Pathogenicity of the hymenolepidid cestode *Microsomacanthus hopkinsi* in its intermediate host, *Hyalella azteca*: implications for transmission, host fitness, and host populations

T. Kokkotis and J.D. McLaughlin

Abstract: Infection by larval parasites can have severe consequences on intermediate hosts that affect transmission, fecundity and fitness of the host, and host population structure. This study examines the pathogenic effects of cysticercoid larvae of the hymenolepidid cestode *Microsomacanthus hopkinsi* (Schiller, 1951) on its amphipod intermediate host, *Hyalella azteca* Saussure, 1858. There was a significant, positive relationship between oncosphere consumption, cysticercoid burden, and age in short-term experiments in which groups of *H. azteca* were exposed individually to single egg packets of *M. hopkinsi* during instars 1, 2, 3, 4, 6, 8, and 9; however, there was no correlation between oncosphere consumption and the intensity of infection in the amphipod hosts within each instar. The mean number of moults over a 14 day experimental period was significantly less in infected amphipods than in their respective controls. In short-term experiments, the greatest mortality appeared to be limited to amphipods exposed during the earliest instars; little mortality was observed in amphipods exposed during instar 4 or later. Long-term experiments revealed a significant negative effect of infection on the overall life span of both male and female *H. azteca* exposed individually to a single egg packet during instar 4. Of 72 females infected during instar 4 and provided with mates during instar 6, only 1 and 4 produced broods in instars 8 and 9, respectively, compared with 58 and 57 of 72 control females. Broods produced by infected females were significantly smaller than those of control females. Infected individuals were less likely to mate successfully. The results are discussed in terms of their consequences for transmission, host fitness, and potential effects on host populations.

Résumé : L'infection par des larves parasites peut avoir des conséquences sérieuses sur les hôtes intermédiaires qui affectent la transmission, la fécondité et fitness de l'hôte et la structure démographique de la population hôte. Notre étude examine les effets pathogènes des larves cysticercoïdes du cestode hyménolépididé *Microsomacanthus hopkinsi* (Schiller, 1951) sur son hôte intermédiaire, l'amphipode *Hyallela azteca* Saussure, 1858. Il y a une relation significative positive entre la consommation d'oncosphères, la charge de cysticercoïdes et l'âge dans des expériences à court terme où des groupes d'*H. azteca* sont exposés un à un à des capsules individuelles d'oeufs de *M. hopkinsi* durant leurs stades 1, 2, 3, 4, 6, 8 et 9; il n'y a pas, cependant, de corrélation entre la consommation d'oncosphères et l'intensité de l'infection chez les amphipodes hôtes au cours de chacun des stades. Le nombre moyen de mues faites par les amphipodes infectés durant la période expérimentale de 14 jours est significativement inférieur à celui des amphipodes témoins correspondants. Dans les expériences courtes, la mortalité la plus forte semble être restreinte aux amphipodes exposés durant leurs tout premiers stades; on observe peu de mortalité chez les amphipodes exposés au stade 4 ou à des stades plus avancés. Les expériences de longue durée indiquent qu'une exposition de mâles et de femelles d'*H. azteca* au stade 4 à une seule capsule d'oeufs a un effet négatif significatif sur leur longévité globale. Des femelles infectées au stade 4 ont été accouplées au stade 6: de celles-ci, une seule de 72 femelles a produit une portée au stade 8 et quatre de 72 femelles au stade 9; par comparaison, respectivement 58 et 57 des 72 femelles témoins ont produit des portées. Les portées des femelles infectées sont significativement plus petites que celles des femelles témoins. Les individus infectés sont moins susceptibles de s'accoupler. Les conséquences de ces résultats sur la transmission et sur la fitness des hôtes ainsi que les effets sur les populations d'hôtes font l'objet de discussions.

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T. Kokkotis[1] and J.D. McLaughlin.[2] Department of Biology, Concordia University, 7141 Sherbrooke Street W, Montréal, QC H4B 1R6, Canada.

[1]Present address: ICON Clinical Research (Canada) Inc., 7405 Transcanada Hwy, Suite 300, Saint-Laurent, QC H4T 1Z2, Canada.
[2]Corresponding author (e-mail: mcljd@alcor.concordia.ca).

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Introduction

Parasitic infections are costly to hosts and are particularly severe in intermediate hosts where transmission to the definitive host occurs by way of predation (Ewald 1995; Poulin 1998). In addition to alteration of host appearance and manipulation of host behaviour, the effects of parasitism on intermediate hosts may be manifested in several ways. These may include, but are not limited to, a shortened life span, adverse effects on growth, and reduced reproductive success.

Freeman (1983) provided an extensive summary of the pathogenesis of larval cestode infections in invertebrate hosts. He noted that little was known about the pathogenicity of metacestode–invertebrate interactions, and it is evident from his review that much of the available information consisted of incidental observations made during the course of other studies. Since then, a number of studies have examined various aspects of the host–parasite interactions in copepod–pseudophyllidean systems (e.g., Rosen and Dick 1983; Shostak and Dick 1986; Dupont and Gabrion 1987; Nie and Kennedy 1993; Pasternak et al. 1995; Wedekind 1997; Wedekind and Rüetschi 2000; van der Veen and Kurtz 2002) and between insects and cyclophyllidean larvae, particularly in the areas of genetics of host susceptibility (Yan and Norman 1995; Zhong et al. 2003), reproductive manipulation of insect hosts (see Hurd 1990, 1998, 2001), and competition (Yan et al. 1998). Much of the cyclophyllidean work has focussed on interactions between *Hymenolepis diminuta* (Rudolphi, 1819) and beetles, however, and comparatively little is known regarding the effects of species whose larval stages infect crustaceans.

Crustaceans serve as intermediate hosts in several orders of cestodes. The cestode larvae typically develop in the haemocoel, where they obtain the resources necessary for growth and development to the cysticercoid stage and for maintenance thereafter. Their biomass is large relative to the size of the host and infections may have a significant effect on the host (Holmes and Zohar 1990). In many cases multiple larvae may be present, further increasing the demands on the host.

Copepods are among the most common crustacean hosts for cestodes, but amphipods also serve as intermediate hosts for a number of species including those belonging to the genera *Cyathocephalus* and *Diplocotyle* (= *Bothrimonus*) (Spathebothriidea), species belonging to several genera of Cyclophyllidea including, among others, *Microsomacanthus*, *Cornacanthus*, *Vaucherilepis*, and *Triodontolepis* (Hymenolepididae), and species of *Anomotaenia* and *Lateriporus* (Dilepididae) (e.g., Denny 1969; McDonald 1969; Podesta and Holmes 1970; Yalynskaya 1980; Valkounova 1985; Tkach et al. 2003). In some cases, amphipods are the only intermediate hosts for a particular species (e.g., Denny 1969; McDonald 1969; Podesta and Holmes 1970); in other cases they may be only one of several types of crustaceans that can serve as intermediate hosts (e.g., McDonald 1969). Amphipod biology has been well studied, yet virtually nothing is known regarding the effect of larval cestode infections on them (see Freeman 1983).

Microsomacanthus hopkinsi (Schiller, 1951) is a small hymenolepidid cestode that infects the caecum and lower large intestine of various dabbling and diving ducks (McDonald 1969; Podesta and Holmes 1970). It belongs to a group of small species within the genus that release the contents of each gravid proglottid as a single egg packet rather than individual eggs. Amphipods serve as the intermediate hosts of these species (e.g., Jarecka 1961; Denny 1969; McLaughlin and Burt 1970; Podesta and Holmes 1970) because the packets are too large for entomostracans to eat (Jarecka 1961). *Hyalella azteca* Saussure, 1858 is the only known intermediate host of *M. hopkinsi* (McLaughlin and Burt 1970; Podesta and Holmes 1970). The number of oncospheres per packet varies (mean = 59 ± 12 in this study), multiple infections are the norm, and heavy infections have been reported in naturally and experimentally infected amphipods (250 and up to 445 cysticercoids, respectively) (Podesta and Holmes 1970).

The *H. azteca* – *M. hopkinsi* system is particularly amenable to experimental studies of host–parasite relations. The egg packets occur in the form of a single string (McLaughlin and Burt 1970) that can be collected easily from caecal droppings (Lee et al. 1992). The number of oncospheres can be counted accurately, allowing a precise estimate of the infective dose. *Hyallella azteca* is easy to culture (de March 1981), and the first nine instars can be distinguished on the basis of head capsule length (Kokkotis and McLaughlin 2002).

In this study we examined the consequences of infection by *M. hopkinsi* on *H. azteca* in terms of their potential impact on transmission, their effects on host fitness, and their potential role in regulation of the host population. We studied the susceptibility of *H. azteca* exposed to *M. hopkinsi* egg packets during each of seven different instars (instars 1, 2, 3, 4, 6, 8, 9) and the effects of infection on the short-term growth and survival of these amphipods. We also examined the effect of the parasite on the long-term survival and reproductive success of individuals infected during the fourth instar (i.e., prior to sexual maturity). We demonstrate that *M. hopkinsi* is pathogenic to *H. azteca* and that infections have negative effects on growth, long-term survival, and reproductive success of the amphipod host.

Materials and methods

Experimental animals

Stock cultures of *H. azteca* were descended from individuals collected in the Delta Marsh, Manitoba, Canada, and were supplemented annually with additional specimens from the Marsh. The cultures were maintained in dechlorinated (culture) water in 23 cm × 36 cm × 10 cm plastic containers. The containers were kept on a specially designed multilevel rack similar to that described by de March (1981). Each level had a bank of 40 W wide-spectrum fluorescent lights controlled by a timer. Each plastic tank contained a small mat of filamentous green algae or a plastic-mesh scouring pad as a breeding substrate. Stock cultures were kept at 23 °C in a photoperiod of 14 h light : 10 h dark and fed TetraMin® ad lib. All experiments were run under these temperature and photoperiod conditions.

Two-day-old domestic ducklings (*Anas platyrhynchos* L., 1758), purchased from Brome Lake Duck Farms, Knowlton,

Quebec, were used as avian hosts. They were kept in the waterfowl holding facilities at Concordia University and were provided with food (18% laying feed, Nutribec Ltd., Montréal, Quebec) and water ad lib. All maintenance and manipulation of experimental animals was performed within the guidelines set forth by the Canadian Council on Animal Care.

Egg packets of *M. hopkinsi* were collected from the caecal contents of black ducks (*Anas rubripes* Brewster, 1902) obtained from hunters near Fredericton, New Brunswick, Canada. About 30 egg packets and 50 amphipods were placed in each of six 10 cm dishes containing culture water for 48 h. The amphipods were then transferred to a plastic container and maintained as described above. At 14 days post exposure (p.e.), each amphipod was placed on a depression slide in a small drop of water, immobilized with a cover glass, and examined with a microscope for cysticercoids. Infected individuals were fed to the ducklings, which then served as the source of egg packets for the experiments. Groups of ducklings were infected at 2- to 3-month intervals to ensure a constant supply of egg packets throughout the study.

Beginning at 11 days p.e., ducklings were placed in wire-bottomed cages and their droppings were collected over periods of up to 4 h in trays lined with moistened paper towels. Caecal droppings (visually distinct from regular droppings) were dissolved in water and filtered through a 150 μm sieve. The sieve was backwashed and egg packets were collected from the sediment under low-power magnification and stored at 5–7 °C in 20 mL vials full of culture water. Although packets can survive for up to 12 weeks at 5–7 °C with no loss of infectivity (Lee et al. 1992), only packets stored for no more than 8 weeks were used in this study.

General procedures

Because amphipods in this study were to be infected during specific instars, the first requirement was to establish the growth curve for the population. Details of this study can be found in Kokkotis and McLaughlin (2002). Briefly, head capsule and body lengths were obtained from amphipods reared from neonate to adult in individual containers. Head capsule length was found to be the better means of determining the instar and was used to determine the stage of each individual when it was exposed (or selected as a control) and, where applicable, at the conclusion of the experiment.

Mortality of intermediate hosts experimentally infected with cestode larvae is well documented. This is generally the result of overexposure to eggs (Freeman 1983; Wedekind 1997) and the resulting trauma due to penetration of the gut and (or) excessive numbers of larvae in the haemocoel (Freeman 1983). Although amphipods commonly support heavy infections (Podesta and Holmes 1970), we restricted exposure to a single egg packet to avoid overexposure, particularly in early instars.

Experimental exposure of amphipods was accomplished by placing one egg packet and one amphipod in each well of a 24-well tissue culture plate that had been filled with culture water. Following exposure (24 h), each amphipod was transferred to a 10 cm × 3 cm plastic vial (Carolina Biological Supply Company, Burlington, North Carolina) full of culture water for the duration of the experiment. Each vial contained a small tuft of filamentous green algae for oxygen and substrate. Amphipods were fed TetraMin® as required and water levels were replenished when necessary. Time zero for each experiment began when the amphipods were transferred from the tissue culture plate.

Experiment 1. Susceptibility, growth, and short-term survival

Individuals in instars 1, 2, 3, 4, 6, 8, and 9 were used in this experiment. Sexes can be distinguished beginning in the fifth instar, so three plates of 24 amphipods each were used for instars 1 to 4 and six plates of 24 amphipods each were used for instars 6, 8, and 9 (three plates of males and three plates of females). Each plate contained individuals exposed on the same day, and exposures were done when sufficient individuals of a particular instar were available to set up both an experimental and a control plate. The oncospheres in each egg packet were counted, and each packet was placed in a well with a single amphipod. Exposure lasted for 24 h. The well was searched for remnants of the egg packet and, where necessary, the number of oncospheres that remained was subtracted from the number placed in the well to give the exposure dose. The amphipod was then removed and placed in an individually numbered vial for the duration of the experiment. An equal number of controls for each instar and (or) sex category were sham-exposed by feeding them egg packets that had been heat-killed at 55 °C. None of the control amphipods became infected. Survival was monitored daily for the first week, when most of the cysticercoid development occurred and most of the mortality was expected, and then on days 9, 12, and 14, when the experiment was terminated. Amphipods that died during the course of the experiment and those that survived were examined for cysticercoids. Unfortunately, the small size of developing larvae and the rapid decomposition of the hosts made detection of infections in individuals that died before Day 6 virtually impossible; however, infections could be detected in individuals that died later. Head capsules of amphipods that survived the full 14 days were measured and susceptibility, infection success (number of infected individuals and intensity of the cysticercoid load), survival, and the number of moults (determined indirectly by head capsule length) were recorded.

Experiment 2. Long-term survival of infected *H. azteca*

Amphipods in the fourth instar were used for this experiment because this was the youngest instar in which short-term survival of infected individuals did not differ significantly from that of control individuals as determined in experiment 1. Amphipods were separated into experimental and control groups. Exposure was accomplished as described for experiment 1, and each amphipod was kept in an individually numbered vial. Controls were sham-exposed. At 6 days p.e., when the amphipods had moulted to the fifth instar, the amphipods were separated by sex. They were examined on Day 10 for cysticercoids. Infected amphipods (three groups of 24 males and 3 groups of 24 females) were selected along with a comparable number of male and female controls. Each individual was held in a separate vial as described above and survival was monitored at 2 day intervals. The experiment was terminated at 56 days p.e. At this time, only 3

Table 1. Percentage of *Hyalella azteca* in each instar that consumed some or all (<50%, ≥50% but <100%, 100%) of the *Microsomacanthus hopkinsi* egg packet offered during experiment 1.

		Percentage of *H. azteca*		
Instar	Sample size (*n*)	<50%	≥50% but <100%	100%
1	72	20.8	18.1	62.1
2	72	5.6	22.2	72.2
3	72	5.6	11.1	83.3
4	72	0	2.8	97.2
6	144	0	0.7	99.3
8	144	0	0.7	99.3
9	144	0	0	100

of 144 infected amphipods were still alive, and these had survived the rest by 2 weeks.

Experiment 3. Effects of infection on reproductive success

Fourth-instar amphipods were exposed individually in 24-well tissue culture plates, pooled after exposure, and held for 12 days before being sexed and examined for cysticercoids. Seventy-two infected females and 72 unexposed females (three groups of 24 amphipods each) from the stock cultures (all in instar 6) were placed in individual vials. Amplexus consists of precopular carrying of females by males and occurs just before the females moult. A male that had been in natural amplexus in the stock culture was added to each vial. Mating occurs as the female moults to the next instar; hence, mating in this study occurred when females entered instar 7. Vials were monitored at 2 day intervals and the number of offspring produced by each female during her eighth or ninth instar was recorded (in *H. azteca* the brood from the previous mating is released when the female moults, hence a mating in the seventh instar results in release of the brood when the female moults into the eighth instar). Broods were removed from the vial and counted. The female's head capsule was measured and she was returned to her mate.

Experiment 4. Effects of infection on mate selection

Fourth-instar amphipods were exposed individually as described above and held until instar 6, when they were sexed. Ten infected and 10 uninfected males (instars 8 and 9) were placed in a 19.5 cm culture dish containing 350 mL of culture water. Ten uninfected females in instar 8 that had been in natural amplexus with males from the stock culture were added. Trials lasted 6 h and observations were made at 10 min intervals. When amplexus occurred, the pair was removed and the infection status of the test individual was determined. Each trial was repeated three times using different individuals. The experiment was repeated a second time using infected and control female amphipods in their eighth or ninth instar and males from the stock cultures that had been in natural amplexus.

Statistical analysis

Preliminary analyses of the data revealed that the replicates for each instar or group were homogeneous. Data were log-transformed before analysis where necessary, but untransformed data are presented in figures. Subsequent analyses included least squares regression analysis, Fisher's exact test, ANOVA, and MANOVA. Duncan's multiple range test was used for a posteriori comparisons following ANOVA. Probabilities < 0.05 were considered significant.

Table 2. Mortality of *H. azteca* exposed to *M. hopkinsi* during each instar and the percentage of deaths that had occurred by Day 4 and Day 7 post exposure.

Instar	Sample size (*n*)	Total no. of deaths	% of deaths by Day 4	% of deaths by Day 7
1	72	39	85	94
2	72	31	61	77
3	72	19	37	78
4	72	8	35	37
6	144	22	45	59
8	144	29	31	72
9	144	26	15	57

Results

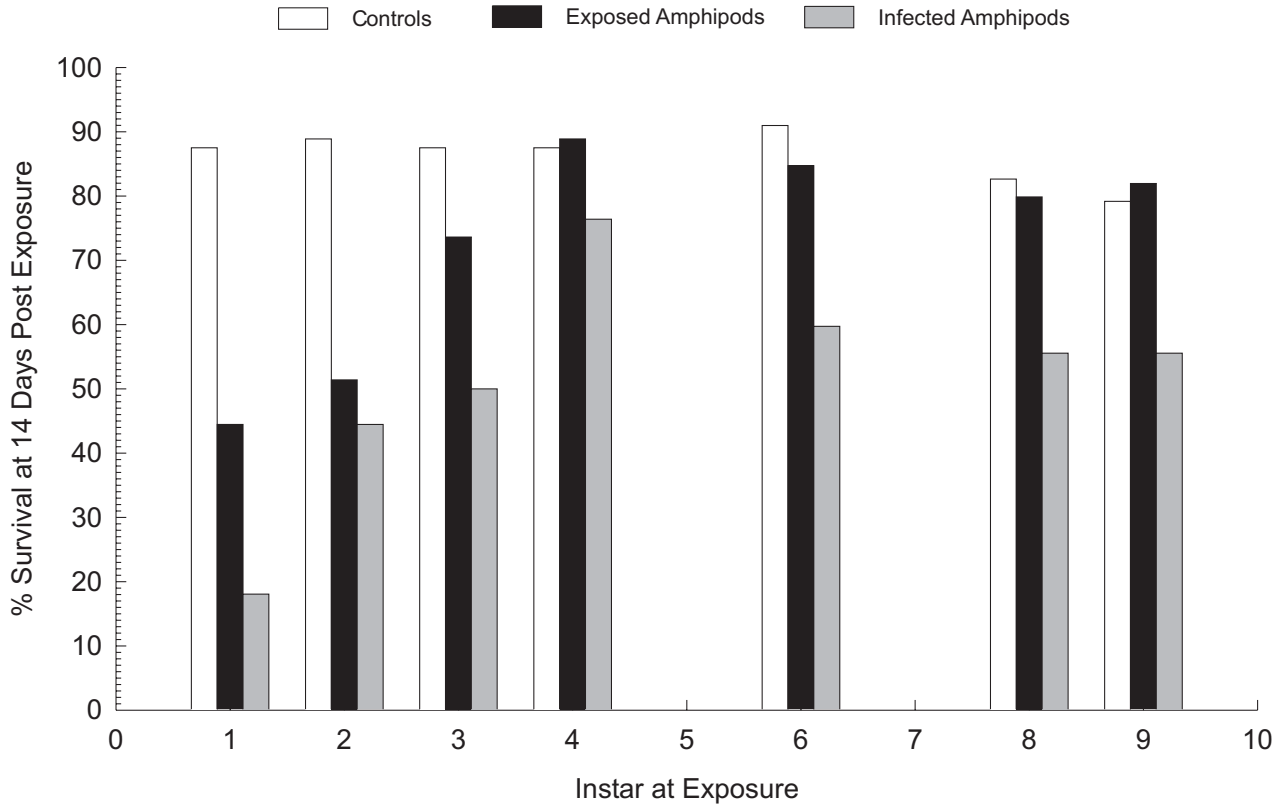
Experiment 1. Susceptibility, growth, and short-term survival

The egg packets fed to amphipods in this experiment contained an average of 59 ± 12 oncospheres. There was no difference in the mean number of oncospheres fed to replicate groups within each instar or between different instars ($F_{[1,718]} = 0.02$; $p = 0.887$). The proportions of amphipods that ate <50%, ≥50% but <100%, and 100% of the packet offered to them are shown in Table 1. Only 62% of amphipods exposed in instar 1 ate the entire packet. The majority of those exposed during instars 2 and 3 ate the entire packet, as did virtually all of the amphipods exposed during the later instars.

There was a significant, positive correlation between the instar at exposure and the number of oncospheres ingested over the 24 h period. Larger amphipods ate, on average, more oncospheres than smaller ones ($F_{[1,718]} = 38.93$; $p < 0.001$), but size (instar number) accounted for little of the variation ($r^2 = 0.050$) found. Amphipods exposed during instar 1 ate significantly fewer oncospheres than those exposed during any other instar, and those exposed during instar 2 ate significantly more than those exposed during instar 1 but significantly fewer than those exposed during instar 8 or 9. There was no difference in the mean number of oncospheres eaten by amphipods exposed during instars 3 to 9, nor was there a difference in the mean number of oncospheres eaten by males and females in instars 6, 8, and 9. Accordingly, the data for males and females in each instar were pooled prior to analysis.

The mortality seen in each instar during the first week p.e. is summarized in Table 2. Mortality was heaviest in amphipods exposed during instars 1 to 3 and was most pronounced during the first week of the infection. Over 75% of the deaths in these three groups had occurred by Day 7 p.e. and, in instars 1 and 2, most of those had already occurred by Day 4. Overall, fewer mortalities were seen among older amphipods and, except for instar 7, roughly half of the total mortality occurred in the first week.

Fig. 1. Comparison of the overall percent survival of unexposed (control), exposed but not infected, and exposed and infected *Hyalella azteca* 14 days after exposure to *Microsomacanthus hopkinsi* during instars 1, 2, 3, 4, 6, 8, and 9. Note that infected individuals are a subset of the exposed group. Sample sizes are as follows: instars 1–4, n = 72; instars 6, 8, and 9, n = 144 (data from 72 male and 72 female *H. azteca*).



The percentages of experimental and control individuals of each instar that survived the 14 day experimental period are shown in Fig. 1. There was a small, but consistent, unexplained mortality among the control individuals, but no difference in mortality was found among control replicates within each instar nor between controls for each instar. The percent survival of amphipods exposed in instars 1 to 3 was significantly lower than that of their respective controls (Fisher's exact test; $p < 0.001$, < 0.001, and < 0.5, respectively). No such differences in survivorship were noted between experimental groups exposed during later instars and their respective controls. The percentage of exposed amphipods in each instar that became infected and that survived the full 14 days is also presented in Fig. 1. There was a significant, positive correlation between the instar during which exposure occurred and the average number of amphipods that survived ($F_{[1,28]} = 11.91$; $p < 0.002$; $r^2 = 0.273$). Among infected amphipods, the average number of survivors was significantly lower within the group exposed during instar 1 than within all other groups except amphipods exposed during instar 2, and it was lower in the group exposed during instar 2 than in the group exposed during instar 4. No difference in the mean number of infected survivors was found among the other groups, nor was any difference in survivorship found between males and females exposed during instars 6, 8, and 9.

Comparison of the mean number of moults that occurred over the 14 day period in controls and amphipods exposed during instars 1, 2, 3, 4, and 6 revealed that infected individuals underwent fewer moults than their respective controls ($t_{[30]} = 3.72$; $p = 0.0227$) (Fig. 2); i.e., there was an increase in the length of the intermoult period. Because instars could be reliably identified by head capsule measurements only to the tenth instar, no attempt was made to study amphipods exposed during instar 8 or 9.

The mean intensity of cysticercoids in amphipods that survived the 14 day period is shown in Fig. 3. There was a significant, positive correlation between the instar during which exposure occurred and the number of cysticercoids found in infected individuals ($F_{[1,386]} = 70.5$; $p < 0.001$; $r^2 = 0.152$). With the exception of amphipods exposed during instar 6, the mean number of cysticercoids present increased progressively in amphipods infected during later instars. Amphipods exposed during instar 1 had, on average, significantly fewer cysticercoids than those exposed during instars 2 to 6. Individuals exposed during instar 8 or 9 had significantly larger cysticercoid populations than the rest. No difference was found in the mean number of cysticercoids present between males and females in instars 6, 8, and 9, so the data for each instar were pooled prior to analysis. There was no correlation between the number of oncospheres eaten by amphipods of a particular instar and the number of cysticercoids that developed.

Experiment 2. Long-term survival of infected *H. azteca*

Effects of infection on long-term survival are shown in Fig. 4. A factorial two-way MANOVA used to assess the effects of infection and host sex on long-term survival of in-

Fig. 2. Mean (+1 SD) number of moults occurring in control *H. azteca* and infected *H. azteca* exposed to *M. hopkinsi* during instars 1, 2, 3, 4, and 6.

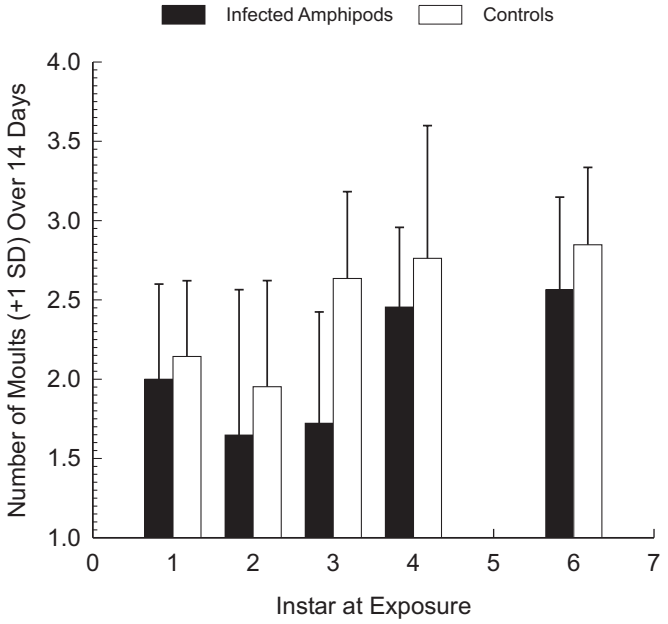
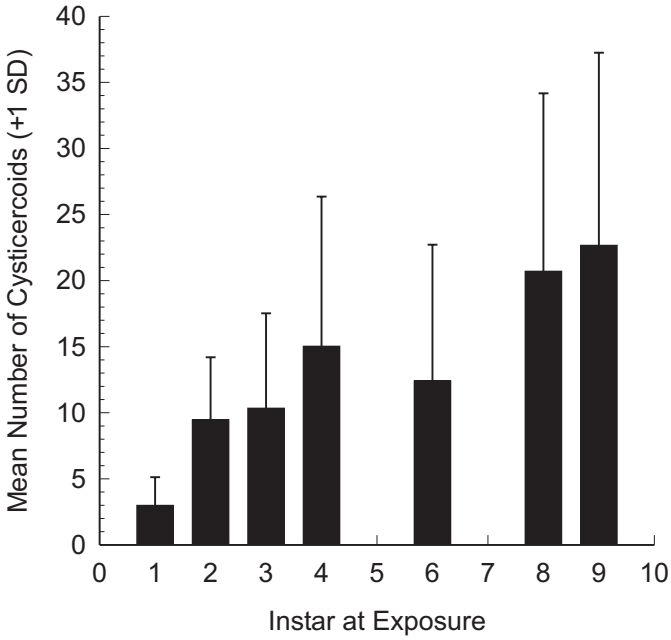


Fig. 3. Mean (+1 SD) intensity of cysticercoids in *H. azteca* exposed to *M. hopkinsi* during instars 1, 2, 3, 4, 6, 8, and 9.



fected amphipods revealed that sex had no significant effect on the mean survival time or the mean number of amphipods that survived the experiment. Accordingly, a one-way MANOVA was performed, which revealed that infection had a significant negative effect on both mean survival time and the number of hosts that survived to the end of the experiment (Wilks' lambda; $\lambda_{[2,9]} = 0.066$; $F_{[2,9]} = 63.18$; $p < 0.001$). Individual ANOVAs confirmed the negative effect of infection on mean survival time ($F_{[1,10]} = 85.07$; $p < 0.001$) and on the number of individuals that survived the 8 week period ($F_{[1,10]} = 63.23$; $p < 0.001$).

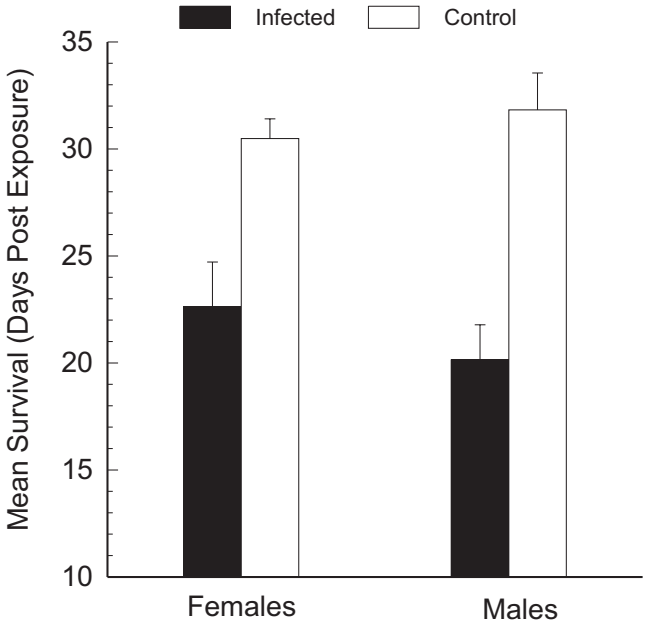
Experiment 3. Effects of infection on reproductive success

Infection had a significant effect on brood production ($F_{[1,142]} = 298.51$; $p < 0.001$). There was no density-dependent effect on fecundity: neither heavily infected nor lightly infected females reproduced successfully. Only 1 of 72 (1.3%) females infected during instar 4 and mated during instar 7 produced a brood in instar 8, compared with 58 of 72 (80.5%) females in the control group. Four (5.5%) of the 72 infected females produced broods during instar 9, whereas 57 in the control group did so. Only 1 infected female produced a brood in both instars 8 and 9, whereas 51 of the control females did so. The average size of broods produced by infected females in instar 9 (6.3 ± 1.4) was significantly smaller than that of broods produced by control females (11.2 ± 3.3) ($F_{[1,142]} = 298.51$; $p < 0.001$).

Experiment 4. Effects of infection on mate selection

In the three trials in which 10 uninfected males were placed with 10 infected and 10 uninfected females, only seven pairings were observed. In all cases, the male paired with an uninfected female. Eleven pairings were observed in the three trials in which 10 uninfected females were placed with 10 infected and 10 uninfected males. Nine of these pairings involved uninfected males. The two infected males that paired had 6 and 26 cysticercoids, respectively.

Fig. 4. Mean (+1 SD) survival times of male and female *H. azteca* exposed to *M. hopkinsi* during instar 4, compared with their respective controls. The experiment was terminated 56 days post exposure.



Discussion

Our results demonstrate that *M. hopkinsi* is highly pathogenic to *H. azteca* and has severe implications for the overall fitness of the amphipod host, at least under laboratory condi-

tions. These effects are consistent with Ewald's (1995) prediction that intermediate hosts that transmit parasites to mobile definitive hosts via predation should be severely exploited by those parasites. Infection appeared to affect short-term survival of young, heavily infected hosts, affected moulting, and reduced both life span and fecundity of female amphipods infected prior to sexual maturity. Early mortality of young amphipods and the generally reduced life span of infected hosts have potential effects on transmission, whereas the effects on survival, growth, and reproduction represent significant fitness costs to the individual host that may affect the population dynamics of the host and, indirectly, community structure (Minchella and Scott 1991; Thomas et al. 2000).

All amphipods ate egg packets, and amphipods of all ages became infected. Infection success (i.e., the number of infected individuals surviving exposure and the mean intensity of the resulting infection) was positively related to instar (host size), but few differences were found between instars, partly because of the variation within each instar. Infection levels in males and females were similar within each instar, consistent with the results of Sheridan et al. (2000), who found no evidence of a general sex bias in their extensive review of parasitic infections of arthropods. Little mortality occurred among amphipods exposed during instar 4 or later, but only about 50% of those exposed during instars 1 and 2 survived the duration of experiment 1. The mortality pattern and the low number of cysticercoids present in the survivors suggest that trauma during the initial stages of infection and cysticercoid development (e.g., Lethbridge 1980; Freeman 1983) may have killed the more heavily infected individuals exposed during the earliest instars. All members of the amphipod population are capable of transmitting the parasite, regardless of age or sex; however, the youngest amphipods seemed less likely to eat an entire egg packet encountered in natural conditions, had smaller numbers of cysticercoids, and were less likely to survive long enough for cysticercoids to develop. Thus, older amphipods appear to play the major role in the transmission of *M. hopkinsi*.

This study is among the few where the exact number of oncospheres eaten by the host was known. Some individuals in each group failed to become infected, and there was no correlation between the number of oncospheres eaten and the number of cysticercoids found in each instar during experiment 1. Intraspecific variability has been demonstrated in the susceptibility of beetles to infection by cestode larvae (Yan and Norman 1995; Zhong et al. 2003) and in the infectivity of coracidia to copepods (Wedekind and Rüetschi 2000; van der Veen and Kurtz 2002). In this study, amphipods came from populations created by pooling broods produced on a specific day, and the egg packets were obtained from different hosts. It is therefore reasonable to expect some degree of heterogeneity in susceptibility among hosts and (or) in infectivity among individual oncospheres or entire packets, which could have resulted in the subset of individuals that failed to become infected.

The interactions that determine infection success in copepods occur during the early stages of infection (Wedekind and Rüetschi 2000; van der Veen and Kurtz 2002), and there is no reason to assume that this should differ in amphipods. Within-packet heterogeneity in the viability or infectivity of oncospheres, damage to oncospheres during ingestion, variability in the activation of oncospheres (Lethbridge 1980), and events occurring during penetration of the gut wall or during establishment of the oncospheres in the haemocoel (e.g., Lethbridge 1980; Yan and Norman 1995; Wedekind and Rüetschi 2000; van der Veen and Kurtz 2002) may have further contributed to variability in the numbers of cysticercoids present in survivors.

Infected amphipods moulted less frequently than controls. The effect was consistent in all groups, suggesting a generalized response to the infection. Cysticercoids grow rapidly during the first week, and their metabolic demands on the host would be greatest during this period. We could not determine whether the moulting frequency was affected over the longer term; however, the metabolic demands of the parasites would continue at some level for the duration of the infection. This could continue to affect the growth rate and likely contributed to the shortened life span of infected individuals.

The reduction in life span of the host also reduces the life span of the parasite. Factoring in the 11 days required for cysticercoid development (Podesta and Holmes 1970), the average window for transmission from the amphipod to the duck host is less than 2 weeks. Yet, despite this limited transmission window, *M. hopkinsi* is a common parasite of surface-feeding ducks (*Anas* spp.). In studies where 40 or more individuals of a particular duck species were examined, the prevalence ranged from 16% to 67% (mean = 38%) (Buscher 1965; Kinsella and Forrester 1972; McLaughlin and Burt 1979; Wallace and Pence 1986; Gray et al. 1989; Fedynich and Pence 1994; Fedynich et al. 1996). The life-history traits of *M. hopkinsi* favour rapid completion of the life cycle. In addition to the rapid development of cysticercoids, the worms produce egg packets within 8 days post infection (Lee et al. 1992). Survival of the oncospheres is temperature-dependent. Lee et al. (1992) found that only 5% of surviving *H. azteca* (n = 60; 63% survival) exposed to packets that had been stored at 20 °C for 4 weeks were infected, whereas 93% of survivors (n = 60; 73% survival) exposed to egg packets stored at 7 °C were infected. Water temperatures typically exceed 20 °C in shallow wetlands during the summer, and survival times of oncospheres would decrease proportionately with increased temperature stress. Given the reduced life span of both oncospheres and infected amphipods, it is evident that transmission depends heavily upon constant contamination of wetlands with egg packets and the continual recruitment of cysticercoids in the amphipod populations, rather than the persistence of either infective stage.

Reduction of host fitness is a frequent outcome of parasitic infections. It may be due to pathological effects induced by the infection or it may be the result of a damage-limiting strategy that enables the host to live longer than it would have had it maintained reproduction at the same level as an uninfected host (Hurd 2001). Infection affected the fitness of *H. azteca* by reducing the number of lifetime mating opportunities, mating success, and the overall fecundity of female amphipods. Cyclophyllidean species are known to reduce fecundity of insects (see Hurd 2001) and crustaceans (see Freeman 1983; Amat et al. 1991), but the host life span is either unaffected (Amat et al. 1991) or prolonged (see Hurd et

al. 2001) rather than reduced. That reduced fecundity of females is coupled with a reduced life span in both sexes suggests that these effects are the result of pathological changes in *H. azteca* induced by the parasite. We did not compare ovaries of infected and control amphipods, so the extent and type of damage caused by *M. hopkinsi* is unknown. Other authors (e.g., Stark 1965; Bollache et al. 2002) have reported the absence of ovaries in amphipods infected with *Bothrimonus* spp. and the partial or complete destruction of the ovaries in amphipods infected with *Pomphorhynchus laevis* (Zoega in Müller, 1776) and *Polymorphus minutus* (Goeze, 1782), respectively.

Our limited observations of mating activity in *H. azteca* are consistent with studies of *Gammarus* spp. infected with acanthocephalan (e.g., Zohar and Holmes 1998; Bollache et al. 2001) and digenean larvae (Thomas et al. 1996) that indicate uninfected males are less likely to pair with infected females than with uninfected ones. In our study, a few infected males mated successfully with uninfected females. Zohar and Holmes (1998) reported that infection by acanthocephalans did not prevent spermatogenesis or alter secondary sexual characteristics of male *Gammarus lacustris* Sars, 1864, which is consistent with the notion that infections may be less costly physiologically in males than in females.

Parasites are known to affect host life-history traits (Thomas et al. 2000). Infection by *M. hopkinsi* had strongly negative effects on survival and reproduction of *H. azteca*; however, we found no convincing evidence of an adaptive response to the infection. There was no accelerated reproductive response such as that reported in amphipods by McCurdy et al. (1999), but it is less clear whether a trade-off exists between present and future reproduction. Brood size in *H. azteca* increases with age (de March 1981), and both the number of infected females that produced broods and the mean brood size increased from instar 8 to instar 9. Conceivably, additional females might have produced broods in later instars, and it is likely that their broods would have been larger. In view of the severe time constraint imposed by the shortened life span, however, a greater proportion of females should have produced broods in instar 9 if delayed reproduction represented an adaptive response to infection. It should be stressed that all of our observations of reproductive activities involved amphipods infected during instar 4 (i.e., before sexual maturation occurred). Whether similar effects occur when older, sexually mature individuals become infected is unknown.

Parasites can regulate host populations (Keymer 1981; Scott 1988; Thomas et al. 2000), and effects that occur at the population level may indirectly affect community structure (Minchella and Scott 1991; Thomas et al. 2000). Our results indicate that *M. hopkinsi* has the potential to regulate populations of *H. azteca* by reducing overall reproductive success. The egg packets, however, are concentrated in the caecal droppings, which are passed infrequently (Sturkie 1965), and therefore are not as widely dispersed as eggs passed in regular feces. This would create foci of infection for amphipods and any impact on the amphipod population would be highly localized. *Microsomacanthus hopkinsi* is one of only a few packet-producing species that regularly infect surface-feeding ducks in North America. Amphipods form a small part of the diet of these ducks and are likely eaten by accident. If the risk of infection is low and (or) the effects of infection enhance predation of the amphipod, there would be little selection pressure for reduced levels of exploitation by the parasite. There would also be little selection for adaptive responses of life-history traits to compensate for reduced fecundity in the amphipod host. Such may not be the case for a number of related species of *Microsomacanthus* that infect the intestine of lesser scaup (*Aythya affinis* (Eyton, 1838)). These species also produce egg packets, and the three most common species require *H. azteca* as an intermediate host (Podesta and Holmes 1970). The prevalence of these species is high in lesser scaup (~97%; $n = 45$) and intensities can be as high as thousands of individuals per species per bird (Bush and Holmes 1986). Egg packets of these species are more widely dispersed because the intestine evacuates much more frequently than the caecum. Exposure opportunities for amphipods should be greater, and the higher prevalence expected in amphipod populations is reflected in the infection levels seen in lesser scaup. If these species have the same effects on *H. azteca* that *M. hopkinsi* does, their impact on amphipod populations could be significant. However, closely related cestode species do not necessarily affect the same host in the same way (Schom et al. 1981). A higher risk of infection might result in selection for reduced pathogenicity, particularly when the amphipod host is a major prey item of the final host (Bartonek and Hickey 1969). This may, in part, account for the heavier infections observed by Podesta and Holmes (1970). A higher risk of infection might also result in the selection of alternative host life-history traits that compensate for reduced fitness caused by the parasite. Comparative studies of the effects of selected *Microsomacanthus* species of lesser scaup on *H. azteca* could provide valuable insights into the role of cestode infection in the evolution of host life-history traits and in structuring host populations.

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