; (2) a specific examination of the relationship between temperature, infection rates and mortality in *C. volutator*; (3) an elucidation of the process of parasite invasion and the resulting pathological effects and behavioural changes induced in the amphipods. The results are discussed in the context of parasite-induced density-dependent regulation of host populations as opposed to environmentally controlled density-independent determination of host abundance (see Scott & Dobson 1989).

## MATERIALS AND METHODS

**Collection and storage of specimens.** Specimens of *Hydrobia ulvae* were collected during spring and summer 1993 on an intertidal mudflat in the southern part of the Danish Wadden Sea (54° 56' N, 8° 39' E), where the collapse in the sympatric *Corophium volutator* population was observed previously (Jensen & Mouritsen 1992). The collected snails were established in the laboratory in aquaria supplied with running sea water and natural substrate containing epipelagic diatoms as the food source. Trematode-infected individuals were identified by the shedding of cercariae. Snails were placed individually in small petri dishes under constant light at 25°C. After 24 h, the petri dishes were inspected through a stereomicroscope, and shed cercariae were identified to species level according to Deblock (1980) using a light microscope. Snails infested with *Maritrema subdolum* (Fig. 1A) and *Himasthla continua* (Fig. 1B) were sorted and stored separately.

Because amphipods living sympatrically with *Hydrobia ulvae* may be naturally infected by larval trematodes, *Corophium volutator* were collected from an artificial saltwater lagoon situated about 500 m from the site of snail collection. *H. ulvae* is very uncommon in this lagoon; consequently, *C. volutator* infected by trematodes using *H. ulvae* as first intermediate host are scarce (authors' pers. obs., and see 'Results'). The amphipods were collected in August 1993, established in the laboratory and held under conditions similar to those of the snails until the start of the experiments.

**Experimental design.** Small glass jars (0.5 l) were used as experimental aquaria (Fig. 2). Each aquarium was supplied with 5 cm of sieved sediment and a 3 cm column of sea water ( $\approx 28\%$ ) aerated via a glass pipette

mounted in a hole in the lid. As a food source for both amphipods and snails, a 3 to 4 mm layer of silty sediment, rich in epipellic diatoms, was distributed on the substrate. A mesh container (3.5 cm in diameter; 1 mm mesh size) enclosing the snails was pressed into the sediment in the centre of the aquarium. The amphipods were established outside the mesh container. This set-up submerged the snails and allowed shed cercariae to escape from the container. To each aquarium 10 trematode-infected *Hydrobia ulvae* and 20 *Corophium volutator* were added, corresponding to 2000 and 4000 specimens  $m^{-2}$ , respectively. Although these densities are below values often encountered in natural populations, the density ratio between *C. volutator* and infected *H. ulvae* is within the range of density ratios previously observed in the field (0.7 to 16; see e.g. Jensen & Mouritsen 1992, Mouritsen 1994).

Closed experimental aquaria without water flow do not resemble the range of conditions in the field, but do resemble restricted conditions under which the main transmission of *Maritrema subdolum* is likely to occur. Release of cercariae from the snails is promoted by high temperatures (see 'Results', Ginetsinskaya 1988, unpubl. data) and, hence, parasite transmission *in situ* is likely to be especially pronounced in intertidal pools during daylight where solar radiation elevates the water temperature considerably (de Wilde & Berghuis 1978, Ginetsinskaya 1988).

The *Corophium volutator* specimens measured  $4.5 \pm 0.5$  mm (mean  $\pm$  1 SD,  $n = 230$ ) in length (rostrum to telson), and the shell of *Hydrobia ulvae* had a mean height (apex to aperture) of  $4.9 \pm 0.5$  mm ( $n = 135$ ). Length measurements on the amphipods were carried out on preserved control specimens (see 'Parasite induced mortality') after experimentation.

**Parasite-induced mortality.** To examine the effect of temperature on infection rates and mortality in *Corophium volutator* exposed to *Maritrema subdolum*-infected snails, 10 aquaria were placed under 24 h constant light in each of 3 windowless rooms at  $15 \pm 1$ ,  $22 \pm 1$  and  $24 \pm 1^\circ C$ , respectively. Two treatments were conducted at each temperature: (1) addition of snails shedding *M. subdolum* cercariae ( $n = 5$  at each temperature), and (2) addition of snails shedding *Himasthla continua* cercariae ( $n = 5$  at each temperature). Distinguishing between infected and non-infected snails

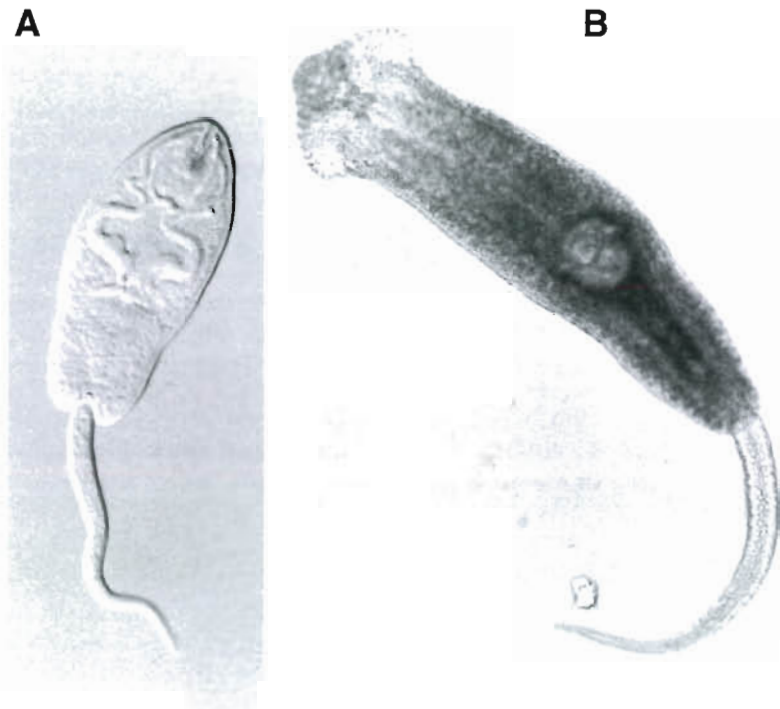


Fig. 1. Micrographs of (A) *Maritrema subdolum* cercaria (Microphallidae) using differential interference contrast (DIC) on formalin preserved material ( $\times 320$ ), and (B) *Himasthla continua* cercaria (Echinostomiidae) using an ordinary objective on live material ( $\times 125$ )

with certainty based on the presence or absence of cercariae emergence can be difficult (Curtis & Hubbard 1990). Consequently, *H. continua*-infected snails were used in control aquaria rather than 'non-infected' snails, as judged by lack of cercariae production. Like *M. subdolum*, *H. continua* is a digenean, but it has a different life cycle, using molluscs instead of amphipods as the second intermediate host. Hence, larval *H.*

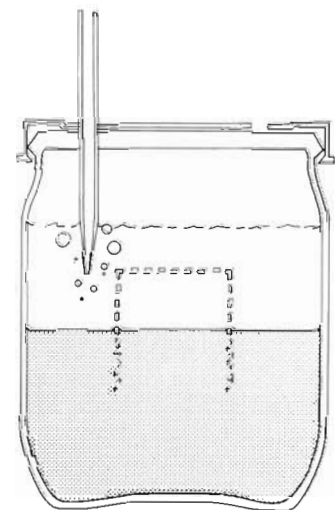


Fig. 2. Glass jar (0.5 l) used as experimental aquarium depicting oxygen supply, water level, substrate and the mesh container enclosing the snails

*continua* are not able to penetrate and encyst in amphipods. Previous experiments have revealed that exposure to cercariae of *H. continua* does not affect the survival rate of *C. volutator* (authors' unpubl. data), thus, *H. continua*-infected snails may be viewed as true controls in the present experiment, as is demonstrated also by the results.

The experimental temperature intervals correspond well to temperatures experienced by the specimens *in situ*. During the period of May through September in the Danish Wadden Sea, temperatures between 20 and 25°C occur regularly in surface sediment and intertidal pools at low water, and temperatures reaching 30°C have occasionally been measured during calm and cloudless days (authors' pers. obs.).

During the 9 d of the experiment, the aquaria were inspected regularly. Dead amphipods on the sediment surface were removed and preserved in 80% ethanol. At the start (Day 2 in order to let added amphipods establish themselves) and at the end of the experiment (Day 9), the number of surface-active specimens (amphipods that had left their burrows completely) were recorded in each aquarium. Knowing the number of amphipods initially added, and the number retained at the end of the experiment, it is possible to calculate the proportion of surface-active animals for each aquarium at Days 2 and 9. It is assumed that no mortality occurred between Days 1 and 2.

At the termination of the experiment, the aquaria were emptied and the remaining amphipods from each container were counted and placed in fresh sea water for an additional 24 h to allow relocation and encystment of recently penetrated cercariae. The amphipods were then preserved in ethanol and subsequently sexed, measured, and dissected under a stereomicroscope for the presence, number, and position of larval *Maritrema subdolum*. During dissection it was noticed that infected animals often possessed more transparent gills than uninfected individuals, apparently due to the presence of only very little haemolymph in the gills of infected specimens. To quantify this, one of the hindmost gills of each *Corophium volutator* specimen was photographed under a light microscope.

**Cercarial emergence.** To estimate average cercarial production per snail per day at different temperatures, another randomly chosen group of *Maritrema subdolum*-infected snails from the same field sample was placed individually in small petri dishes supplied with sea water (≈28‰). The snails were separated into 3 groups and left under constant light for 24 h at 15 ± 1, 20 ± 1 and 25 ± 1°C, respectively. Following removal of the snails and addition of ethanol, the emerged cercariae were counted under a stereomicroscope.

Generally, emergence of larval trematodes from the individual snail host is highly variable on a daily basis

as well as over a longer period of time (e.g. Théron & Moné 1984, authors' unpubl. data). Cercarial production estimated from the above counts may therefore depart somewhat from the number actually released during the main experiment. However, to keep the experimental set-up undisturbed, measurements of cercarial emergence were avoided during experimentation.

**Route of transmission.** In order to elucidate the process of cercariae transmission between snails and amphipods, qualitative observations of the behaviour of cercariae and amphipods during contact were conducted. During a period of 12 to 24 h, recently shed larval trematodes were regularly added to aquaria containing previously established amphipods. Cercariae as well as burrowed and surface active *Corophium volutator* specimens (n = 10) were observed through a horizontally oriented stereomicroscope. The amphipods were subsequently fixed in 80% ethanol, dissected and examined under a light microscope so that newly entered unencysted cercariae could be located within their bodies. To identify the moment of penetration, a few recently killed amphipods were placed in petri dishes with cercariae and observed through a stereomicroscope.

**Data analysis.** Statistical analysis was performed using SPSS (Hull & Nie 1981). Prior to all main analyses, tests for the assumption of homogeneity of variance was conducted. This assumption was violated in the data of cercarial production, and a  $\ln(x + 1)$  transformation was carried out to stabilize the variance.

At termination of the main experiment it became clear that one of the aquaria at 24°C, planned as a control, had been erroneously treated with *Maritrema subdolum*-infected snails, producing slightly unbalanced data. Ordinary ANOVA was nevertheless performed on these data since alternative methods applied in the case of unbalanced data (Shaw & Mitchell-Olds 1993) produced similar results by demonstrating highly significant effects. *A posteriori* planned pair-wise comparisons of means were accordingly performed using Mann-Whitney tests (Day & Quinn 1989) or, regarding infection rates, Student's *t*-test for unequal sample size.

Regarding the data on surface activity, a standard ANOVA performed on the unbalanced data was considered inappropriate because of the relatively high and heterogeneous variances and incomplete independence of observations. Hence, an arcsin square root  $\times$  transformation was performed in addition to random removal of cases thereby balancing the data prior to ANOVA with repeated measures. Although information is lost by removing cases, it was considered a better alternative than addition of cell means. Although statistical analysis was performed on a

reduced set of data, the graphical presentation of the surface activity incorporates all data available (Fig. 8).

## RESULTS

### Cercarial production

The rate of emergence of *Maritrema subdolum* cercariae from the infected snails showed a significant positive relationship with temperature (Fig. 3). The relationship is approximately linear (weakly curvilinear) within the applied temperature range with an almost 9-fold increase in shedding rate per 10°C.

### Route of transmission

When released from the snail, the cercaria strikes vigorously with the tail which keeps the ventrally bent body swimming without any apparent directional transport. However, in the vicinity of a burrowed *Corophium volutator* specimen the swimming cercariae were easily dragged into the burrow by the ventilation current produced by the amphipod's abdominal pleopods. Caught in the main ventilation current, the cercariae were transported backwards mainly along the amphipod's ventral side and were

led out especially at the hindmost thoracical appendages. Direct contact between swimming cercariae and the amphipods' smooth cuticula rarely induced penetration attempts. However, if the larvae were accidentally caught between setae or spines, their swimming behaviour was usually replaced by crawling and penetration movements. Within a few seconds the cercariae would lose their tails, penetrate the cuticula, and move easily around within the amphipod's inner tissue.

Recently penetrated and unencysted cercariae were primarily located in the appendages, whereas meso- and metacercariae were situated in the thoracic/abdominal body segments (Figs. 4 & 5), indicating that the cercariae invade the amphipods through the legs and subsequently occupy the body cavity. This applies particularly to the hindmost thoracical legs and segments harbouring the highest proportion of larval trematodes (Fig. 4). Initial cercarial invasion through the appendages is consistent with the described act of transmission since the appendages, and especially the hindmost pairs, possess high densities of setae in which the cercariae are easily lodged.

### Infection rates and parasite distribution

Increasing temperature exerted a significant positive influence on infection rates among the surviving *Corophium volutator* specimens (Fig. 6). No metacercariae were found in control individuals or in *Maritrema subdolum* exposed *C. volutator* at 15°C, whereas the mean number per specimen per aquaria increased from 3.5 at 22°C to 11.5 at 24°C. Considering together all *Corophium* individuals from each *Maritrema* treatment (5 to 6 aquaria) at 22 and 24°C, the parasite prevalence and range of parasite numbers per specimen were 80% and 0 to 30 ( $n = 73$ ) at 22°C and 97% and 0 to 40 ( $n = 66$ ) at 24°C, respectively.

Also considering together all specimens within each treatment, the coefficient of dispersion ( $s^2/x$ ) of the number of metacercariae per amphipod individual was on average 6.8 and 5.6 at 22° and 24°C, respectively. These figures indicate an overdispersed (= aggregated) parasite distribution at both experimental temperatures (see Anderson & Gordon 1982, Esch & Fernández 1993).

Of the dead *Corophium volutator* specimens collected at the sediment surface during the experiment, only 5 individuals were preserved in a condition adequate for reliable assessment of the pre-mortem meso- or metacercarial numbers. These 5 individuals harboured on average 11.8 cysts (range 4 to 20). Because unencysted cercariae disintegrate rapidly following

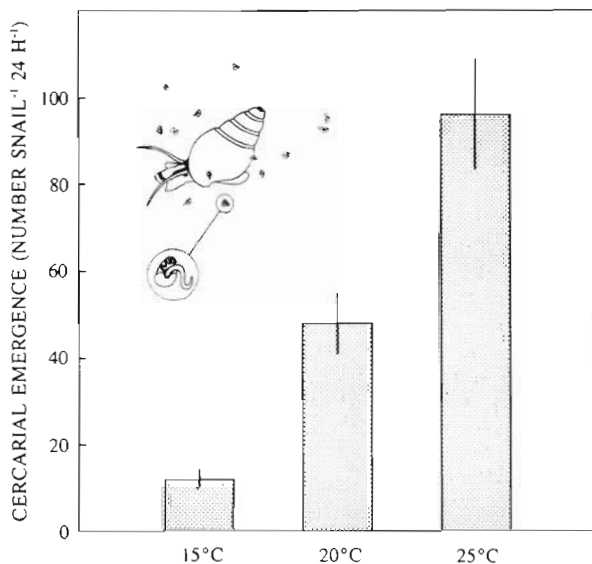


Fig. 3. *Maritrema subdolum*. Mean number ( $\pm 1$  SE) of emerged cercariae per snail per day at different temperatures.  $n = 30$  snails for all values. All mean values differ from each other [1-way ANOVA on  $\ln(x+1)$  transformed data,  $F_{2,87} = 27.29$ ,  $p < 0.0001$ ; LSD *a posteriori* tests:  $p < 0.01$  for all possible comparisons]

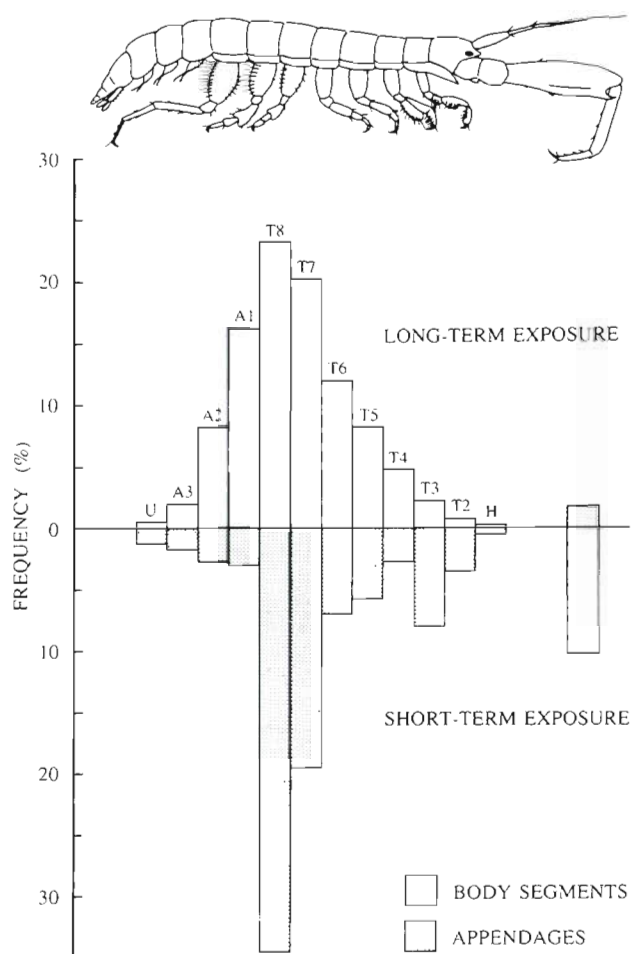


Fig. 4. *Corophium volutator*. Frequency distribution of metacercariae and unencysted tailless cercariae in different body parts of infected amphipods following a long-term exposure (the main experiment,  $n = 122$  amphipods, 955 meso- and metacercariae; columns above x-axis) and a short-term (12 to 24 h) intensive exposure to larval *Maritrema subdolum* ( $n = 10$  amphipods, 374 unencysted cercariae; columns below x-axis). H: head; T2–T8: thoracal segments; A1–A3: abdominal segments; U: urosome

the death of the amphipod (Lauckner 1987), the actual parasite burden of killed specimens may be considerably underestimated.

Infection levels did not differ significantly between sexes, either at 22°C (Student's  $t$ -test,  $t_{60} = 1.1$ ,  $p = 0.28$ ) or at 24°C ( $t_{50} = 0.05$ ,  $p = 0.96$ ). There was, however, a significant positive relationship between infection level and amphipod size both at 22°C ( $r_{66} = 0.44$ ,  $p = 0.0002$ ) and 24°C ( $r_{60} = 0.26$ ,  $p = 0.044$ ) when considering all individuals from each treatment together. These relationships were generally improved if each replicate aquarium was considered separately.

Since the exact lengths of the amphipods were unknown prior to experimentation, the positive rela-

tionship between infection levels and size makes it appropriate to correct for size (number of metacercariae/mm amphipod) when infection levels between different experimental temperatures are compared. Such correction, however, causes no significant departure from the results depicted in Fig. 6. The ratio of infection levels between 24 and 22°C is 3.3 and 3.1 respectively prior to and following correction.

### Pathology and behavioural changes

The bodies, especially the gills, of the amphipods appeared to be considerably more transparent in infected than in uninfected specimens (Fig. 7). Six people were asked to classify the photos of amphipod gills as 'transparent' or 'non-transparent'. This produced very similar results in that 85.7 to 91.4% of the gills from the group of *Maritrema subdolum*-infected specimens ( $n = 35$ ) were declared 'transparent', whereas only 0 to 15.0% of gills from uninfected control individuals ( $n = 40$ ) were classified so. This discrepancy was statistically significant (Mann-Whitney test,  $Z = -2.91$ ,  $p < 0.004$ ) suggesting that the majority of variance in gill transparency can be explained by the trematode infection. Because the transparent gills contained only very little haemolymph, we interpret this transparency as parasite-induced anaemia.

Infection appears to affect the amphipod's surface activity at 24°C (Fig. 8); only 2 *Corophium volutator* specimens remained uninfected in the *Maritrema subdolum*-treated aquaria. The repeated-measures ANOVA for evaluation of the influence of treatment and date on the surface activity on Day 2 and Day 9, showed a marginally significant 2-way interaction between treatment and date ( $F_{1,6} = 5.65$ ,  $p = 0.055$ ). However, because the type II error can be substantial for an  $F$ -ratio based on 1 and 6 degrees of freedom,  $H_0$  should not be accepted if the  $p$ -value for the interaction term is close to 5% (e.g. Underwood 1981). Hence, the proportion of surface-active amphipods exposed to parasites is here considered to increase between Day 2 and Day 9 as opposed to control individuals.

### Mortality

A 2-way ANOVA performed to evaluate the influence of temperature and treatment on the survival of *Corophium volutator*, showed a significant temperature and treatment interaction ( $F_{2,246} = 4.40$ ,  $p = 0.024$ ). As shown in Fig. 9, this interaction is likely to have been accomplished by temperature-independent survival at about 80% among controls, whereas increas-

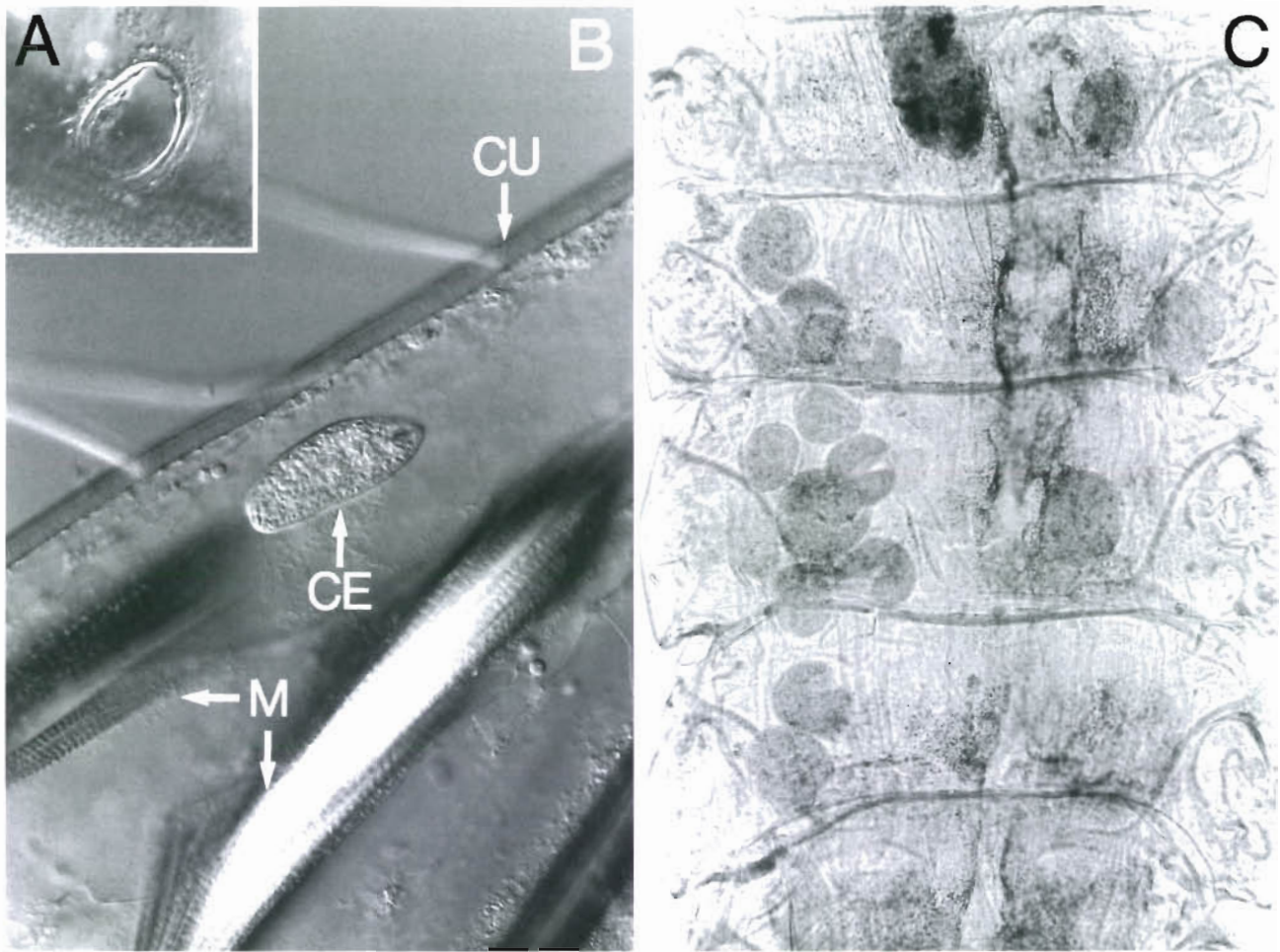


Fig. 5. *Corophium volutator*. (A) Section of a thoracal appendage showing a cercarial penetration site in the cuticula (differential interference contrast, DIC,  $\times 270$ ). (B) Section of a thoracal appendage showing an unencysted tailless *Maritrema subdolum* cercaria (DIC,  $\times 210$ ). CU: cuticula, CE: cercaria, M: muscle fibre. (C) Ventral view of thoracic segments (with appendages removed) containing meso/metacercariae ( $\times 85$ )

ing temperature influenced an increasing mortality of parasite-exposed amphipods. A 1-way ANOVA performed on the controls alone confirmed that temperature had no significant impact on survival ( $F_{2,11} = 2.12$ ,  $p = 0.17$ ). Exposure to *Maritrema subdolum*-infected snails did not affect the survival of *C. volutator* at 15°C (none infected), whereas the parasites seriously compromised amphipod survival at increasing temperatures, inducing a mortality rate at 24°C above 50% in relation to controls.

Inspection of dead amphipods from control aquaria revealed that the majority possessed wounds originating primarily from distortion of the outer segments of various appendages. This damage probably occurred during manipulation by pincers prior to experimentation, and may have contributed to the observed 20% mortality among control individuals.

## DISCUSSION

### Temperature and mortality

Present results demonstrate that larvae of *Maritrema subdolum* are a potential agent of significant additive mortality in *Corophium volutator* in a highly temperature-dependent manner. The similar temperature-dependent pattern in infection rates strongly suggests a causal connection between infection rate and mortality, which is consistent with many other investigated host-parasite associations (see Anderson & May 1978, Anderson 1979, Dobson & Keymer 1990). The temperature-dependent influence of the parasites most likely originates from the positive relationship between cercarial emergence and temperature. However, within the interval of 15 to 25°C this relationship is only slightly

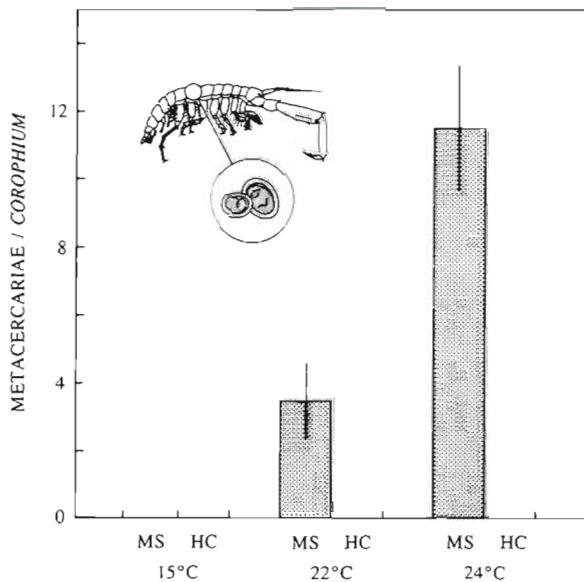


Fig. 6. *Corophium volutator*. Mean number ( $\pm 1$  SE) of metacercariae per amphipod per aquarium as a function of temperature. Treatments: MS = addition of *Maritrema subdolum* infected snails; HC = addition of *Himasthia continua* infected snails (control) ( $n = 5$  aquaria, except for MS 24°C and HC 24°C where  $n = 6$  and 4, respectively). The difference between MS 22°C and MS 24°C was statistically significant (Student's  $t$ -test,  $t_9 = 3.44$ ,  $p < 0.01$ )

curvilinear (see Fig. 3) whereas a threshold temperature seems to occur within the narrow interval of 22 to 24°C, above which the mortality is accelerated disproportionately (Fig. 9). Although the estimates of cercarial production during the main experiment must be taken cautiously (see 'Materials and methods'), it is likely that temperature-dependent factors other than cercarial production affect the transmission success of the parasites. Such factors could be temperature-dependent swimming behaviour, penetration ability of the cercariae, or behavioural changes of the amphipod host facilitating transmission. Although these possibilities are not mutually exclusive, the present study is consistent with the latter possibility. Contact between larval trematodes and amphipods is likely to be accomplished primarily via the ventilation current of the amphipod. Moreover, ventilation rate in marine bottom-dwelling invertebrates generally increases with temperature (see Kristensen 1983, and references therein). Therefore, an additional increase in parasite burden can be expected with increasing temperatures even with a constant density of cercariae in the water. The reason for the observed positive relation between infection rate and size may also be the result of size-dependent ventilation volume.

Infestation seems to induce anaemia in *Corophium volutator* as assessed from the transparent appearance

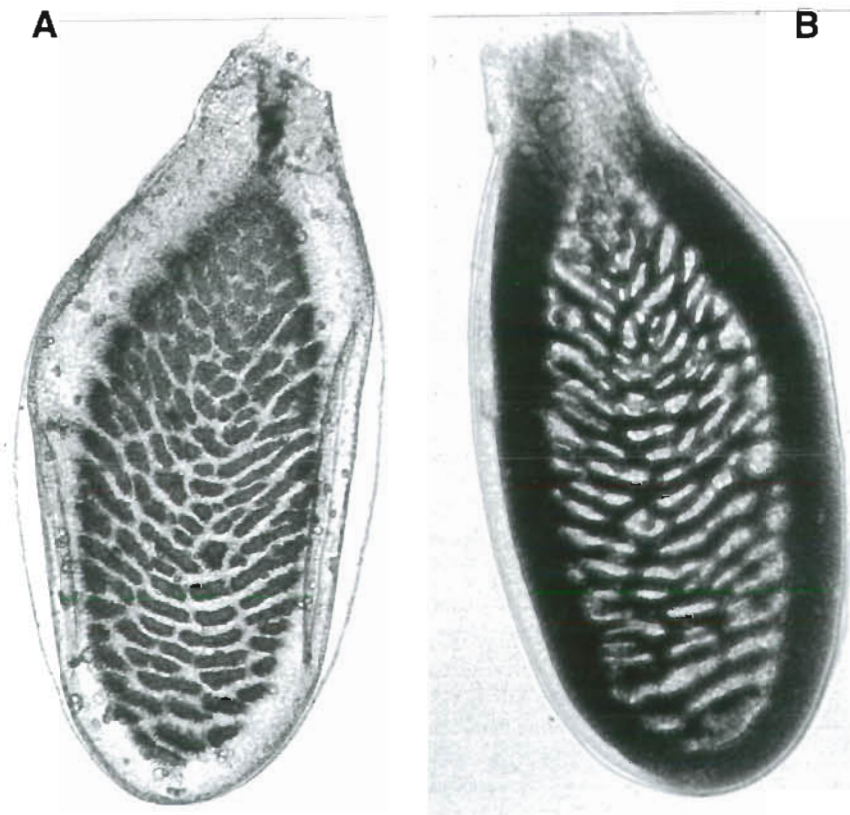


Fig. 7. *Corophium volutator*. Hindmost gill from (A) a *Maritrema subdolum*-infected specimen showing advanced anaemia, and (B) a normal gill from an uninfected individual ( $\times 200$ )

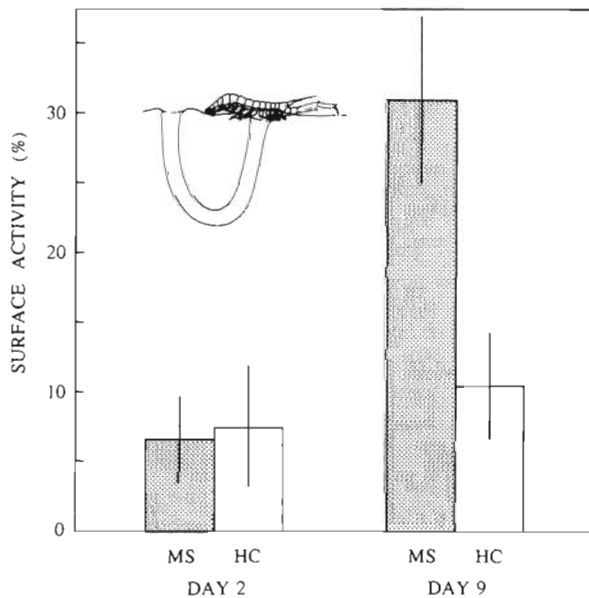


Fig. 8. *Corophium volutator*. Mean (%) surface activity ( $\pm 1$  SE) of amphipods in experimental aquaria at 24°C at initiation (Day 2) and at termination (Day 9) of the main experiment. Number of experimental units (aquaria) and treatment abbreviations as in Fig. 6

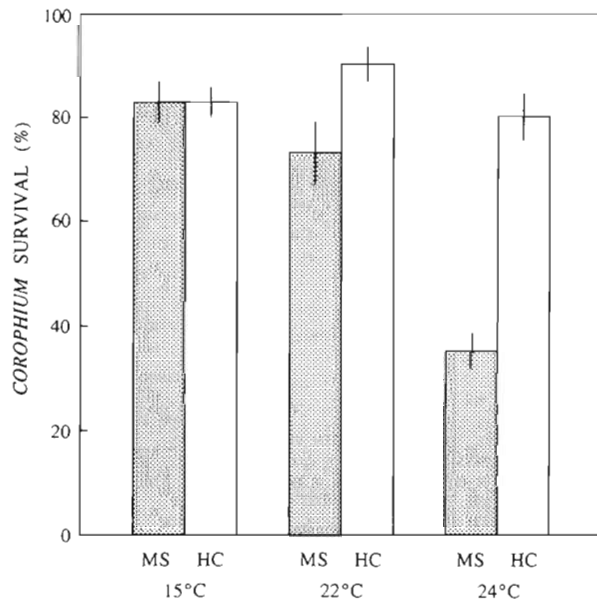


Fig. 9. *Corophium volutator*. Mean (%) survival rates ( $\pm 1$  SE) of amphipods exposed to different experimental temperatures and treatments. n-Values and abbreviations as in Fig. 6. Treatments were compared pair-wise at each temperature by Mann-Whitney tests. 15°C:  $Z = -0.106$ ,  $p = 0.92$  (non-significant); 22°C:  $Z = -2.23$ ,  $p = 0.03$ ; 24°C:  $Z = -2.58$ ,  $p < 0.001$

of the amphipods' gills due to lack of haemolymph. Anaemia causes reduced efficiency of oxygen uptake which, in turn, may further increase ventilation activity and thereby probably also infection rate, as argued above. As would happen during environmentally caused oxygen depletion in bottom waters (e.g. Jørgensen 1980), difficulties of oxygen extraction may ultimately force the animals to leave their burrows in pursuit of higher oxygen tensions. The seemingly higher surface activity among *Maritrema subdolum*-exposed amphipods than among control individuals is consistent with this.

Whether or not infliction of anaemia in the amphipods is a parasite strategy evolved in order to promote transmission of metacercariae to the shorebird final host, it is likely to be advantageous to the parasites. Since most shorebirds prefer to locate their prey by visual means rather than by tactile detection through penetration of the substrate (e.g. Evans 1986, Mouritsen 1993), an elevated surface activity among infected *Corophium volutator* specimens is likely to promote transmission.

#### Type of mortality

Holmes (1982) argued that laboratory experiments on the influence of parasites on host survival are prone

to infer the induced mortality as purely an addition to the mortality in control groups of animals. Yet, the effect of parasites in the field may be compensatory, in that they affect hosts that would have died anyway from other causes. If this applies to the presently studied host-parasite interaction, no population dynamic consequences should be expected from the presence of microphallid trematodes in a natural population of *Corophium volutator*. The present experimental results, however, are supported by a field study describing the extinction of a normally dense, age-structured and reproducing population of *C. volutator* as the likely result of the natural occurrence of these parasites (Jensen & Mouritsen 1992). In both the laboratory and the field, the mortality occurred over a rather short period of time and, regarding the field study, during a season where other conceivable mortality factors, such as bad weather conditions, pollutants, toxic algae, invertebrate and vertebrate predators, were weak or absent (Jensen & Mouritsen 1992, unpubl. data). These circumstances suggest parasite-induced additive rather than compensatory mortality in infected natural *C. volutator* populations. In such populations the parasite-induced mortality can even be expected to have depensatory (sensu Holmes 1982) properties due to the parasite-induced increase in amphipod surface activity. Such elevation of prey availability is likely to attract more feeding shorebirds

during seasons where they are present (Boates & Smith 1989, Mouritsen 1994) which, all other things being equal, must increase the predation pressure on also uninfected amphipods thereby exerting further mortality on the population.

Readers of our previous paper (Jensen & Mouritsen 1992) will know that 2 species of microphallid trematodes (*Maritrema subdolum* and *Microphallus claviformis*) were involved in the above mentioned field mortality of *Corophium volutator*. It should therefore be emphasized that preliminary experimental results do not suggest any important difference between the 2 species regarding their ability to induce mortality in *C. volutator* (Jensen 1996).

### Pathology

Few studies have adequately described the pathological effects of trematodes in marine crustaceans (Meyer 1990), and the cause of death in infested *Corophium volutator* specimens remains somewhat elusive. Although disruption of nervous innervation to vital organs or obstruction of the digestive tract have been suggested (Lauckner 1987, Meyer 1990), the observed gill anaemia, which to our knowledge has not been described in the context of trematode-infection previously, could be an additional mortality factor induced perhaps by loss of body fluid through the penetration holes (Fig. 5A), absorption of these fluids by the growing meso/metacercariae, and/or direct obstruction of haemolymph flow by the cysts. Difficulties in osmoregulation or a secondary invasion of protozoans or bacteria due to the perforated cuticula may have similar deleterious effects.

### Stability of the host-parasite association

The parasites' influence on the host population may be inferred from theoretical considerations (Anderson & May 1978, May & Anderson 1978, 1979, Anderson 1979, 1991, May 1983). A regulatory role of the parasites in stabilizing the dynamics of the host-parasite association is expected to be governed among other things by overdispersion of parasite numbers per host (i.e. few host individuals harbour the majority of the parasite population) and density-dependent host mortality (e.g. malnutrition of host specimens at high densities promoting infection). In contrast, random distribution of the parasites per host, density-independent host mortality, and time delays in the development of infective stages of the parasite are processes that may destabilize the interaction and so cause fluctuations in the host population.

Most known host-parasite associations show stabilizing as well as destabilizing elements (May & Anderson 1978, Anderson 1979), and the present *Corophium volutator*-*Maritrema subdolum* association is no exception. However, the only stabilizing property of the interaction that can be inferred from the present results is the overdispersed parasite distribution. The size-dependent parasite burden with perhaps a size-dependent resistance to parasite-induced pathology may explain this effect. Also the expectation that reinfection is facilitated by parasite-induced increases in ventilation rate could reinforce overdispersion. Regardless of how overdispersion is accomplished, the stabilizing property of overdispersed parasite distribution is based on the assumption that heavily infected hosts exhibit disproportionately high mortality rates causing also the death of the parasites they contain.

Disregarding overdispersion, the life-cycle of the trematodes itself, however, suggests merely an unstable interaction. First, the parasite burden in *Corophium volutator* must depend on the density of sympatric parasitized *Hydrobia ulvae*, the first intermediate host, everything else being equal. Hence, the parasite burden in *C. volutator* need not be related to current amphipod density. Second, the life cycle is completed in intertidal habitats that are utilized by the shorebird primary host mainly during autumn and spring migration (Laursen & Frikke 1984, Smit & Piersma 1989). The parasite prevalence in a given snail population (and hence the infection level in sympatric amphipods) is therefore likely to depend on the transmission between infected amphipods and birds on other sites along the east-atlantic flyway rather than the local amphipod population (K. N. Mouritsen, K. T. Jensen & T. Jensen unpubl.). Third, because of a considerable development time of larval trematodes within the snails (months, see Jensen & Mouritsen 1992), the migratory behaviour of the final hosts cause a destabilizing time-lag between the development of larval stages and parasite reproduction. Finally and perhaps most importantly, the temperature may exert considerable control over the abundance of released infective stages and thus the mortality within the population of the second intermediate host. Even a relatively small temperature increase seems able to overcome the buffering effect of a basically overdispersed parasite distribution and invoke substantial mortality. To the extent that weather conditions are unpredictable, such temperature dependence may in itself give rise to largely unpredictable mortalities or epizootics inherently independent of current host density.

Temperature-dependent infection rates, time delays in the parasite's life cycle, and tendency toward density independence point to at least periodic parasite-induced instability in the host population rather than

stable regulation. In principle, this should increase the probability of trematode-induced epizootics in natural populations of *Corophium volutator*, causing these parasites to have significant population dynamic consequences. We suggest that our finding of a collapse in a local population of *C. volutator* (Jensen & Mouritsen 1992) should be viewed in this context. Although there are many reasons other than parasites why population abundances might show wide fluctuations, parasitic infestation might well be the cryptic mortality agent behind some of the previous more or less unexplained observations of erratic fluctuations in *C. volutator* abundance, including occasional local extinction (see Watkin 1941, Muus 1967, Reise 1978, 1985, Fish & Mills 1979, Möller & Rosenberg 1982, Murdoch et al. 1986, Olafsson & Persson 1986, Wilson 1989, Matthews et al. 1992). We therefore recommend that the influence of parasites also be considered when the population dynamic of amphipods or other soft-bottom invertebrates is investigated in marine habitats.

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