To avoid or eliminate: cestode infections in copepods

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SUMMARY

The outcome of a parasite infection is the result of the interaction between the host and the parasite. In the system we studied, there are 3 critical stages for the outcome of infection of the (intermediate) host, the copepod *Macrocyclops albidus*, with the cestode *Schistocephalus solidus*. During the establishment phase of the parasite, the host may firstly avoid ingesting the parasite and, secondly, may prevent the parasite from entering the body cavity and, thirdly, during the growth phase of the parasite, the host's immune system may eliminate the parasite from the body cavity. We were able to study the growth phase separately from the establishment phase. The establishment phase was influenced by characteristics of the host as well as characteristics of the parasites. Small copepods and males performed poorly; they were more often infected and had a lower survival. Parasites from different sib-groups differed in infectivity. During the growth phase some disappearance of parasites was observed. However, this could not be related to any of the studied characteristics of the host, and the sib-groups of parasites did not seem to differ in their likelihood to disappear. Instead, we suggest that disappearance of parasites, once they have entered the body cavity, may be due to intrinsic mortality of the parasites, independent of the host or the sib-group that the parasites belong to. This indicates that the crucial interactions between host and parasite determining the outcome of infection takes place in the short time-period between ingestion and penetration of the gut-wall.

Key words: *Schistocephalus solidus*, *Macrocyclops albidus*, prevalence, intensity, body size, survival, parasite resistance.

INTRODUCTION

Parasites can have a large impact on their hosts' fitness and hosts can try to decrease the costs of parasite infections in several ways. On the other hand, natural selection will act on the parasite to counteract these adaptations of the host. The coevolution of hosts and parasites can be viewed as an arms race of adaptations and counter-adaptations (Hamilton & Zuk, 1982; Hamilton, Axelrod & Tanese, 1990). As a result, the outcome of a parasite infection will be influenced by characteristics of the host as well as characteristics of the parasite.

In the system we worked with, the cestode *Schistocephalus solidus* and one of its first intermediate hosts, the copepod *Macrocyclops albidus*, there is a clear fitness cost of infection for the host. Infected copepods become more active and are less capable of escaping predator attacks (Wedekind & Milinski, 1996). Furthermore, under risk of predation, infected copepods prefer to be without conspecifics (Jakobsen & Wedekind, 1998). As a result, infected copepods suffer a higher risk of predation by the second intermediate host of *S. solidus*, the stickleback *Gasterosteus aculeatus* (Wedekind & Milinski, 1996). We can therefore expect selection to act on copepods to overcome the detrimental effects of this parasite by favouring the evolution of mechanisms to either avoid or eliminate the parasite.

Both the host and the parasite have several stages of defence and attack. Copepods become infected through ingestion of the parasite larvae and thus the first line of defence during the establishment phase of the parasite might be avoidance of ingestion (Hart, 1997). Parasites could potentially counteract this by becoming more attractive as a food item (Bakker, Mazzi & Zala, 1997). Once the copepod has ingested the parasite, the parasite has to penetrate the wall of the gut to get established in the haemocoel. Hence, the second line of defence of copepods might be to prevent this penetration by means of mechanical barriers (Peters, 1992) or their immune defence (Ratcliffe, 1985). Once the parasite has entered the haemocoel, copepods might use a potential third line of defence during the growth phase of the parasite and try to eliminate the parasite from the haemocoel, for example by encapsulating it (Ratcliffe, 1985; Götz, 1986; Hoffmann, 1995; Gillespie, Kanost & Trenczek, 1997; Söderhäll & Cerenius, 1998). The parasites have to overcome these potential defences and, in addition, compete with other (conspecific) parasites for resources within the host.

How well a host can avoid or eliminate parasites will depend on its genetic and phenotypic quality. Since large female *M. albidus* lay more eggs (Wedekind, 1997), size seems, at least for females, to

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be a good measure of phenotypic quality. However, until now, no effects of body size or developmental stage of *M. albidus* on prevalence or intensity of *S. solidus* infection have been shown (Wedekind, 1997; Wedekind & Jakobsen, 1998).

Male copepods are smaller than females and have a higher risk of becoming infected (Wedekind, 1997; Wedekind & Jakobsen, 1998; Jakobsen & Wedekind, 1998). For vertebrates, such sex differences in susceptibility have been explained as being the result of the male hormone testosterone interfering with the immune function of its producer (Folstad & Karter, 1992; Zuk & McKean, 1996). It has been suggested that this explanation may not apply to invertebrates, since they do not produce testosterone (Zuk & McKean, 1996; Sheridan *et al*. 2000) but, as Wedekind & Folstad (1994) suggested, in invertebrates a similar mechanism could be at work, regulated by other substances. In invertebrates, high parasite prevalence and high mortality of males compared to females have also been linked to higher intersexual competition in males (Wedekind & Folstad, 1994; Zuk & McKean, 1996; Poulin, 1996; Wedekind & Jakobsen, 1998).

The parasite *S. solidus* is a simultaneous hermaphrodite. Very little is known about the relation between characteristics of the parasite and its success at infecting its first intermediate host, except that infection with a group of genetically heterogeneous parasites leads to a higher prevalence but a lower intensity of infection than infection with a group of genetically homogeneous parasites (Wedekind & Rüetschi, 2000). Furthermore, large cestodes allocate more to the female part of reproduction (Schärer *et al*. 2001) and large individuals produce bigger eggs (Wedekind, Strahm & Schärer, 1998), which may affect the performance of their offspring.

In this study, we investigated whether the outcome of an infection can be predicted by characteristics of the copepods and the parasites. We focused on the third line of defence of the copepods, i.e. whether loss of parasites during the growth phase of the parasites can be predicted by characteristics of the copepods (for example size, stage and sex) and parasite sib-group.

MATERIALS AND METHODS

Copepod cultures

The *Macrocyclops albidus* culture was initiated with 20 mated females from a small river (Wedeler Au) in Hamburg, Germany, in June 2000. The culture was maintained by regularly placing about 50 mated females from several aquaria into a new aquarium ($\pm$5 l water) which was initiated with 200 ml of *Paramecium caudatum* solution (about 300 cells/ml). The culturing method was modified from Orr & Hopkins (1969) by Strahm, Wedekind & Christen (unpublished observations). In each aerated aquarium we placed a net with boiled hay. In this hay-bag, bacteria, the food source for the paramecia, could grow, and copepod nauplia could take refuge. Twice a week, 200 ml of *Paramecium* solution was added to each aquarium. The culture was kept at 18 °C and a 16 h light/8 h dark cycle.

Parasites

Field screening showed that cyclopoid copepods from the Wedeler Au were infected with cestodes in the summer of 2000 (Sivars & van der Veen, unpublished observations), although, as yet, the cestode species is unknown. We did not find any sticklebacks infected with *Schistocephalus solidus* in the Wedeler Au and therefore we used *S. solidus* from 8 sticklebacks caught on 21 and 29 September 2000 in the brackish Binnensee in Neustadt for this experiment. The sampling places of the copepods and infected sticklebacks were about 80 km apart. Cormorants (*Phalacrocorax carbo sinensis*), a very common (personal observations) final host of *S. solidus* (Chubb *et al*. 1995) in this region, typically fly about 20 km between feeding sites during the breeding season (Platteeuw & van Eerden, 1995). However, non-breeding birds in particular can fly much longer distances ($>$ 70 km) between feeding sites (van Eerden & Voslamber, 1995; van Eerden, personal communication). This suggests that the population of *S. solidus* in Neustadt and a potential population of *S. solidus* from Wedeler Au are probably not genetically isolated. However, even with low levels of gene flow, local adaptation of hosts and parasites can exist (Mopper *et al*. 2000). How potential local adaptation may have affected our experiment is not clear.

Two of the 8 sticklebacks were doubly infected with *S. solidus*, but only 1 parasite from each stickleback was used in this experiment. The parasites were removed from the fish on 5 November 2000 and bred in pairs in an *in vitro* system modified from Smyth (1954) by Wedekind (1997). The parasites were weighed before onset of breeding. The eggs from each pair of worms were divided into 3 batches and stored at 4 °C. The offspring from each parental pair are called sib-groups in this experiment, even though not all individuals in each sib-group necessarily have to be sibs, since *S. solidus* can produce offspring either through selfing or through outcrossing (Wedeking *et al*. 1998). At 19–24 days before infection, the eggs were placed at 20 °C and covered with aluminium foil. On the day before infection the foil was removed and the light stimulus caused the eggs to hatch (Dubinina, 1966).

The experiment

The experiment was performed in 2 rounds, one from 9 November 2000 to 21 November 2000 and one from 22 January 2001 to 3 February 2001. The

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same sib-groups of parasites were used in both rounds, and thus, in the second round, parasites had been stored longer at 4 °C than in the first round.

Copepods were taken from 3 (round 1) or 6 (round 2) different aquaria 3–7 days before infection. Each copepod was placed individually in a well (water volume ≈ 2 ml) of a 24-well ELISA plate. Each copepod was fed 3 *Artemia salina* 3 times a week. At 1–4 days before infection, copepods were screened under the microscope and their developmental stage (26 C4 and 27 C5 males and 70 C4 and 30 C5 females were used) was determined. Also their body size was measured when lying on the side by measuring the distance from the centre of the eye to the posterior dorsal end of the cephalothorax by means of image analysis performed on a Macintosh computer using Scion Image, which is a modification of the public domain NIH Image program (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). From each copepod 3 size measurements were taken and repeatability of these measurements (calculated as the proportion of the total variance that was due to between individual variance) was very high (99·8 %, $n = 639$ measurements). For further analyses the mean of those 3 measurements was used. In the statistical models relative size, i.e. size subtracted by sex and stage class averages, was used. Only C5 or adult copepods can be accurately sexed, however, already in the C4 stage males are generally smaller than females (in this particular case male size = 350–450 μm, female size = 430–560 μm) and thus C4 copepodites were assigned as being male or female according to their size. Most C4 copepodites moulted to C5 during the experiment, which made a definite determination of sex possible. Copepods in the C4 stage that had a size between 430 and 450 μm could not be assigned to being male or female according to their size and if those copepods did not moult during the experiment they were excluded from the analyses. Males and females were not equally distributed among the copepodite stages ($\chi^2 = 5{\cdot}67$, D.F. = 1, $P = 0{\cdot}017$). Females were over-represented in the C4 stage (72 % females in C4, 54 % in C5) and overall fewer males than females were used in the experiment. This unbalance in the design is due to differences in developmental time and survival between males and females. The over-representation of females in the C4 copepodite stage did not enhance sex or stage effects in our experiment, since females were less often infected than males and on the other hand, C4 copepodites were more often infected than C5 copepodites (see Results section). After the determination of sex, stage and size, the copepods were placed in a new ELISA plate. Half of the 24 wells in each plate were filled with females and the other half with males. Which side contained males or females was alternated between plates.

Infections were carried out by providing each copepod with 6 parasite larvae (coracidia) for ingestion. Each plate received only 1 sib-group of parasites, but each sib-group was used for several plates. In both rounds, infections were done twice a day on 2 consecutive days and at each infection event 3 new plates were infected, i.e. a total of 12 plates were infected in each round (see also treatment effects below). Copepods were last fed 2 days before infection.

Labelling of coracidia

Coracidia were fluorescently labelled with the tracer dye CMAC (7-amino-4-chloromethylcoumarin, CellTracker™ Blue, C-2110, Molecular Probes, Inc.). Coracidia were incubated for 1 h (at 20 °C, in the dark) in 2 ml of aged, sterile filtered tap water containing 20 μM CMAC (Kurtz, van der Veen & Christen, 2002). The dye is chemically modified and bound inside cells, so that it is not transferred to neighbouring cells or tissues. Before use for infection of copepods, coracidia were transferred into 8 ml tap water to dilute surplus dye.

For infection, 6 coracidia were provided to each individual copepod. Two hours after adding the coracidia, a time normally sufficient for the copepods to ingest all coracidia (Wedekind, 1997), copepods were transferred to the dark to prevent the dye from bleaching in the light. Copepods were kept in the dark, except for about 1 h light during feeding every 2 days.

At 1, 3 and 7 days post-infection (p.i.), copepods were examined for the presence of fluorescent procercoids with a Leica DM IL inverted epi-fluorescence microscope at 100 × magnification, using a filter set with emission of 340–380 nm wavelength (UVA) for absorption/emission maxima of 354/466 nm. Screening of the copepods was done on both the left and right side of the copepod. Screening on day 1 p.i. was done 18–22 h after exposure. Eleven days p.i. copepods were screened under normal, brightfield illumination.

Statistical analyses

Prevalence of infection and intensity in infected copepods on day 11 p.i. were modelled with linear logistic models. Prevalence was analysed as a binary response variable and intensity was restricted to 4 levels (1, 2, 3, 4–6 parasites), because of small sample sizes for the high intensities. We also modelled survival until day 11 (0 dead, 1 survived) and loss of parasites between day 1 and 11 (0 or 1 parasite lost; there was never more than 1 parasite lost) as binary response variables in similar models. We used Proc Logistic in SAS V8 (SAS Institute, 1999), with a logit link function in which both continuous as well as class variables could be used as explanatory

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variables. Because of the complexity, only 2-way interactions could be included in the models. The goodness of fit of all models was tested by comparing the likelihood of the current model with that of a fully saturated model using the deviance ($-2\log(Lc/Lf)$) of the current model. All initial and final models fitted the data (all $P > 0{\cdot}6$). Akaikes Information Criterion (AIC) was used to evaluate the significance of omitting or adding variables or interactions, until the most parsimonious model was found (Collet, 1991). Removed interactions are not reported, but removed main effects are reported in the tables. Significance levels of the remaining main effects and interactions were calculated with likelihood ratio tests, i.e. calculating the difference in deviance between the final model and the model omitting the variable of interest. Both the deviance as well as the difference in deviance approximately follows a χ^2 distribution (Collet, 1991).

The power of the main effects in the analyses of parasite loss was estimated by calculating the power of a χ^2 test for each main effect with that particular sample size and degrees of freedom (Cohen, 1988). Although this does not give the true power of our tests, it is a reasonable approximation.

Differences between the observed and calculated expected distribution for loss of parasites were tested with a G-test for goodness of fit (Sokal & Rohlf, 1995).

Controls for treatment effects

We performed 2 controls for the effect of labelling of the coracidia and repeatedly screening of the copepods under UV light on the prevalence and intensity of infection and survival of the copepods. In the experimental treatment we labelled and screened the copepods (LS), in the first control treatment we labelled but did not screen (LN), and in the second control we neither labelled nor screened (NN). Copepods in all treatments were screened under normal light on 11 days p.i., on that day the observer was naive concerning treatment. The 3 treatments were randomly assigned to 1 row in each plate and the remaining row in each of 3 plates was also randomly assigned 1 of the treatments, so that in each set of 3 plates all treatments appeared 4 times. Labelling the coracidia did not significantly affect the prevalence of the infection nor the survival of the copepods (prevalence $\chi^2 = 0{\cdot}72$, D.F. $= 1$, $P = 0{\cdot}40$, $n = 354$; survival $\chi^2 = 0{\cdot}01$, D.F. $= 1$, $P = 0{\cdot}92$, $n = 452$). However, there was a tendency for screening to increase the prevalence of infection and decrease the survival of copepods (prevalence $\chi^2 = 2{\cdot}93$, D.F. $= 1$, $P = 0{\cdot}09$, $n = 354$, survival $\chi^2 = 3{\cdot}59$, D.F. $= 1$, $P = 0{\cdot}06$, $n = 452$). The higher prevalence due to screening cannot be explained by a higher survival of highly infected individuals under screening. Survival was not significantly correlated with the intensity of infection on day 1 (Table 3), and, if anything, highly infected individuals had a lower, instead of a higher survival. It may be that screening the copepods under UV light weakened their immune defence, thus causing a higher prevalence in screened individuals. The effect of screening and labelling on intensity of infection differed between the sib-groups of parasites ($\chi^2 = 19{\cdot}06$, D.F. $= 6$, $P < 0{\cdot}005$). While in 3 sib-groups there seemed to be no clear-cut effects of screening or labelling on intensity, in 1 group screening and labelling seemed to decrease the intensity (Kurtz, van der Veen & Christen, 2002).

RESULTS

Characteristics of copepods

Significantly more males than females got infected (Table 1, 83% and 73% respectively), but the intensity did not differ significantly between males and females (Table 2, 2·27 and 2·35 parasites/copepod respectively). Males also tended to have a lower survival than females, but, this was not statistically significant (Table 3, 70% and 76% respectively). Copepods that were in an earlier stage of development when exposed (C4) had a significantly higher prevalence than copepods that were infected at a later stage (C5) (Table 1, 91% and 57% respectively). However, neither intensity (Table 2, 2·39 and 2·25 respectively) nor survival (Table 3, 70% and 79% respectively) were significantly affected by developmental stage.

Since the sexes and stages of copepods differ in size, we used relative size (see Materials and Methods section) when investigating the effect of size. Copepods that became infected were significantly smaller at the start of the experiment than copepods that were not infected (Table 1, mean $\pm$ S.E.: $-1{\cdot}78 \pm 2{\cdot}57$ μm and $14{\cdot}42 \pm 4{\cdot}56$ μm respectively). Intensity also tended to increase with decreasing relative size (Table 2, Fig. 1A), i.e. smaller copepods tended to have more parasites. The effect of relative size on survival was stronger in males than in females and stronger in early stage (C4) than in later stage (C5) copepods (Table 3, Fig. 1B), but in all these categories surviving copepods were bigger than the ones that died (Fig. 1B).

The experiment was performed in 2 different rounds, separated in time. The intensity was significantly higher in the first round of the experiment than in the second, and also prevalence tended to differ in the same direction (Tables 1 and 2, prevalence 94% and 63% respectively, intensity: 2·64 and 1·95 parasites/copepod respectively), while survival of the copepods was lower in the first round than in the second (Table 3, 63% and 87% respectively). However, there were no significant interactions with round. Thus, although the rounds

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Table 1. The prevalence of *Schistocephalus solidus* in *Macrocyclops albidus* copepods that survived (11 days p.i.), modelled with a logit model and tested with Likelihood-Ratio-Tests for the effect of sex, stage and relative size (corrected for stage and sex) of the copepod at the start of the experiment and the effect of the first or second round of the experiment and the effect of parasite sib-group ($n = 111$)

Factor	χ^2	D.F.	P
Sex	5·41	1	0·0201
Stage	29·63	1	< 0·0001
Size	12·18	1	0·0005
Round	2·76	1	0·0965
Sib-group	16·90	3	0·0007

Table 2. The intensity of infection of *Schistocephalus solidus* in infected *Macrocyclops albidus* copepods (11 days p.i.), modelled with a logit model and tested with Likelihood-Ratio-Tests for the effect of sex, stage and relative size (corrected for stage and sex) of the copepod at the start of the experiment and the effect of the first or second round of the experiment and the effect of parasite sib-group ($n = 85$)

Factor	χ^2	D.F.	P
Sex	0·38	1	0·5381
Stage	1·87	1	0·1712
Size	3·62	1	0·0572
Round	5·63	1	0·0176
Sib-group	11·97	3	0·0075
Sex * sib-group	8·01	1	0·0458

Table 3. The survival of the copepod *Macrocyclops albidus* (11 days p.i.), modelled with a logit model and tested with Likelihood-Ratio-Tests for the effect of sex, stage and relative size (corrected for stage and sex) of the copepod at the start of the experiment and the effect of the first or second round of the experiment and the effect of parasite sib-group and intensity of infection on day 1 p.i. ($n = 154$)

Factor	χ^2	D.F.	P
Sex	2·87	1	0·0902
Stage	0·01	1	0·9287
Size	0·50	1	0·4813
Round	4·63	1	0·0313
Sib-group	3·96	3	0·2656
Intensity day 1 p.i.	6·43	4	0·1691
Size * sex	4·37	1	0·0367
Size * stage	5·67	1	0·0173

differed in infection and survival, all effects we found were similar concerning direction in both rounds, which justifies the use of both rounds of the experiment.

Sib-groups of parasites

Since the outcome of a parasite infection is influenced by the host as well as by the parasite, one could expect differences between parasites in performance in the host. In this experiment we used 4 sib-groups of parasites. These sib-groups differed significantly in prevalence and intensity (Tables 1 and 2, Fig. 2), but high intensity did not seem to coincide with high prevalence, nor did the opposite seem to be the case (Fig. 2). There was a small, but significant difference between male and female copepods in the effect of the sib-groups on intensity of infection (sex * sib-group interaction Table 2); while in one sib-group males had a higher intensity than females, in another sib-group the opposite was the case. The prevalence and intensity of the different sib-groups of parasites did neither seem to be related to the average body mass of the parents or to the difference in body mass between the parents. However, all parents were very similar in weight and a correlation between 4 points has very low power. There were no significant differences in survival of copepods infected with parasites from different sib-groups (Table 3).

Elimination of parasites?

Parasites disappeared from the copepods during the course of the infection (Fig. 3). However, we never clearly observed encapsulations of parasites. Twenty-three % of the infected copepods lost a parasite, but never more than 1 parasite disappeared from a copepod and only 3 % of the infected copepods became completely free of parasites. We could not significantly relate disappearance of parasites from the copepods to characteristics of the copepod, or to sib-groups of the parasites (Table 4). The power of the analyses for characteristics of copepods is relatively good (power = 0·80) for a medium effect size ($w = 0·03$) but low (power = 0·15) for a small effect size ($w = 0·01$), while the power is not very high (power = 0·64) even for a medium effect size for the effect of sib-groups of parasites. This means that small differences could not be detected with our data, but medium differences in loss of parasites for different copepods should have been possible to detect.

The only factor that significantly influences loss of parasites was the amount of parasites the copepods had on the first day of infection (Table 4). Copepods with many parasites had a higher chance of losing 1 parasite during the infection (Fig. 4). The question remains if this is due to competition among the

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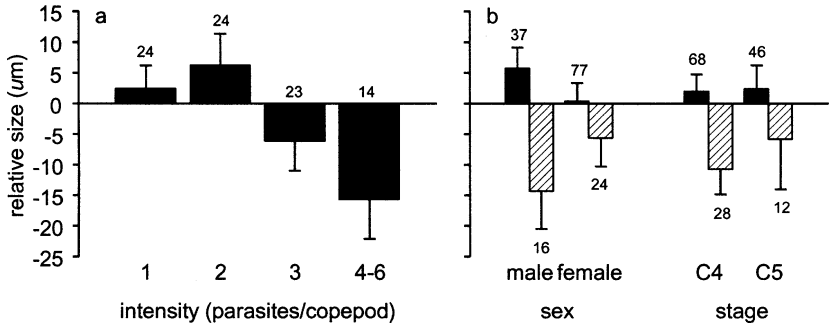


Fig. 1. Average relative size at infection (±S.E.) of *Macrocyclops albidus* copepods with different intensities of infection with *Schistocephalus solidus* (A) and average relative size at infection (±S.E.) of survived (■) and dead (▨) male and female copepods and C4 and C5 copepodites (B). Sample sizes are indicated in the figure.

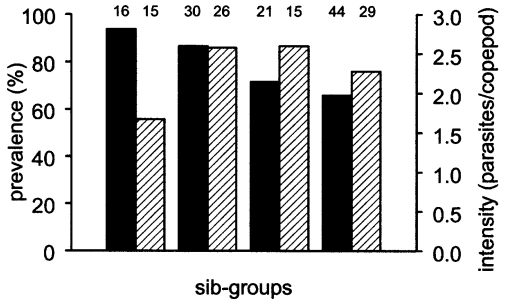


Fig. 2. Prevalence (■) and intensity (▨) of infection in sib-groups of *Schistocephalus solidus* infecting the copepod *Macrocyclops albidus*. Sample sizes are indicated in the figure.

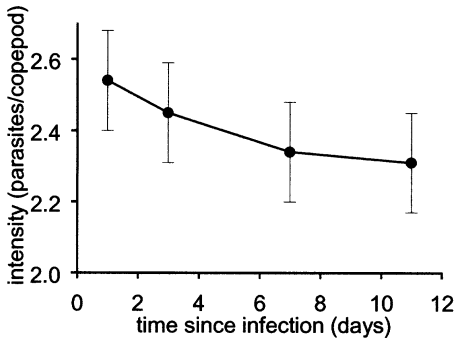


Fig. 3. Intensity of infection of *Macrocyclops albidus* copepods ($n = 87$) with *Schistocephalus solidus* during the course of infection. Bars indicate standard errors.

parasites, an increase in the host's immune response with increasing number of parasites, or intrinsic mortality of the parasites. We calculated the expected number of disappeared parasites for each category of intensity of infection for the case in which every parasite had the same chance of disappearing; i.e. when disappearance was due to intrinsic mortality of the parasites (Fig. 4). When competition between parasites plays a role or when copepods with more parasites would mount a stronger immune response, one would expect the observed disappearance to

Table 4. The disappearance of *Schistocephalus solidus* parasites throughout the infection in the copepod *Macrocyclops albidus*, modelled with a logit model and tested with Likelihood-Ratio-Tests for the effect of sex, stage and relative size (corrected for stage and sex) of the copepod at the start of the experiment and the effect of the first or second round of the experiment and the effect of parasite sib-group and intensity of infection on day 1 p.i. ($n = 87$, only copepods without missing values were included)

Factor	χ^2	D.F.	P
Sex	1·36	1	0·2441
Stage	0·96	1	0·3283
Size	0·41	1	0·5240
Round	1·77	1	0·1834
Sib-group	2·79	3	0·4257
Intensity day 1 p.i.	4·69	1	0·0303

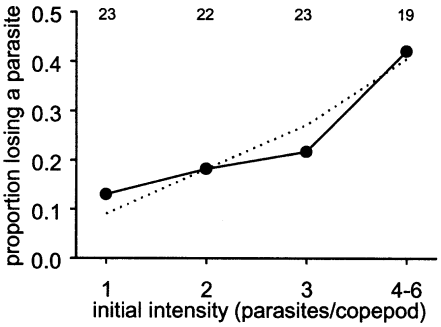


Fig. 4. Observed (—●—) and expected (·····) proportion of *Macrocyclops albidus* copepods that lost *Schistocephalus solidus* parasite during the course of infection for groups of copepods differing in initial intensity of infection. The expected values were calculated by assuming equal probability of a parasite being lost across intensities. Sample sizes are indicated in the figure.

increase faster with increasing intensity of infection than the expected disappearance. The observed disappearance of parasites resembles the expected

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distribution very closely (G-test for goodness of fit: $G = 0{\cdot}69$, D.F. $= 3$, $P = 0{\cdot}88$). The parasites seem to have a 10% intrinsic mortality during the course of the 11 days of infection, while we found no evidence for competition among parasites or increasing immune response with increasing intensity of infection to influence disappearance of parasites.

DISCUSSION

As expected, we found that the outcome of the infection differed among parasite sib-groups and was related to various characteristics of the copepods. We found no evidence for elimination of parasites during the growth phase of the parasite.

It seems likely that genetic differences between the sib-groups have played a role during the establishment phase of the parasite, which is already suggested by Wedekind & Rüetschi (2000), who showed that genetic homogeneity of a group of parasites within a single copepod affects the outcome of the infection. However, due to the small number of sib-groups we were unable to relate the outcome of infection to characteristics of the parent parasites.

We showed that male copepods were more often infected than females, and that C4 copepodites were more often infected than C5 copepodites. We also showed that small individuals performed poorly: they were more often infected, tended to have more parasites and were more likely to die compared to large individuals. Such size effects on prevalence were not found in previous studies on *Macrocyclops albidus* infected by *Schistocephalus solidus* (Wedekind, 1997, Wedekind & Jakobsen, 1998). A study on the copepod *Cyclops strenuus* infected by *Diphyllobothrium* parasites did not reveal any size effects either (Pasternak, Huntingford & Crompton, 1995). Contrary to these studies, we corrected size for stage and sex. However, overall size effects should have been even stronger than the relative size effects found in our study, because the categories of copepods with the (absolute) smallest body size (males and C4 copepodites) also had the highest prevalences. A more plausible reason for the difference in results may be that in the other studies most individuals were adults (Wedekind, 1997, Wedekind & Jakobsen, 1998), while we only used copepodites. Size effects may be less prominent among adults than among juveniles, because selection may already have acted against small individuals by the time they reach adulthood (for example, van der Jeugd & Larsson, 1998).

Relatively small individuals seem to be of inferior quality with respect to parasite resistance and survival. The effect of size on survival was stronger in males than in females, and one could therefore expect selection to act more strongly on body size in males than in females. Yet, males are much smaller than females. This might indicate that different selective forces act on males and females when it comes to reproduction. Large females seem to have a reproductive advantage over small females (Wedekind, 1997), but for males this may be the opposite. Males develop fast and mature at a small size. A possible advantage of such a strategy in males may be that by maturing before the females they maximize the number of females they can mate with. However, when generations overlap, as in *M. albidus* (Einsle, 1993; Laybourne-Parry, Abdullahi & Tinson, 1987) the importance of this is not clear.

Under natural circumstances, the overall survival advantage of females over males may be decreased considerably through increased predation risk of egg-bearing females (Hairston, Walton & Li, 1983; Svensson, 1992, 1997; Maier *et al*. 2000), although this may partly be counteracted by poorer escape abilities of males (Svensson 1992).

As yet, very little is known about sexual selection and reproductive strategies in male *M. albidus*. This lack of information makes it difficult to explain the difference in prevalence of infection between males and females (but see Wedekind & Jakobsen, 1998). Sex differences in susceptibility have been explained by the suppressive effect of androgenic hormones, like testosterone, on several immune functions (Folstad & Karter, 1992, Lindström *et al*. 2001). However, instead of being an immunosuppressive substance, testosterone may be a regulatory substance, mediating an optimal resource allocation between immune function and reproductive investment (Wedekind & Folstad, 1994). Similar regulatory systems could be at work in invertebrates, which do not produce testosterone. Also, behavioural trade-offs may result in sex differences in prevalence and intensity of infections (for example, McKean & Nunney, 2001). If males cover large distances in order to search for receptive females, or spend energy on finding and attracting mates at the expense of their immune function, one may expect higher parasite prevalence and intensity in males than in females (Zuk & McKean, 1996, but see Sheridan *et al*. 2000). In this experiment it is plausible to assume that males and females encountered the parasites at the same rate, due to the restricted space available, and males not being sexually active yet. This would suggest that males and females make different trade-off decisions concerning how many resources to allocate to their immune defence.

Disappearance of parasites seemed not to depend on competition between parasites or increased immune response of the host with increasing number of parasites. However, we were not able to significantly relate disappearance of parasites to the parasite sib-groups or characteristics of the copepods. Especially for effects of sib-groups, the power of our analysis was not particularly high.

If loss of parasites from the haemocoel does not correlate with host characteristics but is simply due

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to intrinsic mortality of the parasites; this implies that a potential influence of the host on the outcome of exposure to this infection is restricted to a short period of ingestion and penetration of the gut wall. Ingestion has to take place within a few hours after hatching of the coracidia (Dubinina, 1966, personal observations), but it is not quite clear how much time is available for penetration of the gut wall. Procercoids can be observed in the body-cavity already a few hours after ingestion of coracidia which, incidentally, can be retained in the hindgut for up to 3 days (personal observations). However, whether these retained parasites are still capable of penetration is unclear. If they are, this may explain the tendency for a higher prevalence when screening under UV light. In vertebrates, it has been shown that UV light can suppress the immune function (Kripke *et al*. 1992). When copepods have to re-allocate resources to repairing damage caused by the UV light, they may have fewer resources available for an immune response, making it possible for retained parasites to penetrate after the first day of screening. We observed an increase in parasites in the haemocoel from days 1 to 3 in 13 % of the cases. However, to what extent this is due to under-estimation of the number of parasites on the first day or to delayed penetration is not clear.

Since this parasite has serious fitness consequences for its host, namely increased predation risk (Wedekind & Milinski, 1996), one can expect selection to favour a defence of the copepod against this parasite. It may seem surprising that we never clearly observed any encapsulation of the parasites in the body cavity, which is a very common defence mechanism observed in many invertebrates (for example, Kraaijeveld, Emmett & Godfray, 1997, Siva-Jothy, 2000). Copepods seem to be unable to eliminate the parasite once it is in the haemocoel. This might be due to the fact that selection has acted on the parasite to overcome immune attack, for example, by camouflaging itself or suppressing the immune functions of the host (Loker, 1994). However, it should be kept in mind that the immune system of the copepods could have been impaired due to the UV light used in this study, and elimination of parasites from the haemocoel could therefore be more important under more natural conditions. Even so, we think that the major lines of defence of the host lie in the early stages of infection. They could avoid ingestion, i.e. by selective foraging, or prevent the parasite from entering the haemocoel. The peritrophic membrane, a chitinous layer surrounding the gut contents, might constitute a shield against penetrating parasites (Yoshikoshi & Ko, 1988; Peters, 1992). Furthermore, immunological elimination of the parasite by encapsulation (see Thomas, Guldner & Renaud, 2000) or some other mechanism (Gillespie *et al*. 1997) might occur early when a parasite tries to penetrate the gut wall. We sometimes observed brown spots along the gut surface, which might contain encapsulated parasites (*cf*. Dimopoulos *et al*. 2001 and Shahabuddin & Costero, 2001 for *Plasmodium* parasites in *Anopheles* mosquitoes).

In conclusion, the outcome of the infection of *M. albidus* with *S. solidus* is affected by the phenotypic quality of the copepods as well as probably the genetic background of the parasites. Our results suggest that copepods cannot eliminate the parasite during the growth phase of the parasite, i.e. once it has entered the haemocoel and the outcome of the infection is therefore probably determined in the establishment phase; in a short time-period of ingestion of the parasite and its penetration of the host's gut wall.

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