The infectivity, growth, and virulence of the cestode *Schistocephalus solidus* in its first intermediate host, the copepod *Macrocyclops albidus*

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SUMMARY

In an experiment to study the infectivity, growth and virulence of *Schistocephalus solidus* in their first intermediate host, copepods of the species *Macrocyclops albidus* were kept singly and exposed to up to 9 coracidia. Eleven or 14 days post-infection (p.i.) the presence and growth of the cestode larvae relative to survival, growth and reproduction of their host was determined. As expected, the probability of a copepod becoming infected increased with increasing numbers of parasites administered. However, the chances of a single coracidium establishing in a copepod also increased with increasing numbers of coracidia administered, which indicates that the parasites profit from a dilution effect of the host's defence. Copepod size or developmental stage had no significant effect on the infection, but 14 days p.i., constraining effects of copepod size on the growth of the parasites were apparent. Moreover, procercoids in multiple infections grew smaller and developed their cercomer at a smaller size than those in single infections. No significant effect of the parasite on host mortality was found within the observation period. However, growth between the 5th copepodid stage and adult stage was negatively affected by infection. An infection with *S. solidus* was also strongly linked with host reproduction: infected females were more likely to bear an egg sac at the end of the experiment than non-infected ones. These egg sacs, however, contained fewer eggs.

Key words: *Schistocephalus solidus*, cestode, copepod, infectivity, growth, virulence, life-history.

INTRODUCTION

Schistocephalus solidus is a pseudophyllidean cestode which matures in the gut of fish-eating birds. The eggs pass out into water with the birds' faeces. After several weeks of development a free-living larva, the coracidium, hatches and is ingested by a cyclopoid copepod. Development of the procercoid stage and growth occurs in the body cavity of the crustacean and, if the latter is swallowed by a stickleback, the parasite develops into the plerocercoid stage and grows in the body cavity of the fish. The life-cycle is completed when the infected stickleback is swallowed by a bird and the plerocercoid develops to the adult hermaphroditic worm, mates (with another worm or with itself) and produces eggs (e.g. Hopkins & Smyth, 1951; Clarke, 1954).

The interaction between *S. solidus* and its second intermediate host, the three-spined stickleback, is well studied. Infection rate in a population can approach 100% (Smyth, 1946; Arme & Owen, 1967; Lester, 1971; McPhail & Peacock, 1983; Godin & Sproul, 1988), with most fish having 1–4 plerocercoids (Chappell, 1969; Meakins, 1974; Reimchen, 1982), but up to 140 worms/fish have been reported (Smyth, 1946). The parasites can become nearly twice as heavy as their host (Clarke, 1954; C. Wedekind, own observation), since they are more efficient in energy transformation than their hosts (Walkey & Meakins, 1970; Pascoe & Mattey, 1977). For this and for other reasons, plerocercoids of *S. solidus* are well known to cause severe fitness reduction in their second intermediate host (see review by LoBue & Bell, 1993).

In contrast, not much is known about the interaction between *S. solidus* and its first intermediate host, the copepod. Copepods have been successfully infected in the laboratory by several authors (e.g. Callot & Desportes, 1934; Clarke, 1954; Orr & Hopkins, 1969; Urdal *et al.* 1995; Wedekind & Milinski, 1996). Clarke (1954) reported that an infected 4th-stage nauplius needed about twice the time to grow to the adult stage, Urdal *et al.* (1995) demonstrated that infected copepods show a different activity behaviour, and Wedekind & Milinski (1996) found that infection causes a reduced swimming ability and increased predation risk.

The present paper reports the results of an experiment designed to investigate the infectivity of coracidia of *S. solidus*, the growth of procercoids in the copepods, and the virulence this parasite causes to its first intermediate host.

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MATERIALS AND METHODS

Host and parasite

The parasite *Schistocephalus solidus* was cultured *in vitro* using a technique modified from Smyth (1954). Plerocercoids were removed aseptically from sticklebacks (Smyth, 1946) and immediately placed into seamless semi-permeable tubes (1–3 worms/tube). Every tube was hanging in a 250 ml bottle filled with sterilized culture medium based on Minimum Essential Medium with Earle's salts, L-glutamine, with 25 mM HEPES-buffer (distributor Sigma (Nr. M2645)) and additives (per l of medium: 1 g penicillin/streptomycin, 6·5 g D-glucose, and titrated with NaOH to pH 7·5). These 250 ml bottles were placed in a water bath at 40 °C and shaken continuously with a horizontal motion (frequency: 80/min) throughout the 3 days incubation period in darkness. The worms were then removed and the eggs collected by rinsing the tube with tap water into a Petri dish. After the eggs had settled the water above them was replaced by clear tap water to remove the remaining culture medium and waste products of the adult worms. The eggs were kept at room temperature until hatching. Coracidia of 11 different clutches were used for the experiments.

Cultures of the copepod species *Macrocyclops albidus* have been maintained in our laboratory for several years following techniques described by Orr & Hopkins (1969).

Experimental set-up and infection

Before infection, copepods were filtered from the culture tanks and caught singly with a pipette (opening 2·5 mm). They were then placed on a slide in a small drop of water and filmed under the microscope. These film sequences were later used to determine the sex of the copepods, their copepodid stage, and their body size. Then, each copepod (only those without egg sacs, since I had observed in previous studies that nauplii fed on coracidia soon after hatching) was transferred into a well of a 24-well ELISA-plate (well volume 2 ml) where they stayed during the experiments. After they had stayed in the well without food for 1 day, either 0, 1, 3, 5, 7 or 9 coracidia (caught with a micro-pipette) were added to the wells. This procedure was randomized with respect to both the order of capture of the copepod and the origin of the coracidia. All coracidia added to a given well were from the same clutch, and the number of copepods infected by 1, 3, 5, 7 or 9 coracidia was balanced out per clutch (as far as possible, depending on the availability of coracidia). Spot-checks revealed that the coracidia were taken up by the copepods within 1 or 2 h after introduction.

After infection, the copepods were kept under constant conditions (20 °C, 12 h light/12 h dark) and fed every 2 days with about 20 paramecia. Eleven days ($n = 193$) or 14 days ($n = 96$) p.i. the copepods were filmed again under the microscope to determine their growth. Then, the number of procercoids in the haemocoel was recorded and, after dissecting them from the copepod, they were filmed to determine their size. If a copepod had developed an egg sac at this time, these egg sacs were gently squeezed and the number of eggs counted.

The body size of procercoids (excluding the cercomer) was measured in μm^2 as the sectional area filmed at 100× magnification. Their contour was drawn from the screen on transparent sheets. These images were cut out and weighed to the nearest mg from which the μm^2 of area could be calculated. Body size of copepods was measured as the length of the overall part of the 'body' including the 4th thoracic segment. Measured this way, the mean size of the copepods at the beginning of the experiment was 0·865 mm (S.D. = 0·174).

The data were analysed with SYSTAT (SYSTAT, 1992).

RESULTS

Growth and mortality of copepods

Of 289 copepods at the beginning of the experiment, 35 died for unknown reasons during the course of the experiment (= 12·1 %). The dead copepods did not differ from the surviving ones with respect to size at the beginning of the experiment ($t = -1{\cdot}17$, $P = 0{\cdot}25$), nor by their developmental stage ($\chi^2 = 1{\cdot}31$, D.F. = 3, $P = 0{\cdot}73$) or the number of coracidia administered (Fig. 1; 6 of the 52 control copepods died (11·5 %), while 29 of the 237 copepods (12·2 %) that were exposed to coracidia died ($\chi^2 = 0{\cdot}02$, D.F. = 1, $P = 0{\cdot}89$)). However, there was a tendency for males experiencing a higher mortality than females (males: 20·4 %, $n = 49$, females: 10·5 %, $n = 228$, $\chi^2 = 3{\cdot}66$, $P = 0{\cdot}056$, two-tailed).

At the beginning of the experiment, 57 copepods were copepodids (1 in the 3rd, 7 in the 4th and 49 in the 5th copepodid stage). Of these copepodids 44 developed into the next stage during the course of the experiment. The probability of this moult did not seem to be affected by infection ($\chi^2 = 0{\cdot}076$, $P = 0{\cdot}78$) or number of procercoids developing (only infected copepods: Mann–Whitney $U = 240{\cdot}5$, $P = 0{\cdot}13$). However, the difference between first and second measurement of body size of copepods that changed from the 5th copepodid stage to adult during the experiment was affected by infection: the more procercoids developing in the haemocoel, the smaller the growth of the copepods (all individuals: $r_s = -0{\cdot}275$, $n = 36$, $P = 0{\cdot}03$, directed; only copepods measured 11 days p.i.: $n = 25$, $r_s = -0{\cdot}127$; only copepods measured 14 days p.i.: $n = 11$, $r_s = -0{\cdot}527$).

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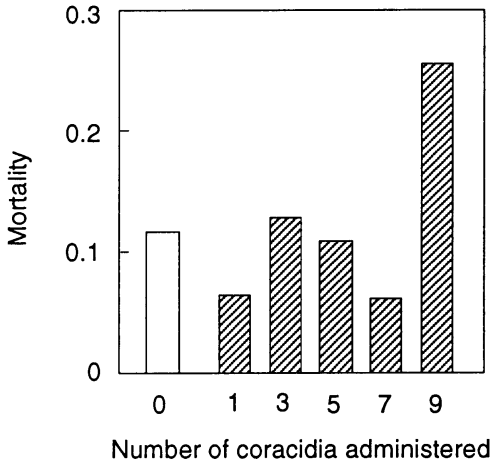

Fig. 1. The mortality rate of copepods infested by different numbers of coracidia (males and females pooled; over all: directed G-test for heterogeneity: G = 10·22, $P_h = 0{\cdot}07$, $r_s P_c = 0{\cdot}13$, $k = 6$, $P > 0{\cdot}10$).

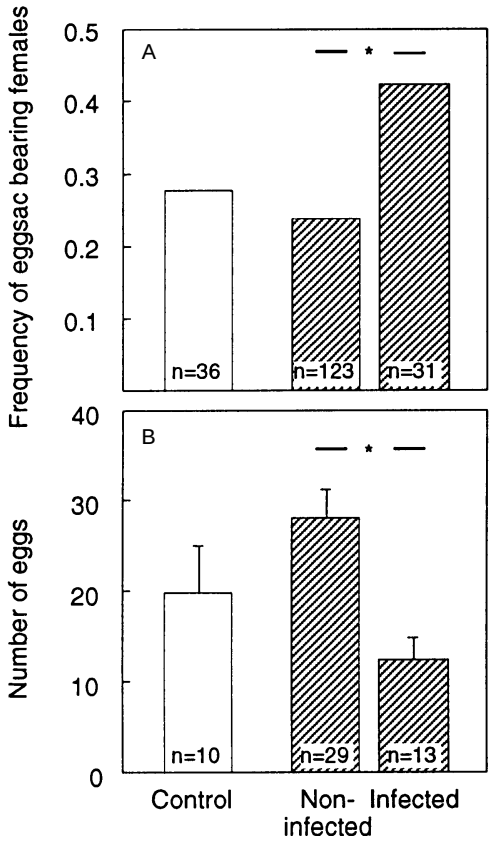

Fig. 2. Reproduction in non-exposed (control), exposed but non-infected and infected adult females. (A) Frequency of egg sacs (overall: $\chi^2 = 3{\cdot}95$, D.F. = 2, $P = 0{\cdot}138$; comparison between infected and non-infected exposed females: $\chi^2 = 3{\cdot}95$, $P = 0{\cdot}047$). (B) Mean + S.E. number of eggs of egg sac bearing females (overall: ANOVA, F = 4·43, D.F. = 2, $P = 0{\cdot}017$; comparison between infected and non-infected exposed females: Tukey HSD, $P = 0{\cdot}015$).

Reproduction of copepods

In general, larger females produced more eggs than smaller ones (all adult females: $r = 0{\cdot}144$, $n = 179$, $P = 0{\cdot}05$). Although infected females and non-infected females were not significantly different in size at the end of the experiment ($t = -0{\cdot}027$, $P = 0{\cdot}98$), they differed greatly in their reproductive output: infected females were more likely to produce an egg sac than uninfected ones (Fig. 2A) but their egg sacs contained fewer eggs (Fig. 2B). Of those females that were still in their 5th copepodid stage when infected but reached adulthood during the experiment, 1 was infected and produced an egg sac, while only 1 of the other 24 non-infected copepods did so (Fisher exact test, $P = 0{\cdot}08$, two-tailed). The exposure to coracidia itself did not seem to have an influence on whether or not the copepods produced an egg sac (only non-infected copepods: $\chi^2 = 1{\cdot}85$,

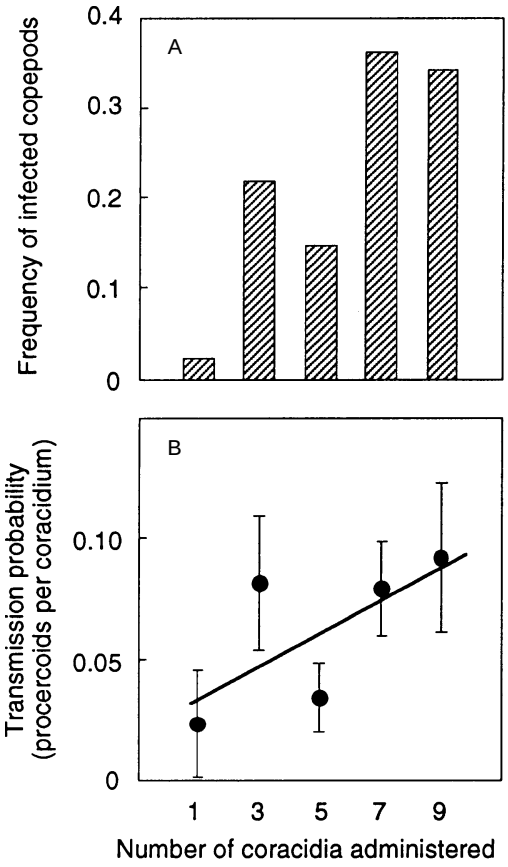

Fig. 3. Success of infestation. (A) Frequency of infected copepods in relation to the number of coracidia administered. The probability of infection increases with increasing number of coracidia administered: directed G-test for heterogeneity (Rice & Gaines, 1994): G = 108·5, $r_s P_c = 0{\cdot}8$, $k = 5$, $P < 0{\cdot}001$. This was true for female ($r_s P_c = 0{\cdot}79$, $P < 0{\cdot}01$) and male copepods ($r_s P_c = 0{\cdot}81$, $P < 0{\cdot}01$). (B) The ratio of procercoids/coracidium per copepod plotted against the number of coracidia administered (means ± S.E.; the means were used to calculate the regression line). The probability of a single coracidium becoming established in a copepod increases with increasing number of coracidia administered (all surviving copepods: Spearman's $r_s = 0{\cdot}236$, $n = 208$, $P < 0{\cdot}001$; only females: $r_s = 0{\cdot}206$, $n = 163$, $P < 0{\cdot}01$; only males: $r_s = 0{\cdot}340$, $n = 34$, $P = 0{\cdot}05$, two-tailed).

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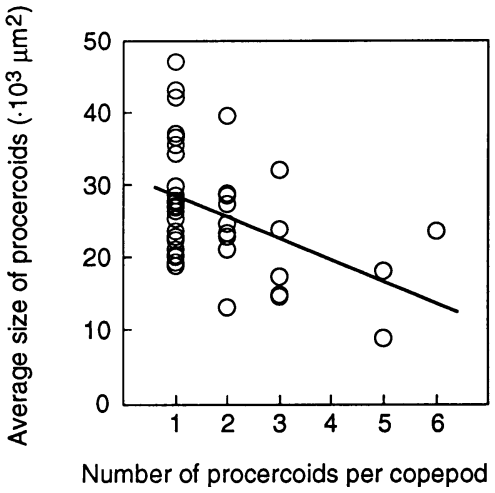

Fig. 4. The size of the procercoids (measured as the sectional area seen in the microscope, cercomer not included) relative to the number of procercoids per copepod (procercoids of copepods dissected 11 days p.i.: $r = -0{\cdot}51$, $n = 25$, $P = 0{\cdot}009$; procercoids of copepods dissected 14 days p.i.: $r = -0{\cdot}41$, $n = 17$, $P = 0{\cdot}10$; pooled: $r = -0{\cdot}44$, $n = 42$, $P = 0{\cdot}004$).

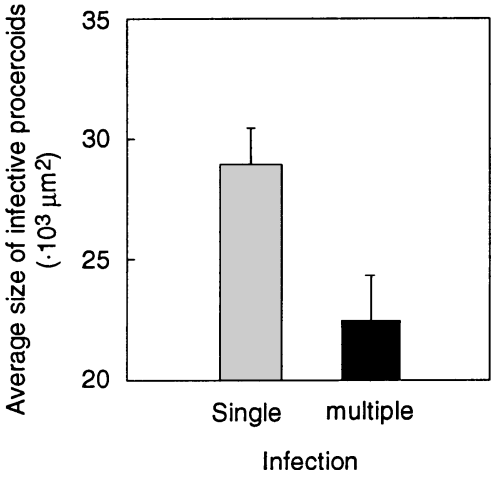

Fig. 5. Average size of infective procercoids, i.e. procercoids with a cercomer, in singly and multiple infected copepods ($n_1 = 17$, $n_2 = 25$, $t = 2{\cdot}67$, $P = 0{\cdot}01$). means + S.E.

D.F. = 5, $P = 0{\cdot}87$), or on the number of eggs these egg sacs contained at the end of the experiment (Kruskal–Wallis = 6·2, $P = 0{\cdot}29$).

Infection and success of coracidia

As would be expected, the frequency of infected copepods rose with the number of coracidia administered (up to 36%; see Fig. 3A). The same pattern could be observed in male and female copepods (see legend in Fig. 3). Furthermore, the chances of a single coracidium becoming established in a copepod increased with increasing numbers of coracidia administered (Fig. 3B). This was again true for males and females (see Figure legend). Males tended to be more susceptible to infection, especially when higher numbers of coracidia had been administered (transmission probability in males with 7 coracidia: 0·10; with 9 coracidia: 0·13). However, this difference was not significant (Mann–Whitney U tests, P always $> 0{\cdot}05$).

Whether the copepods were adult or still in a copepodid stage had no significant influence on the probability of infection ($n_{\text{(copepodids)}} = 46$, $n_{\text{(adults)}} = 156$, $\chi^2 = 1{\cdot}39$, $P = 0{\cdot}24$), the number of procercoids that developed (Mann–Whitney $U = 3322$, $P = 0{\cdot}29$), or on the transmission probability of coracidia ($U = 3300{\cdot}5$, $P = 0{\cdot}23$, two-tailed). Also, copepod size at the beginning of the experiment did not appear to affect the probability of infection ($t = -0{\cdot}96$, $P = 0{\cdot}36$), the number of procercoids developed ($r_s = -0{\cdot}07$, $P = 0{\cdot}29$), or the transmission probability of coracidia ($r_s = -0{\cdot}04$, $P = 0{\cdot}52$). The occurrence of multiple infections was not significantly different between copepodids and adults ($\chi^2 = 0{\cdot}114$, $P = 0{\cdot}74$).

Growth of procercoids

Procercoids grew less in multiple infections (Fig. 4), and developed their cercomer at a smaller size than procercoids in single infections (Fig. 5). It even seemed as if procercoids in singly infected copepods tended to develop their cercomer at a later stage than procercoids in multiple infected copepods (Fig. 6).

The size of the copepod at the time of infection correlated with the size of its procercoids 14 days p.i. (Kendall partial rank order coefficient, effect of multiple infection partialled out: $T_{xy.z} = 0{\cdot}25$, $n = 31$, $P = 0{\cdot}05$, directed), but not 11 days p.i. ($T_{xy.z} = 0{\cdot}008$, $n = 38$, $P > 0{\cdot}5$).

DISCUSSION

Infection and transmission rate

Cyclopoid copepods like *M. albidus* are obligate intermediate hosts of *S. solidus* (Clarke, 1954; Orr & Hopkins, 1969). Transmission of this cestode is achieved by means of a free-living infective stage (the coracidium) which has to be taken up by the copepod by predation (Clarke, 1954; Orr & Hopkins, 1969; C. Wedekind, own observation). The rate of encounter between copepod and cestode is influenced by the spatial and temporal distribution of the coracidia, and the number of coracidia that actually penetrate the intestine is expected to depend on the number of coracidia eaten. This could be confirmed in the present study, which supports similar findings in comparable parasite–host systems with free-living infective stages (e.g. Keymer & Anderson, 1979; Nie & Kennedy, 1993).

A possibly more surprising result is that the number of cestode larvae that establish themselves in

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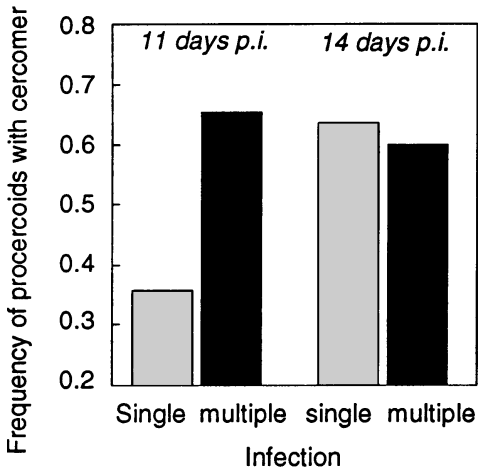

Fig. 6. Frequency of infective procercoids, i.e. procercoids with a cercomer, 11 and 14 days p.i., and in single and multiple infections (difference between single and multiple infections after 11 days: Fisher exact test, $P = 0{\cdot}10$; after 14 days: Fisher exact test, $P = 1{\cdot}0$, two-tailed).

the copepod host was more than linearly dependent on the number of coracidia administered: the transmission rate/parasite increased with increasing parasite exposure. This indicates that the parasites profit from a dilution effect of the host's defence. It remains unclear, however, on which level of host defence this dilution effect acts, e.g. on damaging with the copepods' mouth-parts upon ingestion, on the resistance of the intestine wall against penetration, or on any form of humoral or cell-mediated immune defence after penetration (Roitt *et al*. 1996).

Wedekind and Jakobsen (unpublished observation) found that male and female *M. albidus* differ in their susceptibility to *S. solidus*. A similar sex difference could be observed here, however, the difference was not statistically significant. This could be due to the lower parasite transmission rate observed in this study compared to Wedekind and Jakobsen which reduces the power for a statistical analysis of sex-dependent infectivity. This difference in transmission rate may be connected to the fact that the parasites used were from different years, and that the experimental procedure differed in some aspects. Wedekind and Jakobsen, for example, used *M. albidus* caught in Bielefeld (Germany), i.e. close to Bochum where the cestodes originate, while here copepods from an old laboratory culture originally founded by individuals caught near Glasgow, Scotland (O. Lassière, personal communication) have been used. The observed difference in transmission probability fits into the general pattern that parasites are normally more virulent to the hosts they are locally adapted to (Ebert & Hamilton, 1996). However, *S. solidus* uses birds as final host and vector. Therefore, eggs of this cestode are likely to be spread to new regions where they encounter new copepod hosts.

There are further factors that could influence transmission of this parasite and that are not studied here, e.g. parasite and host genetics, size and age of the coracidia, or abiotic factors like temperature etc. At the moment, there are no data available on infection rates and number of *S. solidus* procercoids that can be found in copepods in the wild.

Growth of procercoids and competition between them

In this study up to 6 procercoids could be found within a single host. With mass infection, Callot & Desportes (1934) could find copepods with up to 60 procercoids. The present study shows that growth and size of procercoids depend on the number of competitors within a single host: with increasing intensities of infection the procercoid size was smaller at the end of the experiment. This confirms other studies on cestode infection in copepods (e.g. Rosen & Dick, 1983; Nie & Kennedy, 1993) or on other hosts, e.g. *Tribolium* (Keymer, 1980). Growth and size of procercoids also depended on the size of the host, however, apparently only after the procercoids reached a certain threshold size: 11 days p.i. host size did not show a significant effect on parasite growth, while 14 days p.i. this effect became apparent. It appears that growth is maximal during the first days p.i. and becomes reduced in older infection due to the fixed size of the copepod. In contrast to this, *S. solidus* can reach nearly twice the weight of its host in the fish, whose shape is not fixed by a rigid exoskeleton.

The time at which the mature infective procercoid stage is reached not only depends on the size of the procercoid but also on other factors. Procercoids in multiple infections tended to develop their cercomer earlier than procercoids in singly infected copepods, although the latter grew faster and bigger. This could reveal a life-history response to multiple infection, because multiple-infected copepods may suffer more from their infection and could therefore be more susceptible to predation by the next host. This may especially be so because the procercoids are not clonally related to each other (Frank, 1993, 1996; Nowak & May, 1994; May & Nowak, 1995) since nearly all coracidia used stem from parents that had the opportunity to outcross, the relation coefficient between procercoids in multiple infection may be around 0·5 rather than near 1·0. However, it has not yet been tested whether there is a relationship between infection levels and the probability of predation by sticklebacks. If such a relationship exists, the parasites in multiple infections are expected to be prepared for transmission to this next host earlier than parasites in a single infection. The cost of such a life-history response may be the smaller size at transmission to stickleback since cercomer formation appears to negatively affect procercoid growth (Clarke, 1954). The size of the

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infective procercoid may be a relevant factor for establishment and growth in the next host, the three-spined stickleback (Clarke, 1954). This seems obvious, given that the procercoids grow rapidly and can actually reach relatively large sizes in the copepods. Rosen & Dick (1983) found in another cestode that larvae from crowded infections grew slower and reached smaller sizes in their copepod host. When fed to fish, these parasites actually achieved lower transmission success than those from less crowded infections.

Virulence

A significant difference in mortality was found neither between non-exposed and exposed copepods nor between low and high numbers of coracidia administered. This is in contrast to other studies in which significant effects on mortality of copepods infected with other cestode parasites have been found. Normally, mortality increased with increased dose of infection (e.g. Rosen & Dick, 1983; Nie & Kennedy, 1993; Ashworth *et al*. 1996). A possible explanation of this contradiction could be that in the present study the copepods were kept singly and were well fed. In this way, they did not suffer from intra-specific food competition and other social stress factors that may amplify a viability difference between infected and non-infected individuals. Moreover, the experimental period in this study was short, and the infection doses achieved here are very low compared to the other studies on mortality effects of cestodes (Rosen & Dick, 1983; Nie & Kennedy, 1993; Ashworth *et al*. 1996). Figure 1 suggests that the mortality tended to increase only with the highest infection dose administered (9 coracidia). Such a parasite-induced host mortality would prevent the parasite from being transmitted to the next host, which is of course non-adaptive to the parasite. However, in copepods that got 9 coracidia multiple infections were more frequent, and adaptive reactions of procercoids to competition within an individual host may lead the parasites to over-exploit the resources of their host (Frank, 1993, 1996; Nowak & May, 1994; May & Nowak, 1995).

Indirect mortality effects could be demonstrated in other studies on our species. *M. albidus* infected with *S. solidus* show reduced motility and a behaviour that makes them more likely to be preyed upon by three-spined sticklebacks, the second intermediate host of *S. solidus* (Wedekind & Milinski, 1996; Jakobsen & Wedekind, unpublished observations). This corresponds to comparable studies on other cestodes and other copepod species (Poulin, Curtis & Rau, 1992; Pasternak, Huntingford & Crompton, 1995).

An infection with *S. solidus* also correlates with a reduced growth of its host. The increase in length during the moult from the 5th copepodid stage to adult was smaller in infected than in non-infected copepods. This confirms findings of Clarke (1954) who observed that an infected 4th-stage nauplius needed about twice the time to grow to adult state. It is likely that infection has caused this reduction in growth, given the extraordinary increase in biomass of this parasite. However, since the transmission probability was far from 100 %, I cannot exclude the possibility that the causal chain is the other way around, i.e. copepods that grow less for other reasons are easier to infect.

The term 'virulence' is often not simply defined as the parasite's effect on host mortality and growth, but in a more evolutionary sense as the effect a parasite has on its host's fitness (e.g. Read, 1994; Bull, 1994). *S. solidus* appears to be very fitness-relevant to its copepod host when egg production is compared between infected and non-infected females: infected copepods have a strongly reduced egg number under the food constraints given by the experimental procedure in this study. Again, the large size and biomass of the procercoids compared to its copepod host make it very plausible that the parasite's resource requirements contribute largely to this aspect of its virulence. However, infected copepods developed an egg sac more often than non-infected ones at the end of the observation period. This could, on the one hand, be the result of a manipulation by the parasite, because ovigerous females may be more susceptible to predation by fish than females not bearing egg sacs (Vuorinen, Rajasilta & Salo, 1983). On the other hand, this could reveal a life-history response of the copepod to infection (Stearns, 1992): infection may lead the copepod to value current reproduction more compared to future reproduction than non-infected copepods would (Minchella & Loverde, 1981; Minchella, 1985). As a consequence, infected females could start reproduction earlier despite the cost of producing fewer eggs per egg sac. However, the possibility that females who will produce an egg sac with few eggs in the near future are easier to infect can still not be excluded.

The reproductive behaviour of exposed but non-infected females could reveal another interesting life-history response to *S. solidus*. The contact to the parasite could also lead the exposed but non-infected copepods to value current reproduction compared to future reproduction more than non-exposed copepods would (Minchella & Loverde, 1981; Minchella, 1985), but the consequence of this appears to be different to that of infected copepods. Instead of producing small egg sacs with few eggs soon after exposure and infection (like infected copepods do), they appeared to delay the development of egg sacs and produce larger ones that contained more eggs, even more than egg sacs of non-exposed copepods. The cost of such a changed reproductive strategy could be a reduced future

reproduction, but the strategy could be adaptive if a contact to *S. solidus* implies to the copepods that actual infection is more likely in the future. However, this hypothesis remains to be tested.

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