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Host-parasite interactions between the cysticercoid larvae of the cestode *Microsomacanthus hopkinsi* and the amphipod intermediate host *Hyalella azteca*

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ABSTRACT

Host-parasite interactions between the cysticercoid larvae of the cestode *Microsomacanthus hopkinsi* and the amphipod intermediate host *Hyalella azteca*

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This study examined host-parasite relations between cysticercoids of *Microsomacanthus hopkinsi* and the amphipod intermediate host *Hyalella azteca* throughout the latter's life history. There was a significant positive relationship in oncosphere consumption, parasite loads, and short-term survival in amphipods exposed during Instars 1, 2, 3, 4, 6, 8 and 9. Amphipods exposed during Instars 1 and 2 ate significantly fewer eggs and over a 14 day period, experienced significantly greater mortality and had significantly lighter infections than later instars. Oncosphere consumption, short-term survival, and intensity of infection were generally similar among older amphipods. No correlation was found between oncosphere consumption and cysticercoid levels in any instar. Infection had no effect on short-term survial in amphipods infected during Instar 4 or later but long-term survial times of amphipods infected during Instar 4 were significantly shorter than that of

controls. Infected amphipods moulted less frequently than controls regardless of the instar at exposure. No differences were found in oncosphere consumption, short- or long-term survival, moult frequency, or intensity of infection among males and females. Infection during Instar 4 (i.e. prior to sexual development) had significant negative effects on reproduction. Only one of 72 females, infected during this instar, produced offspring during Instar 8 and four of 72 produced offspring during Instar 9 compared to 58 and 57 of 72 control females, respectively. In pairing experiments, uninfected male *H. azteca* never paired with infected females. Infected male *H. azteca* paired with uninfected females, but less frequently than uninfected males.

Overall, infections had a significant negative effect on short-term survival of young *H. azteca*, on the long-term survival of *H. azteca* infected during Instar 4, and on the number of moults over a 14 day period in *H. azteca* of all ages. Fecundity was markedly reduced in females infected before sexual maturity and uninfected males did not pair with infected females. The few infected males that paired suggests that they may be at a reproductive disadvantage relative to uninfected males.

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INTRODUCTION

Many parasitic worms (helminths) have indirect life cycles that require at least two hosts for completion. The definitive host, in which the helminth develops to sexual maturity, is typically a vertebrate. The intermediate host, which is infected by eggs or larvae from the definitive host, supports development of the parasite to an infective stage that can be transmitted back to the definitive host. While vertebrates may serve as intermediate hosts, most helminth groups use invertebrates.

The relationship between parasite and host is complex regardless of whether it involves a definitive or an intermediate host. The host is the environment for the parasite, supplying its physical habitat, nutriments, and the cues necessary for establishment, growth, and development. The parasite obtains all its resources from the host, in a constant demand for the energy and nutriments required to support its own biomass.

In three of the major helminth groups, many cestode, acanthocephalan and nematode genera use arthropods as intermediate hosts; crustaceans in aquatic systems, insects in terrestrial ones. The other major group, the digeneans, uses molluscs as the first intermediate host. Many digeneans also require a second intermediate host which, in

some genera, may be an arthropod.

A substantial amount of information exists regarding the relationship between adult helminths and their definitive host, especially those of medical or veterinary importance. The relationships between larval stages and their intermediate host have also been examined, particularly in the life cycles of those species where vertebrates (fish, livestock, or humans) may be affected. However, with the notable exception of digeneans and their effects on molluscan hosts, comparatively little is known about the host-parasite interactions between larval parasites and invertebrate intermediate hosts. No study has ever examined the relationship between a larval cestode and a particular intermediate host throughout the host's life history. It is this gap in our knowledge that this work seeks to address.

LITERATURE REVIEW

Much of the information on host-parasite relationships involving invertebrates and parasite larvae comes from studies on digenean-snail interactions. These have provided numerous examples of morbidity, conspicuousness, gigantism, and parasitic castration (see reviews: Reinhard, 1956; Hurd, 1990). Arthropods serve as second intermediate hosts for many digeneans. They become infected following penetration

of cercariae that quickly encyst to form metacercariae, which persist as encysted forms in the invertebrate host. The relationship between digeneans and arthropods has received much attention recently (e.g. Jensen and Mouritsen, 1992; Thomas *et al.*, 1995; Thomas *et al.*, 1996a; Thomas *et al.*, 1996b). However, except for effects on crustacean reproductive behaviour, digeneans are not considered further because the host supports rapid transformation of one stage to another rather than the full ontogeny of it.

Cestodes, acanthocephalans and many nematodes produce eggs or larvae that must be consumed by an intermediate host. Once ingested, they penetrate the gut wall and migrate to a parenteral site where they develop, over a period of time, into an infective stage that can be passed on to the next host via the food chain. Consequently, the host must support the parasite through an extended developmental process. Because intermediate hosts tend to be small, the eventual biomass ratio of parasite to host can become quite large and a number of detrimental effects can result. These can include: initial trauma associated with penetration of the gut, nutrient depletion, a decreased assimilation efficiency of nutriments, physical damage, and organ malfunction. These effects can culminate in the death of the host in severe cases and, in survivors, can entail additional, more long-term effects, including decreased stamina, altered appearance, altered behaviour, reduced

growth, and decreased reproductive abilities (reviews: Reinhard, 1956; Holmes and Bethel, 1972; Freeman, 1983; Rosen and Dick, 1983; Hurd, 1990).

The effects of larval parasites on arthropods in general, and on crustaceans in particular, have attracted little attention. Studies on arthropods infected with larval parasites have concentrated on several areas, including sex- or age-related differences in susceptibility and parasite loads, host-immune responses, pathology, host survival following infection, host growth and development, reproduction, and behavioural modification and its implications for survival.

Freeman (1983) provided an extensive review of the relationship between larval cestodes and invertebrate intermediate hosts. He found little information on the interaction between cestode larvae and their hosts. Existing information is badly fragmented, often occurring as observations or anecdotal reports included in life cycle studies. In fact, comparatively few studies have focused directly on the relationship between cestode larvae and their intermediate hosts.

Effects of age and sex on infection success:

Among some cestodes, age of the intermediate host at exposure may influence infection success. Calentine (1965a) showed that the caryophyllidean cestode *Biacetabulum* spp.,

which uses tubificid worms rather than a crustacean as the intermediate host, could only infect young *Tubifex* spp.; the parasites die when eaten by older worms. Field observations on *Cyathocephalus* spp. (Spathebothridia) suggest that only young *Gammarus* spp. are susceptible to infection (Wiśniewski, 1932). Copepodid stages of *Cyclops* spp. are highly susceptible to infection by *Schistocephalus* spp. larvae whereas adults are not (Smyth 1963). Nie and Kennedy (1993) found that in *Cyclops vicinus* infected with *Bothriocephalus claviceps* (Pseudophyllidea), young copepods had heavier infections than gravid females suggesting that age, or developmental-stage related differences in susceptibility to infection, exist. In contrast, Boyce (1974) found that susceptibility of two of three species of *Cyclops* to *Eubothrium salvelini* was age or size-dependant, and of those species, only individuals in copepodite stage IV or older could be infected experimentally.

Work on insect hosts has revealed that cyclophyllideans like *Dipylidium caninum* can only infect larval fleas due to changes in mouthpart anatomy resulting from metamorphosis (Chen, 1934). *Hymenolepis diminuta* has far more success establishing in adult *Tenebrio molitor* than in larvae (Voge and Graiwer, 1964). Lethbridge (1971) subsequently demonstrated that the difference in susceptibility of *T. molitor* was due to the greater thickness of the gut wall in larval beetles.

There is relatively little information available on the effects of host sex on the susceptibility of invertebrates to infection by larval cestodes. Gruber (1878) found that female cyclopoid copepods had heavier infections of proteocephalid pleurocercoids than males. There are also reports from field studies that suggest females may acquire heavier infections than males. Wagner (1917), for example, found that in natural conditions, female *Diaptomus* sp. had heavier infections of *Proteocephalus torulosus* than males.

Laboratory studies showed that female *Tribolium confusum* exposed to *H. diminuta* oncospheres developed heavier cysticercoid infections than males (e.g. Stallard, 1975; Mankau, 1977; Schom *et al.*, 1981). Stallard (1975) found similar results in beetles exposed to *H. citelli* and *H. peromysci*. Yan and Norman (1995) studied the susceptibility of different strains of *T. confusum* and *T. castaneum* to *H. diminuta* and, while observing significant between-strain and between-species variation, also found that in all but one instance, females developed the heavier infections.

Host Mortality:

Little is known regarding the pathogenesis of cestode larvae in invertebrates (Freeman, 1983). Unfortunately, much of the information available is anecdotal and rarely quantified. While some data comes from naturally infected

hosts, much of the information comes from life cycle studies where potential hosts are exposed to relatively large numbers of eggs. Many become heavily infected and die, however, such infections are unlikely to occur under natural conditions (Freeman, 1983).

During ingestion, infective larvae (oncospheres) are liberated from the egg. They penetrate the gut wall and migrate to a parenteral site, where they develop to the next stage. There is some debate regarding the details of the penetration process. Wardle and McLeod (1952) suggest that the oncospheres claw their way through the gut wall to reach the haemocoel. Thomas (1941) suggested that the oncospheres use their hooks to push intestinal cells aside and then use an amoeboid-like movement to slide between the cells into the haemocoel. Freeman (1964), studying the penetration of oncospheres of *Proteocephalus parallacticus* through the gut wall of *Cyclops vernalis*, concluded that entry was by intestinal cell displacement rather than cell rupture; a form of penetration he believed minimized trauma to the host. Regardless, some trauma occurs during the process. The pathology associated with migrating oncospheres in the gut and developing cestode larvae in parenteral sites may result in mechanical, physiological, or immunological changes in the host, which may in turn vary with the species, the age and sex of the host, the number of larvae present, and their location within the host.

Most caryophyllidean cestodes are pathogenic to their annelid intermediate host (Kennedy, 1972). Mackiewicz (1972) found that less than 3% of suitable wild annelids were infected with caryophyllaeids, normally with less than 5 - 10 parasites per annelid. Higher intensities could be obtained experimentally, but heavily infected oligochaetes died quickly (Mackiewicz, 1972). Calentine (1965a) found only single infections with *Biacetabulum* spp. in nature, and showed experimentally that more than one pleurocercoid per annelid usually killed the host. Annelids with more than four pleurocercoids of *Archigetes iownesis* died within 100 days while those with one or two pleurocercoids survived up to two years (Calentine, 1964). Other caryophyllaeid species may also be lethal to their hosts if present in sufficient numbers (Calentine 1965b; Calentine and DeLong, 1966). Calentine *et al.* (1970) were able to demonstrate experimentally that, for a number of caryophyllaeid species, infected intermediate hosts survived only half as long as controls under similar conditions.

Varying degrees of pathogenesis and mortality have been observed in copepods infected with pseudophyllidean procercoids. Extensive studies on *Diphyllobothrium* spp. infections in copepods have produced conflicting results. Several authors found that pseudophyllideans, in general, caused little mortality among their copepod hosts regardless of infection levels (Michajlow, 1958; Watson and Price,

1960; Guttowa, 1961; Kuperman, 1973; Freeman, 1983). An almost equal number assert that heavy infections are deleterious and are potentially lethal to the host (see Freeman, 1983). Nie and Kennedy (1993), studying the infection dynamics of *Bothriocephalus claviceps* procercoids in *Cyclops vicinus*, found that survival among control copepods was better than in infected groups. Increased mortality was related to the dosage and the duration of exposure. Younger copepods had heavier infections but survived longer than gravid females. Freeman (1983) suggested that there is a limit to the number of pleurocercoids that can develop without seriously or fatally damaging the host, and that the severity of the damage is related to a combination of the size, number, and location of the parasites. Rosen and Dick (1983) proposed that mortality in *Cyclops bicuspidatus thomasi* following infection by the cestode *Triaenophorus crassus*, was related to a combination of factors including: penetration by the oncospheres, the mechanical pressure of procercoids on the internal organs, and nutritional stress on the host. They suggested that a higher parasite burden would result in a higher host mortality and infected hosts would not survive as long as uninfected ones.

Several authors studying proteocephalidean life cycles have observed that heavy infections are rapidly lethal to copepod intermediate hosts (e.g. Wagner, 1917; Hunter, 1929;

Herde, 1938; Thomas, 1941; Fischer, 1968). Typically, copepods with light infections survived, whereas those with heavy infections usually died within a week (Thomas, 1941; Fischer, 1968).

Keymer (1980) demonstrated that increased numbers of *H. diminuta* cysticercoids resulted in a significant reduction in the survival of *T. confusum* and suggested that host mortality was related to the damage of the midgut wall following penetration of the parasite into the haemocoel.

Tribolium confusum can also serve as the intermediate host for *Hymenolepis nana*, *H. microstoma*, and *H. citelli*. Although all species cause some mortality during the first 15 days of infection, mortality is most pronounced in *H. citelli* infections (Voge, 1956; Rothman, 1957; Voge and Heyneman, 1957; Dvorak *et al.*, 1961; Stallard, 1975). Schom *et al.* (1981) examined the mortality patterns caused by *H. citelli* and found that the heaviest mortality occurred during the first 14 days of infection. Exposure conditions influenced mortality. Mortality was higher in beetles starved for six days than those starved for one day. Regardless of starvation time, the majority of beetles died between 8 and 11 days postexposure, although those starved longer died sooner. Survival time was negatively correlated with parasite load. The authors state that with few exceptions beetles that survived until day 15, when the experiments were terminated, contained 14 or fewer

cysticercoids, whereas those that died earlier had 20 or more. Mortality was equally high in both sexes but the mean survival time was significantly shorter for females. Starvation had similar effects on the mortality of beetles exposed to *H. diminuta* (Dunkley and Mettrick, 1971) and it is clear that availability of food, or the extent of fasting, may influence infection levels and their consequences.

Mortality and Sex of Infected Hosts:

There are reports that mortality due to larval cestodes may be influenced by the sex of the intermediate host (Freeman, 1983). Gruber (1878) observed that female copepods had heavy infections of proteocephalid pleurocercoids but no infected males were found, leading him to suggest that the comparatively smaller males had been killed before the parasites had reached their full size. Wagner (1917) found that in a wild population, female copepods were more heavily infected with proteocephalid larvae than the smaller males, and suggested that more heavily infected smaller males were dying. Schom *et al.* (1981) found that female *T. molitor* infected with *H. citelli* developed heavier infections and died sooner than males.

The only study to address this issue specifically in crustaceans is that of Uznanski and Nickol (1980) who studied the effect of host sex on the survival of *Hyalella*

azteca (Amphipoda) infected with larvae of the acanthocephalan *Leptorhynchoides thecatus*. There was a marked difference in survival between the sexes. Infection intensity did not seem to affect survival in male amphipods, but curiously, surviving infected females actually had a higher mean parasite burden than those that died.

Effects on Host Growth:

There is evidence that larvae of some cestode species may affect the growth of the intermediate host. Several species of caryophyllaeid cestodes cause reduced growth in their annelid intermediate hosts (review: Freeman, 1983). Similar effects have been reported anecdotally in arthropods (principally crustaceans). Clarke (1954) observed that a *Cyclops serrulatus* nauplius larva infected with *Schistocephalus solidus* exhibited a distinct lag in growth. A fourth stage-infected nauplius was still a nauplius three weeks later, well after sufficient time had elapsed for it to have completed development to adulthood. Subsequent studies revealed that uninfected nauplii completed all the moults to become adults within 15 days whereas infected nauplii had only attained the second copepodid stage by that time. Mueller (1966) also observed that *Cyclops vernalis* infected with the pseudophyllidean *Spirometra mansonoides* frequently failed to moult to the adult stage.

Recent experiments by Thomas *et al.* (1995, 1996b) have

shown that the intermoult duration in *G. insensibilis* infected with metacercariae of the digenean *Microphallus papillorobustus* is prolonged, resulting in slower growth and a reduced probability of infected males pairing successfully later in life.

Reduced host growth is not always the outcome of parasitic infections however. In some associations, particularly in digenean-snail systems, accelerated growth or gigantism occurs (Reinhard, 1956). Gigantism is frequently associated with the timing of infection and reductions in host fecundity (review: Baudoin, 1975). The belief is that energy normally apportioned to reproduction is diverted to host and parasite growth (Baudoin, 1975; Freeman, 1983; Hurd, 1990). A possible example of this in cestode infections may occur in female gammarids infected with *Diplocotyle* sp. In this case infected individuals are substantially larger than uninfected ones (Stark 1965).

Effects on Reproduction:

Parasites may affect host reproductive ability either by direct castration or by a reduction in fecundity. Parasitic castration is common in molluscs infected with digenean sporocysts or rediae (reviews: Reinhard, 1956; Baudoin, 1975; Hurd, 1990) but has also been reported in arthropods (e.g. Hanström, 1939; Wülker, 1964; Keymer, 1980) and annelids infected with other parasites (e.g. Reinhard,

1956; Baudoin, 1975; Hurd, 1990). Strictly speaking, parasitic castration implies some damage to the sex organs of the host, impairing their function (Reinhard, 1956; Baudoin 1975). Hurd (1990) categorized the pathobiology associated with parasitic castration as mechanical, where the parasite physically interferes with the structure and function of the gonad (Cheng *et al.*, 1973), and chemical, where chemicals produced by a parasite impede gonad development or function (Crews and Esch, 1987). As with gigantism, it has been suggested that the energy normally allocated to reproduction is diverted to host and parasite growth (Baudoin, 1975; Freeman, 1983; Hurd, 1990).

The effect of parasitic infections on host reproductive potential has become a major area of interest in the study of host-parasite interactions. Castration or a parasite-induced reduction in host fecundity can have serious implications for the reproductive potential of the host population (Weatherly, 1971; Keymer, 1980). Michalakis and Hochberg (1994) point out that the outcome of host-parasite interactions can be complex if the effects of parasitism are stage-dependent or if fecundity increases with body size, as is the case in many of the arthropod species that serve as intermediate hosts. The authors postulate that animals which successfully avoid parasitism will grow relatively faster, usually be larger, and have a higher potential fecundity.

There is evidence that infection by the larvae of some cestode species may reduce host fecundity or castrate the host completely (review Freeman, 1983). Several species of caryophyllaeid cestodes have been shown to inhibit sexual development in their annelid hosts (Reinhard, 1956; Freeman, 1983). Similar effects have been reported in arthropods (principally crustaceans) and these seem to be more serious in females, where the gonad may be severely affected, than in males. The ovaries of female gammarids infected with *Diplocotyle* sp. are reduced or absent (Sandeman and Burt, 1972; Scott and Bullock, 1974) and the oostegites lack bristles; a feature characteristic of immature females (Stark, 1965).

Experimental studies by Gruber (1878) and Meggitt (1914) and observations of naturally infected copepods by Wagner (1917) suggest that ovaries atrophy when female copepods become infected by various proteocephalidean cestodes. Freeman (1983) claimed to have repeated Meggitt's study with similar results but, unfortunately, failed to comment further on observations related to the ovary. Mueller (1966) observed that *Cyclops vernalis* infected with the pseudophyllidean *Spirometra mansonoides* seldom developed egg sacs. Castration of a copepod by a cyclophyllidean cysticercoid has also been reported (Bayly, 1963). Amat *et al.* (1991) collected individuals from a wild population of *Artemia* (brine shrimp) and isolated those bearing

cysticercoids of the hymenolepid *Flamingolepis* sp. Infected individuals were classified based on reproductive state as: immature, empty ovisac, ovulating, or fully ovated (gravid) and the number in each category was counted. They found that although survival was not impeded by the parasites, reductions in host fecundity were obvious. Significantly fewer infected *Artemia* were gravid. Although empirical evidence is lacking, these observations are suggestive of compromised reproductive capabilities.

Adverse effects of infection on the fecundity of intermediate hosts have been examined in more detail in beetles infected with *Hymenolepis diminuta* and related species. In such situations, a reduction in egg production rather than outright cessation tends to occur as a result of the infection. Keymer (1980) found that female *T. confusum* infected with *H. diminuta* continued to lay eggs but the infected beetles laid significantly fewer eggs over a 24 hour period than the controls. There was no difference in the viability of the eggs from either group and Keymer suggested that the reduction in fecundity might be related to the biomass of the parasite larvae in the host. Hurd (1990) demonstrated that infection with *H. diminuta* also resulted in decreased fecundity in another beetle, *Tenebrio molitor*.

Reductions in the fecundity of *H. diminuta*-infected beetles coincide with the decreased uptake of a fat body

protein by the ovaries of infected individuals (Hurd and Arme, 1986b ; Webb and Hurd, 1996). This is apparently due to an inability of developing oocytes to accumulate sufficient amounts of vitellin (egg yolk protein), which results in retarded egg development and ultimately in decreased egg production (Hurd and Arme, 1986a; Maema, 1986; Webb and Hurd, 1996). The precursor of vitellin, vitellogenin, is synthesized in the fat body of mature females (Hurd and Arme, 1986 a,b). After being secreted into the haemolymph, vitellogenin is taken up by the oocyte and stored as vitellin. Webb and Hurd (1996) found that while both the vitellogenin titre in the fat bodies and the vitellin content of the ovaries were significantly reduced in infected female beetles, there was a pronounced increase in haemolymph vitellogenin. They suggested that this may be the result of some hindered passage of the vitellogenin into the oocyte and argued that since the parasite does not take up vitellogenin, it may somehow inhibit vitellogenin uptake in the host.

Studies on acanthocephalan infections provide some insight into the range of the effects of parasitism on reproduction induced by different parasite species. *Leptorhynchoides thecatus* apparently does not interfere with the normal function of the gonads of *H. azteca*, nor does it alter any secondary sex traits (Spaeth, 1951). Zohar (1993) found no evidence that *Polymorphus paradoxus* or *P. marilis*

castrated male or female *Gammarus lacustris*. However, brood sizes of *P. marilis*-infected females were reduced by 3-5 eggs compared to brood sizes of similar-sized uninfected females. In contrast, female *Gammarus pulex* parasitized by the larvae of *P. minutus*, suppress oogenensis, and lose the marginal hairs on the lamellae of the brood pouch (oostegites) (LeRoux, 1931; Reinhard, 1956). Spermatogenesis in the males was unaffected.

While host reproductive fitness can be lowered by partial or complete sterilization (Reinhard, 1956; Baudoin, 1975; Hurd, 1990), it can also be affected by a modulation of host behaviours, including those critical to courtship and dominance (Hamilton and Zuk, 1982; Rau, 1983, 1984; Hurd, 1990; Zohar, 1993). Holmes and Bethel (1972), Moore (1984a, 1984b), and Moore and Gotelli (1990) provide excellent reviews of host behavioral morbidity induced by parasites. Whether or not changes in host behaviour are adaptive remains a point of controversy, however (Dobson, 1988; Moore and Gotelli, 1990; Poulin, 1995). Interest here will focus on the behavioral changes that may influence host reproductive function.

Little is known of the effects of parasites on the reproductive behaviour of crustaceans. The best studied example of this phenomenon involves crabs and *Sacculina*, a parasitic barnacle. *Sacculina* sp. castrates its crab host. In the process, secondary sex traits in males and females

are modified. True crabs normally show pronounced sexual dimorphism, but when "sacculinized", a broadening of the male abdomen occurs so that it more closely resembles that of the female. Perhaps more significantly, infected males do not engage in courtship or any of the aggressive displays characteristic of normal, uninfected males (Reinhard, 1956).

Reproductive behaviour in male crustaceans is complex and they are often the initiators of courtship. Helluy and Holmes (1990) provided evidence suggesting that larvae of the acanthocephalan *P. paradoxus* alter the behaviour of *Gammarus lacustris* by affecting a serotonin-sensitive pathway, possibly the one involved in the precopular hold of males. Spaeth (1951) found that both male and female *G. lacustris* parasitized by *P. paradoxus* display significant reductions in pairing success. Zohar (1993) confirmed this effect on the pairing of gammarids infected with *P. marilis*, and also found that infected females had reduced clutch sizes. Thomas *et al.* (1996b) studied the pairing success in wild populations of *G. insensibilis* infected with metacercariae of *M. papillorobustus*, and found that infected individuals were more likely to mate among themselves; the uninfected gammarids having a higher probability of pairing with other uninfected individuals. Within the infected group, infected males paired with females in a size-assortative manner, regardless of parasite intensity. In organisms like amphipods, that display complex mating

behaviour including a period of courtship or mate assessment, behavioral modification may have serious implications for host reproductive success.

EXPERIMENTAL SYSTEM

Microsomacanthus hopkinsi is a small cyclophyllidean cestode that infects the caeca of several species of surface feeding ducks (McLaughlin and Burt, 1970; Podesta and Holmes, 1970). Details of the life cycle (Figure 1) can be found in McLaughlin and Burt (1970) and Podesta and Holmes (1970). Eggs, containing fully developed oncospheres, are passed intact from the duck host in the form of a long, slender egg packet. Each packet may contain up to 80 oncospheres, and represents the entire production of a single gravid proglottid. The packet is the natural infective unit and may be consumed in whole or in part by the intermediate host. If ingested, each oncosphere within the egg packet has the potential to develop into a cysticercoid and heavy infections can develop in the haemocoel of the intermediate host.

The amphipod, *Hyalella azteca* is the only known intermediate host of *M. hopkinsi* (McLaughlin and Burt, 1970; Podesta and Holmes, 1970). It occurs in wetlands throughout most of North America (Bousfield, 1958; de March, 1977, 1978, 1981). Populations survive the winter, but

individuals are inactive below 10°C (de March, 1977, 1978, 1981). The morphology, moulting patterns, sexual development, and mating behaviour of *H. azteca* are well known (Wilder, 1940; Geisler, 1944; Pennak, 1953; de March, 1977, 1978, 1981). Reproduction is obligately sexual (Embody, 1911; Geisler, 1944; Pennak, 1953; Cooper, 1965; Kruschwitz, 1972; Strong, 1972, 1973; de March, 1977, 1978, 1981), and the amphipods are sexually dimorphic. Adult males have larger second gnathopods (walking legs) which are used to grip the female in precopula (amplexus) and during copulation (Pennak, 1953; de March, 1977, 1978, 1981). The second gnathopods of the female retain the juvenile form although they continue to grow with age (de March, 1981). Sexual dimorphism is apparent by the fifth instar. *Hyalella azteca* attain sexual maturity at about the eighth instar (Strong, 1972, 1973; de March, 1978, 1981).

Courtship and reproductive behaviour in amphipods are complex. Males and females amplex (pair) as the opportunity for copulation approaches (Dunham *et al.*, 1986). During amplexus, the males grip females in a precopular hold during which they assess the quality of the female and her readiness to mate (Pennak, 1953; de March, 1978, 1981). This mating strategy is common in crustaceans where ovulation and fertilization are restricted to a brief interval following the female moult (Birkhead and Clarkson, 1980; Dunham *et al.*, 1986).

As in gammarids, *H. azteca* is categorised as a Bayesian decision maker (uncertainty hypothesis) (Hunte *et al.*, 1985; Wen, 1992). Males will attempt to amplex with a female even when they are unsure of her reproductive state. An unreceptive female can usually escape an attendant male within a few seconds. In *Gammarus* spp., gravid females close to mating acquiesce to male advances and appear to be more valuable to males the longer they have been amplexed (Hunte *et al.*, 1985; Dunham *et al.*, 1986; Hunte and Myers, 1987).

In *H. azteca*, pairing success is strongly mediated by male-male competition and females can be stolen away by stronger, marauding males (Wen, 1992). Only tactile contact appears to initiate amplexus in *H. azteca* (Strong, 1972, 1973). Males will amplex with injured males, dead amphipods, discarded exuvia, and large food items suggesting an absence of any chemical cues in reproductive behaviour (Strong, 1972).

The first clutch of eggs is produced at the end of the eighth female instar (Pennak, 1953). In the final stages of amplexus, the female moults and ovulates. The male, having held on to the female during her moult, fertilizes the clutch and the two separate. The eggs and young are carried in the marsupium (brood pouch) located under the abdomen of the female where they are held until the next moult when they are released. The young grow through approximately

seven immature instars before becoming adults. After each mating, the female moults and releases the young from the previous mating. Sexually mature females mate regularly, as often as once every six days at 26°C (de March, 1978, 1981) and can produce between 1 - 30 offspring at each moult (de March, 1981). The number of offspring produced increases in successive broods and is related to the size and geographic origin of the female (Strong, 1972; de March, 1978).

OBJECTIVES

The fragmentary nature of our knowledge of host-parasite relationships between crustaceans and cestode larvae is due in large measure to practical difficulties of exposing the host to a natural number of infective stages. The comparatively large size of *H. azteca*, its well-known biology, the fact the an egg packet constitutes the natural infective unit of *M. hopkinsi*, the ease with which the infective dose can be determined and the fact that *H. azteca* can, under natural conditions, ingest an entire packet and sustain the cysticercoid population that develops, makes this system an attractive one with which to examine the host-parasite relations between cestodes and crustacean intermediate hosts.

This study examines several parameters of the relationship between *M. hopkinsi* and its intermediate host,

H. azteca. Specifically, the objectives were: i) to determine if host age, size, or sex at the time of exposure influences susceptibility to infection and cysticercoid burden; ii) to determine if infections affect the short- or long-term survival of *H. azteca*; iii) to determine whether infections acquired before sexual maturity, affect the reproductive success of female *H. azteca*; and iv) to determine whether infections acquired before sexual maturity affect the mating success of male or female *H. azteca*.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Hyalella azteca:

The stock culture of *H. azteca* originated from specimens collected at Delta Marsh, Manitoba, in 1990 and was augmented with specimens from the Marsh at yearly intervals. Stock cultures were maintained in dechlorinated water in 23 x 36 x 14 cm plastic tanks, supplied with a tuft of filamentous algae and/or a plastic mesh scouring pad as a breeding substrate, and provided with TetraMin® *ad lib*. The tanks were held on a specially designed multilevel rack similar to that described by de March (1981). Each level had a bank of 40W wide-spectrum fluorescent lights controlled by a separate timer. The stock cultures were kept at 23°C under a photoperiod of 14L:10D.

Ducklings:

Domestic ducklings *(Anas platyrhynchos domesticus)* were purchased from Brome Lake Duck Farms, Knowlton, Quebec. They were kept in the waterfowl holding facilities at Concordia University, and were provided with food (18% laying feed, Nutribec Ltd., Montreal) and water *ad lib*.

Microsomacanthus hopkinsi:

The original material was obtained from the caeca of black ducks (*Anas rubripes*) shot by hunters near Fredericton, New Brunswick. The viscera were removed, and the caeca were detached, opened, and rinsed. The rinse water was allowed to settle and most of it was decanted. The sediment was examined under low power magnification with a dissecting microscope and the egg packets were removed by Pasteur pipette.

Laboratory Maintenance of *M. hopkinsi*:

Hyalella azteca were exposed to the egg packets in 24-well tissue culture plates. Two ml of dechlorinated water, a single *H. azteca* and a single egg packet were added to each well. Exposure lasted 24 to 48 hours, then the amphipods from each plate were transferred to plastic containers and maintained on the rack as described above. At 14 days postexposure, each amphipod was placed on a depression slide in a small volume of water, immobilized with a coverglass, and examined with a microscope for cysticercoids. Infected amphipods were fed, by pipette, to ducklings.

At 11 days postexposure, individual ducklings were placed in wire-bottom isolation cages and their fecal droppings were collected over a period of several hours. The trays were lined with moistened paper towels to prevent desiccation of the droppings. Caecal droppings (visually

distinct from regular droppings) were diluted in tap water and filtered through a 150-μm sieve. The egg packets were collected from the backwashed sediment under low power magnification using a dissecting microscope.

Groups of ducklings were infected at regular intervals to provide a constant supply of egg packets. Egg packets were stored at 5-7°C in 20 ml vials full of water. Although the egg packets can be stored at 5-7°C for at least 12 weeks with no loss in infectivity (Lee *et al.*, 1992), only packets less than eight weeks old were used in this study.

EXPERIMENTAL PROCEDURES

Hyalella azteca Growth Curve:

As most of the experiments were performed on individuals of known age or instar, the first step in this project was to establish the growth curve of the population under the conditions of the study (23°C; 14L:10D). Females bearing broods were obtained from stock cultures and placed individually in *Drosophila* vials (Carolina Biological Supply Co.). They were examined at daily intervals and the offspring produced on any one day were pooled.

The head capsule length and total body length of 137 newly-hatched *H. azteca* were obtained by placing each individual in a depression slide in a small drop of water, immobilizing it with a coverglass and measuring it on a

compound microscope (Figure 2). Each amphipod was then placed in an individually labelled *Drosophila* vial. Measurements were repeated at four day intervals, until the amphipods died. Means, standard deviations, and 95% confidence intervals were calculated for head capsule lengths and total body lengths for each instar. Head capsule measurements were chosen to distinguish the various instars (see results for justification).

Exposure of Amphipods:

For some experiments it was necessary to know the number of oncospheres eaten by individual amphipods. This required that each amphipod be exposed to a known number of oncospheres and that each individual be isolated following exposure. Individual amphipods were exposed in 24-well tissue culture plates as described above but with two modifications. Only amphipods of the appropriate instar (as determined by head capsule measurement) were exposed and the number of oncospheres in each egg packet was counted before it was placed in the well. Exposure lasted 48 hours then the wells were examined with a dissecting microscope to verify consumption of the egg packet. If the entire egg packet was not eaten, the remnants were recovered and the remaining oncospheres counted to determine the exact number ingested. Controls were treated as above except they were sham exposed by feeding them egg packets that had been heat-

killed at 55°C.

When observations on individuals were necessary, each amphipod was placed in a separate *Drosophila* vial after exposure and kept separately for the duration of the experiment. Thus, the number of oncospheres consumed by an individual could be compared with the number of cysticercoids that developed. Each vial was filled with dechlorinated water, a small tuft of filamentous algae was added as a substrate and for oxygenation, and a few flakes of TetraMin® were added. Otherwise, amphipods were pooled following exposure and held in plastic tanks until needed. Exposed and control amphipods were maintained on the rack described above under the same conditions (23°C; 14L:10D) as the stock cultures.

Live amphipods were examined in well slides six days postexposure to identify infected individuals. At the end of the experiment, the amphipods were killed and either dissected in well slides, or mounted in Hoyer's medium (Humason, 1979) on slides and the number of cysticercoids present determined using a microscope.

Oncosphere Consumption, Susceptibility, Cysticercoid Burden and Survival of *H. azteca* Exposed at Different Instars:

Individuals in instars 1, 2, 3, 4, 6, 8, and 9 were used in this experiment. Three groups of 24 *H. azteca* in Instars 1 through 4 were exposed. Sexes are readily

discernable from the fifth instar on, so six groups of 24 individuals (three groups of males and three groups of females) were exposed in Instars 6, 8, and 9. Individual amphipods were exposed to a known number of oncospheres as described above and following exposure (48 hours), the wells were searched for egg packet remnants to determine the exact number of oncospheres ingested by each individual. Each individual was maintained separately in a numbered *Drosophila* vial, prepared as above, for the duration of the experiment. An equal number of controls for each instar and/or sex category was sham exposed.

Cysticercoids complete their development by day 14 and preliminary studies showed that little mortality occurs immediately thereafter. Accordingly, survival was monitored at 3, 6, 9, 12, and 14 days postexposure, when the experiment was terminated. Amphipods that died during the experiment were examined using a compound microscope to determine the presence and magnitude of infection. Unfortunately, the small size of the developing larvae and the rate of decomposition made detection of infections in those amphipods that died within six days postexposure virtually impossible. After six days, the developing cysticercoids could be identified in the haemocoel of amphipods recovered in reasonably good condition. Amphipods that survived the full 14 days were placed in depression slides. The head capsules were measured and the animal was

dissected to determine the number of cysticercoids present.

Survival, susceptibility, mean parasite burden, and the number of moults (based on head capsule measurements) that had occurred over the 14 day period were compared between each instar and its respective control, and between groups exposed during different instars.

Long-term Survival of Infected *H. azteca*:

The effect of cysticercoid infection on long-term survival was examined by exposing fourth instar *H. azteca* to egg packets as outlined in Experiment 1. This instar was selected because it is the youngest instar in which the short-term survival of infected individuals did not differ significantly from that of older instars. An additional advantage was that both host sex and the presence of infection could be confirmed at six days postexposure.

Fourth instar *H. azteca* were separated into experimental and control groups. Amphipods were placed individually in wells of 24-well tissue culture plates and exposed to a known number of oncospheres, as described above for 48 hours, then placed in individually labelled *Drosophila* vials. Controls were sham exposed. At six days postexposure, amphipods exposed to live oncospheres were examined in depression slides and the infected individuals were retained. By this time, the amphipods had moulted to the fifth or sixth instar and the sexes could be

distinguished.

Trials consisted of three groups of 24 infected individuals of each sex and an equal number of controls for each sex. The amphipods were maintained individually in *Drosophila* vials as described above and monitored at two day intervals, for eight weeks, until all of the infected individuals had died.

Effects of Infection on Host Fecundity:

To determine whether female amphipods infected before sexual maturity produce fewer offspring than uninfected females, fourth instar amphipods were exposed individually to single egg packets in the wells of 24-well tissue culture plates as described above. The amphipods were pooled after exposure. Infected females, identified by examination in well slides, were selected at 12 days postexposure, when they had reached the sixth instar. Seventy-two infected females were placed individually in *Drosophila* vials. The control group consisted of 72 unexposed sixth instar females from the stock cultures. Larger (eighth or ninth instar) uninfected males were obtained from stock cultures. A male was added to each vial once the females had moulted to the seventh instar. The vials were monitored every two days. Amplexus was recorded when observed and the number of offspring produced by each female during the eighth and ninth instars was determined. In *H. azteca*, the brood of

the previous mating is released at each moult. Thus, offspring found in the presence of a ninth instar female were produced during the eighth instar, while offspring found in the presence of a tenth instar female were produced during the ninth instar. Broods were removed from the vial and counted, and the head capsule of the female was measured again. Following collection of the first brood, the female was returned to her vial along with her attendant male and the monitoring was resumed.

The experiment was terminated when females attained the tenth instar. The females were removed and their head capsules measured. They were then dissected to determine the cysticercoid burden.

Effects of Infection on Mate Selection:

To determine whether male and female amphipods select uninfected individuals over infected ones, fourth instar *H. azteca* were exposed to single egg packets in 24-well tissue culture plates as above. Controls were sham exposed. The sex of the individual and presence of infection were determined under low power magnification 14 days later, when individuals were at the sixth or seventh instar.

To determine whether infection influences male pairing success, 10 infected and 10 control ninth instar males were placed in a round, tapered bottom, 19.5 cm x 7 cm high culture dish, containing 350 ml of dechlorinated water. Ten

uninfected eighth instar females that had been in natural amplexus were taken from stock cultures, separated from their attendant mates and added to the culture dish. Trials were run for six hours and visual inspections for amplexed pairs made at 10 minute intervals. Once amplexus occurred, the pair was removed, the time was recorded, and the pair was examined to determine which individual, test or control, attached. Each trial was repeated three times using different individuals.

A similar test was performed to determine whether males would select uninfected over infected females. Ten infected and ten control eighth instar females were placed in a 19.5 cm x 7 cm culture dish containing 350 mL of dechlorinated water. Ten uninfected ninth instar males, that had been in natural amplexus, were obtained from stock cultures, separated from their females and added to the culture dish. Each trial was repeated three times using different individuals and mating activity was monitored as above.

STATISTICAL ANALYSES

All statistical analyses were performed using the NCSS (Number Cruncher Statistical System, version 5.03; Dr. Jerry Hintze, Kaysville, Utah 84037). Replicates were tested for homogeneity and then pooled for subsequent analysis. Analyses included least-squares regression

analysis, analysis of variance, Duncan's multiple range tests, *t*-tests and MANOVA. Probabilities of 0.05 or less were considered significant.

TERMINOLOGY

For simplicity, the term Instar 1, 2, 3,...etc. is used throughout the Results and Discussion sections to designate all of the *H. azteca* exposed during that instar.

RESULTS

Growth and Development of *Hyalella azteca*:

Head capsule lengths and overall (total) body lengths obtained from specimens of *H. azteca* in Instars 1 through 10 are presented in Table 1 and in Figures 3 and 4. Regression Approach ANOVAs of head capsule and total body length on instar number revealed significant positive relationships in each case *[$p < 0.001$ for each; $r^2 = 0.984$ and 0.974, respectively]*. The relationship between head capsule length and instar number was linear *[head capsule length (μm) = $104.28 + 61.05$ (instar)]*, whereas that between body length and instar number was not *[body length (μm) = $1339.28 + 55.82$ $(instar)^2$]*. A regression of head capsule length on overall body length (not shown) was also highly significiant *[$p < 0.001$; $r^2 = 0.990$]*. Head capsule length was chosen as a means of identifying a particular instar because of the linear relationship, greater r^2 value, and the comparative ease and accuracy with which it could be measured.

Under our rearing conditions, sexes could be separated by the fifth instar. Fifth instar males had larger second gnathopods (walking legs) and the developing marsupium could be seen in females. Adult sizes and time to maturity corresponded with those reported by de March (1978) for *H. azteca* reared at 20°C.

Oncosphere consumption, short-term survival, cysticercoid burden, and moulting patterns in infected *H. azteca*

Oncosphere Consumption:

The average egg packet offered to amphipods in this experiment contained 59.7 ± 12.01 oncospheres. There was no significant difference in the mean number of oncospheres in egg packets fed to replicate groups of *H. azteca* within each instar nor between the replicates of different instars $[F_{1,718}= 0.02; p \geq 0.887]$.

During the course of this study, no differences were detected in oncosphere consumption, cysticercoid burden, moult frequency, or in short- and long-term survival between the sexes. Results for Instars 6, 8, and 9 were therefore pooled.

Table 2 shows the percentage of *H. azteca* in each instar that ate little (<50%), most (≥50%, <100%), or all (100%) of the egg packet during the exposure period. The percentage of the packet eaten was positively correlated to the instar at the time of exposure. Larger *H. azteca* ate a greater proportion of the egg packet than smaller ones $[F_{1,718}= 96.05; p < 0.001; r^2= 0.12]$.

Regression Approach ANOVA showed a significant, positive correlation between instar number and the number of oncospheres ingested by the amphipods; larger amphipods, on average, ate more oncospheres than smaller ones $[F_{1,718}=38.93;$

$p < 0.001$; $r^2=0.050$] (Figure 5). *Hyalella azteca* exposed during Instar 1 ate significantly fewer oncospheres than any other instar. Instar 2 ate significantly more oncospheres than Instar 1 but fewer than Instars 8 and 9. *Hyalella azteca* exposed at Instar 3 or later ate significantly more oncospheres than those in Instar 1 (Duncan's Multiple Range Test) and normally consumed the entire egg packet during the 48 hour exposure period.

Short-term Survival:

The percentages of control and test individuals in each instar that survived to the end of the experiment are presented in Figure 6. Data for the control group, the exposed group, and the infected group are presented separately. The "exposed" group contains all the individuals exposed. The "infected" group is the subset of the exposed group that became infected. Regression Approach ANOVA revealed no significant difference in percent survival among the controls for the various instars [$F_{1,28}=3.99$; $p \geq 0.055$; $r^2=0.094$], but there was a significant, positive correlation between instar number and the percent survival of the exposed group [$F_{1,28}=11.91$; $p \leq 0.002$; $r^2=0.273$]. The percentage of *H. azteca* exposed in Instars 1, 2, and 3 that survived the 14 day test period was significantly lower than in the respective controls [$t_{16}=3.95$; $p \leq 0.003$]. No differences between the survival of exposed and control

groups were observed in the other instars. The percentage of Instars 1 and 2 that survived was similar but survival in these instars was significantly lower than in other instars (Duncan's M.R.T.).

Within the infected group, the percent survival of Instar 1 was significantly lower than in all other instars except Instar 2. Survival in Instar 2 was, in turn, significantly lower than that of Instar 4. Survival was similar among the other instars (Duncan's M.R.T.).

Mortality patterns in each instar are summarized in Table 3. Over 75% of the mortalities in Instars 1, 2, and 3 occurred by Day 7 postexposure, and most of the mortality observed in Instars 1 and 2 had occurred by Day 4. The total number of mortalities declined in Instars 4, 6, 8, and 9. Except for Instar 4, over half of the mortalities observed in these instars had also occurred by Day 7. After Day 7, mortalities in all instars were evenly distributed over time.

Cysticercoid Burden:

The mean intensity of cysticercoids (the mean number of cysticercoids/infected amphipod) in surviving *H. azteca* is shown in Figure 7. Regression Approach ANOVA revealed a significant positive correlation between the instar at the time of exposure and the number of cysticercoids found in infected individuals 14 days later $[F_{1,386}=70.50;\ p < 0.001]$,

however, this only explained a small amount of the variability in the data *[r^2=0.152]*. Amphipods infected during later instars harboured significantly more cysticercoids than those infected at earlier instars. With the exception of amphipods exposed during Instar 6, the mean number of cysticercoids increased in progressively older instars. *Hyalella azteca* infected during Instar 1 had significantly fewer cysticercoids than the others. There was no significant difference in the mean intensity of cysticercoids in amphipods exposed during Instars 2, 3, 4, and 6, nor between those exposed during Instars 8 and 9. Instars 8 and 9 had significantly heavier infections than all other instars (Duncan's M.R.T.). There was no correlation between oncosphere consumption and cysticercoid number in any of the instars.

The degree of aggregation of the cysticercoids in surviving amphipods exposed at different instars was examined by studying the variance to mean ratios of the respective populations. Theoretically, this parameter can take on any value from zero to infinity. A variance to mean ratio greater than 1 indicates a parasite population that is overdispersed or aggregated. The degree of aggregation can increase theoretically to infinity, where a single host harbours the entire parasite population. A ratio of 1 describes a random (Poisson) distribution. Values less than 1 indicate a uniform or even distribution. The extreme

value (0) would reflect a situation where all hosts harbour the same number of parasites. The variance/mean ratios for the cysticercoid populations in the survivors of different instars are given in Table 4. The populations were all aggregated and the variance/mean ratios increased progressively with each instar.

Moulting Patterns:

Figure 8 shows the mean number of moults that occurred in infected and control amphipods, exposed during Instars 1, 2, 3, 4, and 6 over the 14 day experimental period. Infected individuals underwent significantly fewer moults than their respective controls $[t_{30}=3.72,\ p \leq 0.027]$. Because instars could only be identified reliably by head capsule measurements to the tenth instar, no attempt was made to study those exposed during the eighth or ninth instars.

Long-term Survival of Infected Amphipods:

The previous experiment provided data on the effects of infection by *M. hopkinsi* on the short-term survival of *H. azteca*. To study the effects of the parasite on long-term survival, fourth instar *H. azteca* were exposed to egg packets. The fourth instar was selected because it is the youngest instar in which the short-term survival of infected

individuals was comparable to that of older instars. The mean survival times (days postexposure) of infected and control females and males are shown in Figure 9. The experiment was terminated at 80 days postexposure. At this time only 2.1% of infected amphipods (3/144) were still alive, and these had survived the rest by two weeks.

A Factorial (2-way) Multivariate Analysis Of Variance (MANOVA) was used to examine the effects of two independant variables: infection (IV_1) and host sex (IV_2) on long-term survival. Long-term survival was assessed as the mean survival time of the amphipods (days postinfection) (DV_1) and the number of amphipods that survived to the end of the experiment (DV_2). Host sex had no effect on long-term survival so the data for both sexes was pooled. A 1-way MANOVA showed that infection had a significant negative effect on both mean survival time and the relative number of *H. azteca* that survived to the end of the experiment *[Wilk's* $\lambda_{2,9}$*: 0.06649;* $F_{2,9}$*=63.18;* $p < 0.001$*]*. Individual ANOVAs confirmed the significant negative effect of infection on both mean survival time *[*$F_{1,10}$*=85.07;* $p \leq 0.001$*]* and the number of individuals per treatment surviving to eight weeks postinfection *[*$F_{1,10}$*=63.23;* $p \leq 0.001$*]*.

Fecundity of Infected and Uninfected Females:

Table 5 summarizes the number of infected and control female *H. azteca* that produced broods during Instars 8 and 9. Fewer females infected during the fourth instar (before sexual development begins) produced offspring than control females (Figure 10).

Regression Approach ANOVA showed that infection had a significant negative effect on the mean number of broods produced by females over the two consecutive instars *[$F_{1,142}$= 298.51; $p < 0.001$]* (Figure 11). Overall, four infected females and 64 control females produced broods during Instars 8 and/or 9. Only one and four of the 72 infected females produced offspring during the eighth and ninth instar respectively, whereas 58 and 57 of the 72 control females did so. Fifty-one control females, but only one infected female, produced broods during both instars.

The proportions of infected and control females producing: no broods; producing a brood in the eighth instar only; producing a brood in the ninth instar only; or producing broods in both the eighth and ninth instars are shown in Figure 11.

A MANOVA, performed to examine the effect of infection (IV_1) on the brood size of female *H. azteca* in Instars 8 (DV_1) and 9 (DV_2), revealed that the mean brood sizes for the two instars were negatively correlated with infection

[Wilk's $\lambda_{2,141}$: 0.39113; $F_{2,141}$=109.74; $p < 0.001$]. As only one infected female produced a brood in the eighth Instar, an individual ANOVA performed on the ninth instar data showed that the mean brood size produced by ninth instar females was significantly less than that in controls *[$F_{1,59}$=9.43; $p \leq 0.003$]* (Figure 12). Infected ninth instar females produced 6.3 ± 1.3 young per brood while corresponding controls produced 11.2 ± 3.2 young per brood. The single infected eighth instar female produced three young while corresponding controls produced 5.3 ± 2.4 young.

Effects of Infection on Mate Selection

The purpose of this experiment was to determine whether male and female amphipods, presented with potential mates, will select uninfected individuals over infected ones.

Pairing Success of Infected and Control Females:

The objective of this experiment was to determine whether males will pair selectively with uninfected rather than infected females. Ten uninfected ninth instar males were presented with 10 infected and 10 control eighth instar females. Should no preference exist, an equal number of pairings with infected and uninfected females should occur. Seven pairings were observed, none of which involved an infected female (Table 6).

Pairing Success of Infected and Control Males:

The objective of this experiment was to determine whether uninfected males pair more frequently than infected males when presented with uninfected females. Ten uninfected eighth instar females were presented to 10 infected and 10 control ninth instar males. Of the 11 pairings observed, nine involved control males but two infected males also paired (Table 7). The 30 infected males used in the experiment had between one to 46 cysticercoids (mean 22.6 ± 14.5). The two infected males that paired had six and 26 cysticercoids, respectively.

DISCUSSION

The purpose of this study was to assess the host-parasite relationship of a cestode (*Microsomacanthus hopkinsi*) and its intermediate host *Hyalella azteca*, infected at various stages throughout its life history. A growth curve was established in order to reliably identify *H. azteca* under the rearing conditions used. Adult sizes and time to maturity corresponded with those reported by de March (1978) for *H. azteca* reared at 20°C.

Infection by *M. hopkinsi* had significant effects on short-term and long-term survival, growth, fecundity, and pairing success in *H. azteca* exposed during different instars. The effects were generally related to the instar at exposure and earlier instars were the most severely affected.

AGE-RELATED DIFFERENCES IN TRANSMISSION AND SURVIVAL

Oncosphere consumption:

The variability in oncosphere consumption by amphipods in different instars reflected the variation in the number of oncospheres (25 - 80) in each packet. *Hyalella azteca* exposed during Instars 1 and 2 typically ingested fewer oncospheres and, although they were less likely to eat the

whole egg packet than those exposed at older instars, many of them did so. *Hyalella azteca* exposed during Instar 3 and older generally ate the entire packet regardless of the number of oncospheres it contained.

The packets are potential food items and observations on feeding *H. azteca* indicate that larger amphipods will eat the entire packet when they encounter it (Lee *et al.*, 1992). Smaller individuals likely become satiated more quickly and eat less of the packet. This may be more common than the data suggests. Under natural conditions, individuals are free to eat some or all of the packet when they encounter it and then move on. Given the restricted space of the exposure chamber (2 ml) and the 48 hour exposure period, it is probable that some of the smaller individuals that ate the entire packet did so during separate feeding sessions. The implication is that smaller individals may not consume the entire packet under natural conditions due to satiation or perhaps loading constraints, thereby limiting exposure to a smaller number of oncospheres.

In contrast with previous findings in copepods (Smyth, 1963; Boyce, 1974) and insects (Voge and Graiwer, 1964), *H. azteca* was susceptible to infection during all instars tested. Unlike copepods and insects which undergo complete metamorphosis, development in amphipods is gradual. While the instars differ in size, morphological, and presumably physiological differences between instars are less

pronounced in amphipods than in copepods or in insects. Consequently, amphipods provide a more homogenous environment for parasites over a greater portion of their life-history than hosts that undergo more extensive metamorphosis. This leaves them susceptible to infection throughout their lives.

Short- and long-term survival of infected H. azteca:

Experimental studies have shown that heavy infections of larval cestodes are usually lethal to intermediate hosts (Freeman, 1983; Schom *et al.*, 1981; Nie and Kennedy, 1992; Dupont and Gabrion, 1987; Shostak and Dick, 1985; Poulin *et al.*, 1992). There is a limit to the number of cestode larvae that an intermediate host can carry and it varies with the particular host-parasite system (Freeman, 1983). Most mortalities that occur in laboratory studies are the result of exposure to numbers of eggs well beyond what an individual host would encounter in nature (Freeman, 1983). Most cestode species with aquatic life cycles produce eggs that disperse quickly in the environment. Under natural conditions, potential hosts are seldom exposed to more than one egg, and multiple infections are rare (Freeman, 1983). With *M. hopkinsi*, the eggs are enclosed in a packet, which is the natural infective unit for this species, and ingestion of a single packet can produce a heavy infection in the host.

Heavy mortality was restricted to groups exposed during Instars 1 to 3. Over the 14 day period, survival in the exposed groups was significantly less than in controls. Further, the number of individuals infected during Instars 1 and 2 that survived was significantly less than in the other groups. Although damage to oncospheres occurring during ingestion, the amount of food present in the gut and the rate of food passage through the gut may have had some role in limiting the number of oncospheres that established in Instars 1 and 2, it is more probable that heavily infected individuals died. Unfortunately, the rapid decomposition of the amphipod and the small size of the developing cysticercoids made it impossible to determine whether the individuals that died were infected. The lack of a correlation between the number of oncospheres eaten and numbers of cysticerciods that developed precludes any attempt to assess mortality indirectly by oncosphere consumption.

Anderson and Gordon (1982) suggested that the variance to mean ratio of parasite abundance was a useful indicator of parasite-induced mortality provided that: i) there may be age-related changes in the average rate of infection; ii) there may be decreased overdispersion with age due to a decrease in the heterogeneity in the infection rate within older age classes or due to an increase in acquired immunity with age; or iii) chance effects could produce bizarre

patterns when the sample size is small. Conditions i and iii apply to our system; ii is less applicable because the amphipods were only exposed once although it may apply more closely if repeated exposures occurred.

The low survival of *H. azteca* exposed during Instars 1 and 2, the small cysticercoid populations that developed and the low variance to mean ratios in each suggest that larger populations were lethal to the young amphipods. While many *H. azteca* in Instars 1 and 2 ate entire packets, the mean intensity of infection in early instars remained low and no large infections were found in those that survived. The situation is less clear for Instar 3, but infection seems to have had little or no effect on the short term survival of older instars.

Most of the mortality seen in Instars 1 and 2 occurred by day 4, and in other instars, by day 7 postinfection. Schom *et. al*, (1981) reported two periods of mortality in infected beetles. The first occurred between 0 and 5 days postinfection due to damage resulting from the trauma associated with the infection process. The second period, with peak mortality on day nine or ten postinfection, was believed to be due to the effects of the developing cysticercoids on internal organs. The initial mortality seen in all instars in this study was likely due to trauma inflicted during penetration of the gut or the aftermath of it. The effects would be dose-dependant, reflecting the

number of oncospheres eaten, the number that survived ingestion and the number of those that established successfully. It appears that few amphipods in Instars 1 and 2 can survive the initial effects of large infections. Later mortality likely reflects the effects of increased parasite biomass on the host. Most of this occurred by day 7 postexposure, around the time the developing cysticerciods begin to encyst (Podesta and Holmes, 1970).

Conversely, infection by *M. hopkinsi* had a significant negative impact on the long-term survival of *H. azteca*. Survival times for infected males and females were significantly shorter than those observed for controls. The cause of mortality was not determined nor was the number of cysticercoids due to the extent of the decomposition that had occurred. It is assumed, however, that death was due to either a general interaction between the cysticercoid population and the individual host or due to effects on a particular organ or organ system. Freeman (1983) has also suggested that the host immune response may be more of a drain on host resources than the parasite.

The shortened lifespan of infected individuals has a potentially negative impact on transmission. Factoring in a developmental time of roughly 11 days for the *M. hopkinsi* cysticercoid population (Podesta and Holmes, 1970), infected individuals would be available for approximately 2 weeks before being eliminated from the population. This seems

counterintuitive and, as nothing is known of the infection levels and transmission dynamics of this parasite under natural conditions, any interpretation of this would be highly speculative. Compensatory factors might include behavioral modifications such as those reported for amphipods infected with acanthocephalans (Bethel and Holmes, 1973) that might increase chances of predation by the definitive host. Behavioral modifications have been reported for some cestode-beetle intermediate host systems (Keymer, 1980, 1981; Hurd 1990; Hurd and Fogo, 1991; Yan and Norman, 1995; and others). Anecdotal accounts of altered behaviour or behavioral morbidity in cestode-infected crustaceans also exist (see Freeman, 1983). Infected *H. azteca* in this study appeared hyperkinetic but these observations have not been quantified.

It is also important to acknowledge that the effects of infection on long-term survival may vary with instar. While *H. azteca* exposed during the fourth instar died prematurely, this may not necessarily be the case for amphipods infected during older instars. In older amphipods, where more of their growth has occurred, the effect of the parasites may not be as severe. Survival times in such individuals might approach that of the normal lifespan.

Cysticercoid burdens

A large percentage of the amphipods that survived the

14 day postexposure period was infected. However, there were differences in the numbers of individuals that were infected and in the numbers of cysticercoids present in the infected survivors of groups exposed during different instars. There was a significant relationship between the instar number at exposure and the resulting cysticercoid burden. The degree of aggregation in the cysticercoid populations within each instar also increased with age of exposure. Curiously, there was no correlation between the number of oncospheres eaten and the resulting number of cysticercoids present in any instar. Despite the fact that only packets well within the viability range were used (Lee *et al.* 1992), consumption of the entire packet did not guarantee that an infection would result. Individual variation in viability of oncospheres within a packet or in the viability of entire packets likely exists and this may have affected the number of oncospheres that established in each individual.

Alternatively, damage to oncospheres during ingestion, variability in the rate of food passage through the gut, (possibly influenced by recent feeding activity), degree of satiation or variability in hatching rates of oncospheres once in the gut may have also influenced the numbers that established. In particular younger *H. azteca*, with their smaller mouthparts, could damage oncospheres while eating the packet and their shorter intestines could limit the

window of opportunity for undamaged oncospheres to hatch and penetrate the gut. Once established, individual host responses, competition between developing larvae or parasite-induced host mortality may have also contributed to the overall variability in the numbers of cysticercoids seen. One, or any combination of the above, could account for the patterns observed.

Interestingly, *H. azteca* exposed during Instar 6 ate similar numbers of oncospheres as those exposed in Instars 4, 8 and 9 yet had fewer cysticercoids. The reasons for this are unclear. Sixth-instar *H. azteca* are in the early stages of sexual development. Host-parasite interactions may become more complex during periods of increased host energy investment in growth and/or reproduction (Baudoin, 1975), and this may have influenced infection success. This aspect is worthy of further study.

Host Growth:

Cestode infections are known to retard the growth and development of intermediate hosts. Tubificids infected with caryophyllaeid pleurocercoids fail to mature (see Freeman, 1983) and both Clarke (1954) and Mueller (1966) reported that cestode infections had a negative impact on moulting and development in copepods. The number of moults observed in infected individuals in each group was consistently less than that of controls. Based solely on moulting frequency,

it appears that infected individuals grew more slowly during the 14 day experimental period than contols regardless of when they were exposed. An important consequence is that sexual maturation may be delayed. Thomas *et al.* (1995, 1996a,b) found that the intermoult duration in *G. insensibilis* infected by *M. papillorobustus*, was prolonged. They suggested that by increasing duration of the intermoult period, *M. papillorobustus* reduced the probability that small or medium sized males will have access to the larger (and more fecund) females in the population later in life. Field studies revealed that a high proportion of the smaller, heavily infected males remained unpaired.

The consequences of parasite-reduced growth are potentially significant for male *H. azteca*. First, it may be difficult for an infected male to establish and maintain amplexus with a larger female. Secondly, reproduction in *H. azteca* is mediated by male-male competition in a size-assortative manner and larger males may displace smaller amplexed ones (Wen, 1992). Smaller infected males that successfully paired would be at risk of losing the female to larger, uninfected males.

Sex differences:

Despite reports that female copepods (e.g. Gruber, 1878) and female beetles (eg. Keymer, 1980; Schom *et al.*, 1981; Hurd 1990; Yan and Norman, 1995) were more heavily

infected than males, there were no differences in oncosphere consumption, susceptibility, parasite loads or growth, among male and female amphipods exposed during Instars 6, 8 and 9 and both short- and long-term survival of the two sexes was similar. It is generally believed that the differences in infection levels in copepods are due to sexual size dimorphism. Larger females can apparently harbour more parasites (Gruber, 1878; Shostak *et al.*, 1984; Shostak *et al.*, 1985). In beetles, the differences in infection levels between the sexes have been attributed to eggs being less palatable to males (Dunkley and Mettrick, 1971). A particular advantage of the *H. azteca* - *M. hopkinsi* system lies in the fact that the number of oncospheres fed to, and consumed by, each individual can be determined accurately. This permits a more stringent control over the infective dose than was possible in the preceeding studies. Males and females received similar dosages and developed similar infection levels within each instar.

EFFECTS ON REPRODUCTION

Host Fecundity:

Infection by *M. hopkinsi* affected fecundity and pairing behaviour in *H. azteca*. Infections by larval cestodes are known to affect reproduction in tubificids and crustaceans (see Freeman, 1983) and in beetles (eg. Keymer, 1980; Hurd

and Arme, 1986a, 1986b). Effects range from a reduction in fecundity to complete castration. Infections acquired in the fourth instar, before *H. azteca* began to develop sexually, had a significant negative effect on reproduction. Most infected females failed to produce broods. Those that did produced fewer offspring than controls. A concomitant effect was that the pairing success of infected females was severely reduced.

Several studies report a reduction or inhibition of ovarian development in amphipods as a consequence of infection (Reinhard, 1956; Zohar, 1993; Freeman, 1983). It is possible that infection prior to sexual development, during a period when the amphipod is growing rapidly, creates too great a metabolic strain on the animal for normal ovarian development to occur. Infection had a negative effect on growth and it seems reasonable, therefore, to assume that ovarian development would also be affected. This is consistent with Baudoin's (1975) views on the significance of the timing of infection and its effect on host development (Hurd, 1990). Given that some of the infected individuals eventually produced broods, it is likely that ovarian development was reduced or delayed rather than inhibited completely.

Alternatively, work on beetles infected with *H. diminuta* suggests that the parasites may reduce fecundity of the host by impeding the synthesis and/or uptake of

essential components by the eggs in the ovary thereby limiting the number of eggs produced (Hurd and Arme, 1986a; Maema, 1986; Webb and Hurd, 1996). Resolution of this question would require histological studies to assess the effects of infection on the development of the ovary and depending on the results, biochemical studies might be warranted. Although the infection seems to be the proximate cause of the decreased fecundity in *H. azteca*, the ultimate cause remains unknown.

It is also important to note that effects were observed in individuals exposed prior or the onset of sexual development and it is possible that *H. azteca* exposed in a more advanced state of sexual development would be less severely affected. It is also unclear whether the infected individuals would have improved reproductive success had the experiment been extended beyond the ninth instar. More infected females in the ninth instar had broods than in the eighth and their broods were larger. If the results reflect a delay in the onset of ovarian activity rather than inhibition, more females might produce broods at later instars although it is unlikely brood size would be as large as that of uninfected individuals.

Reproductive Behaviour:

The limited data on pairing success of infected individuals indicates that they are less likely to mate than

uninfected ones. While there is no data available for cestode-infected amphipods, field data suggest gammarids parasitized by acanthocephalans (Spaeth, 1951; Zohar, 1993) and digeneans (Thomas *et al.*, 1995, 1996b) have reduced pairing success.

In *H. azteca*, males initiate reproduction. There is no evidence that *H. azteca* uses chemical cues as a precursor to reproduction. Only tactile contact is required to initiate amplexus (Strong, 1972). Males will attempt to amplex with any female they encounter regardless of her reproductive state. Once in amplexus, males assess the readiness of the female to mate (Pennak 1953; de March 1978, 1981). Females close to mating will acquiesce whereas unreceptive females can usually escape within a few seconds (Wen, 1992).

In the pairing assays, no infected females were ever found in amplexus. As infected females were rarely capable of reproduction it seems likely that unreceptive females are too fast, or perhaps too strong for males to pair with successfully. Infected males can pair successfully and on this basis, the consequences of infection seem less severe than for females. That few infected males paired successfully suggests that they may have been under some disadvantage. Because both the infected and control males were in the same instar and of similar size in this experiment, size differences should have had little influence on the results. Infected males have decreased O_2

levels in the haemolymph which could seriously affect male tenacity and endurance (Reinhard, 1956; Freeman, 1983; Hurd, 1990). This could place them at a physiological disadvantage with uninfected males of comparable size.

Again, all individuals tested were infected before onset of sexual development. Effects of infection in more mature individuals therefore, may not be as severe as those reported here.

SUMMARY AND CONCLUSIONS

This is the first study to examine the effects of a cysticercoid infection over the lifespan of the intermediate host and, with the exception of the study by Lee *et al.* (1992), is the only study in which the infective dose given to each host was known. *Hyalella azteca* are susceptible to infection by *M. hopkinsi* throughout their lifespan. Heavy infections appear to reduce short-term survival in early instars but not in later ones. Most of the mortality in earlier instars seems to be due to the initial trauma caused by the establishment of the oncospheres. Long-term survival is compromised, however. Amphipods infected during the fourth instar die prematurely. Infection levels increase with age but there is no correlation between the number of oncospheres eaten and the numbers of cysticercoids that develop in each instar. Growth of infected individuals was

reduced regardless of the instar during which they were exposed. Infection levels, growth and survival were similar in males and females. Infection of males and females during the fourth instar had adverse effects on reproduction. Infected females seldom reproduced and those that did produced smaller broods than controls. Uninfected males did not pair with infected females. Only a few infected males paired successfully with uninfected females.

It is evident that infection by *M. hopkinsi* does have a negative impact on *H. azteca*. Some of the effects are stage specific with younger instars being more severely affected; others affect the host regardless of when it was infected. Infection before sexual maturity significantly affects fecundity in later instars. At this point, however, it is not known whether the effects on fecundity or long-term survival are consistent over all instars or whether they are specific to individuals infected during Instar 4.

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Table 1

Mean, standard deviation (± *SD*), and 95% confidence intervals of head capsule and body lengths for Instars 1 through 10 of *H.azteca*. All measurements in micrometers (μm).

	Head Capsule Lengths		Total Body Lengths	
Instar	length μm ± *SD*	Lower/Upper 95% C.I.	length μm ± *SD*	Lower/Upper 95% C.I.
1 n = 137	186.99 ± 8.67	185.52 / 188.45	1151.12 ± 75.97	1138.29 / 1163.96
2 n = 123	227.00 ± 12.65	224.75 / 229.26	1460.29 ± 50.21	1451.33 / 1469.26
3 n = 109	275.85 ± 11.59	273.65 / 278.05	1883.45 ± 68.09	1870.52 / 1896.38
4 n = 103	323.84 ± 11.58	321.57 / 326.10	2348.04 ± 108.85	2326.77 / 2369.31
5 n = 87	404.44 ± 13.85	401.49 / 407.39	2952.31 ± 149.64	2920.42 / 2984.20
6 n = 78	465.02 ± 21.70	460.13 / 469.92	3518.31 ± 246.17	3462.81 / 3573.81
7 n = 83	530.31 ± 21.33	525.65 / 534.96	4298.66 ± 234.92	4247.37 / 4349.96
8 n = 69	593.06 ± 23.45	587.43 / 598.69	4939.83 ± 123.07	4910.26 / 4969.39
9 n = 58	663.36 ± 13.71	659.76 / 666.97	5646.26 ± 158.41	5604.61 / 5687.91
10 n = 39	741.05 ± 22.21	733.85 / 748.25	6619.15 ± 269.90	6531.66 / 6706.64

Table 2

Egg packet consumption (less than half, over half, or all of the packet) by *H. azteca* of various instars during the 48 hour exposure period.

	Percentage of *Hyalella*		
Instar	<50% consumption	≥50%, <100% consumption	100% consumption
1 $_{n = 72}$	20.83	18.06	61.11
2 $_{n = 72}$	5.56	22.22	72.22
3 $_{n = 72}$	5.56	11.11	83.33
4 $_{n = 72}$	0	2.78	97.22
6 $_{n = 144}$	0	0.69	99.31
8 $_{n = 144}$	0	0.69	99.31
9 $_{n = 144}$	0	0	100

Table 3

Percentage of mortalities occurring in *H. azteca* by day four and day seven postexposure.

		Percent Dead By	
Instar	Total Deaths	Day 4	Day 7
1 $_{n = 72}$	39	85	94
2 $_{n = 72}$	31	61	77
3 $_{n = 72}$	19	37	78
4 $_{n = 72}$	8	35	37
6 $_{n = 144}$	22	45	59
8 $_{n = 144}$	29	31	72
9 $_{n = 144}$	26	15	57

Table 4

Variance to mean ratios for cysticercoid populations in *H. azteca* that survived to the end of the 14 day trial.

Instar at Time of exposure	Population Variance to Mean Ratio ($s^2/\mu x$)	Distribution
1 $_{n = 33}$	3.903/ 1.182 = 3.30	Aggregated
2 $_{n = 41}$	31.478/ 7.854 = 4.01	Aggregated
3 $_{n = 55}$	58.295/ 6.964 = 8.37	Aggregated
4 $_{n = 65}$	138.110/12.723 = 10.86	Aggregated
6 $_{n = 124}$	106.878/ 8.702 = 12.28	Aggregated
8 $_{n = 115}$	217.261/14.409 = 15.08	Aggregated
9 $_{n = 121}$	256.517/15.364 = 16.70	Aggregated

Table 5

The number of infected and control females producing broods in the eighth, the ninth, and both the eighth and ninth instars.

Group	Instar 8	Instar 9	Both Instars
Infected	1	4	1
Control	58	57	51

Table 6

Summary of pairings of infected and control female *H. azteca* with uninfected males.

	Females		
Pairings	All	Infected	Control
Total	7	0	7
Pairings/Trial	2.333 ± 0.577	0.0 ± 0.0	2.333 ± 0.577

Table 7

Summary of pairings of infected and control male *H. azteca* with uninfected females.

	Males		
Pairings	All	Infected	Control
Total	11	2	9
Pairings/Trial	3.667 ± 0.577	0.667 ± 0.577	3.0 ± 0.0

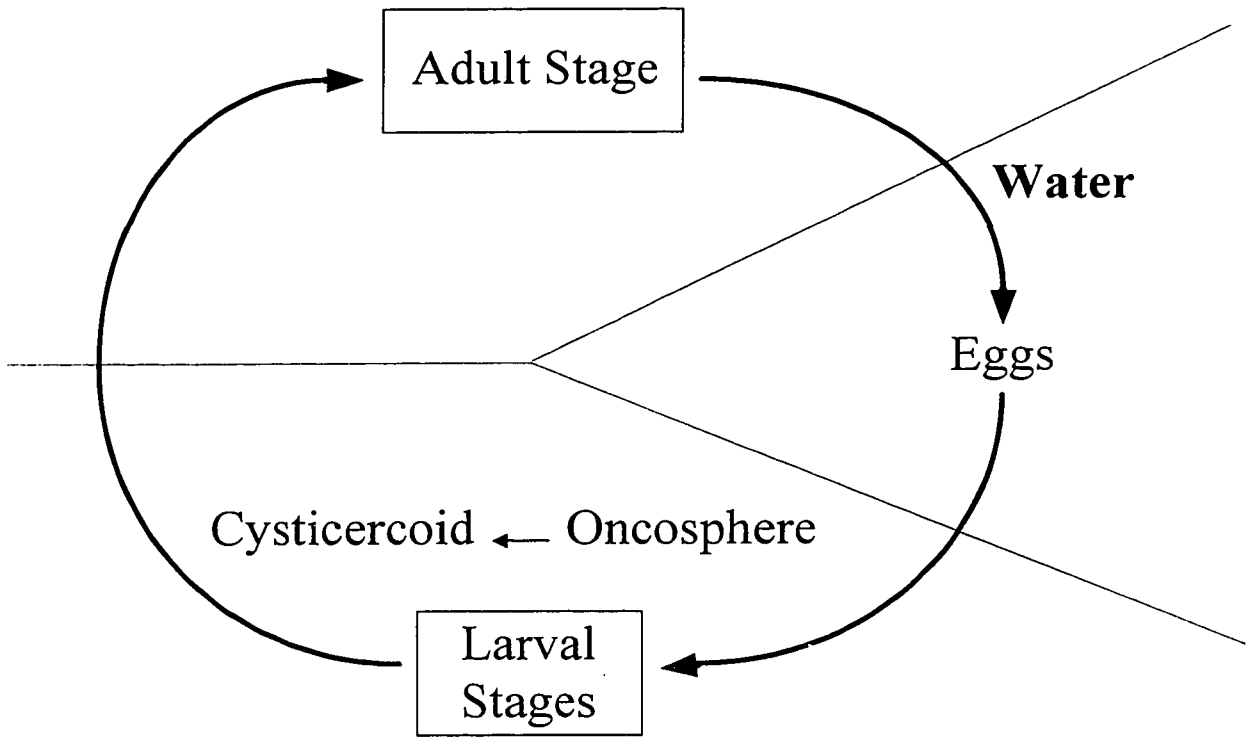

Figure 1: The life cycle of *Microsomacanthus hopkinsi.*

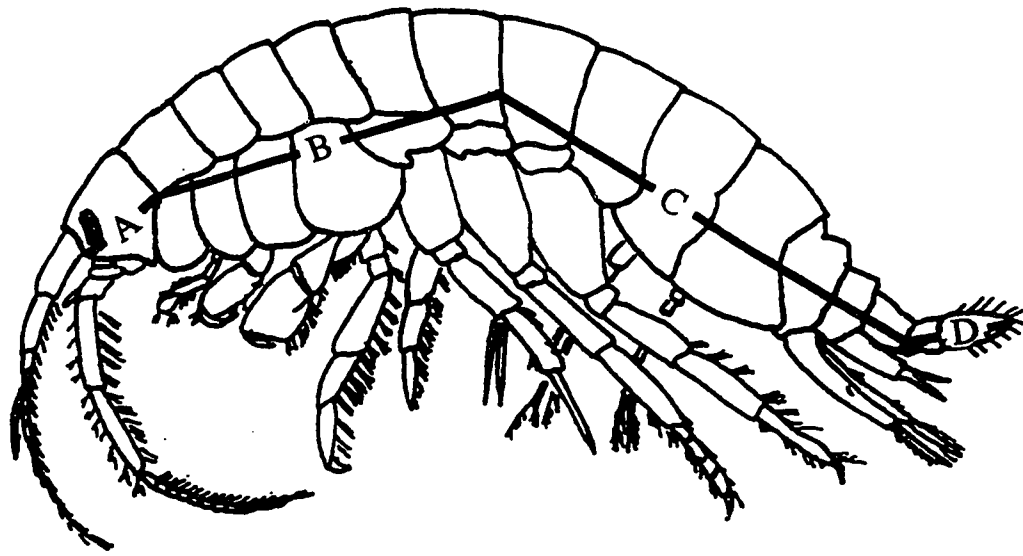

Figure 2: An amphipod, showing measurements taken to determine head capsule length (A) and body length (A+B+C+D).
(Modified from Pennak, 1953)

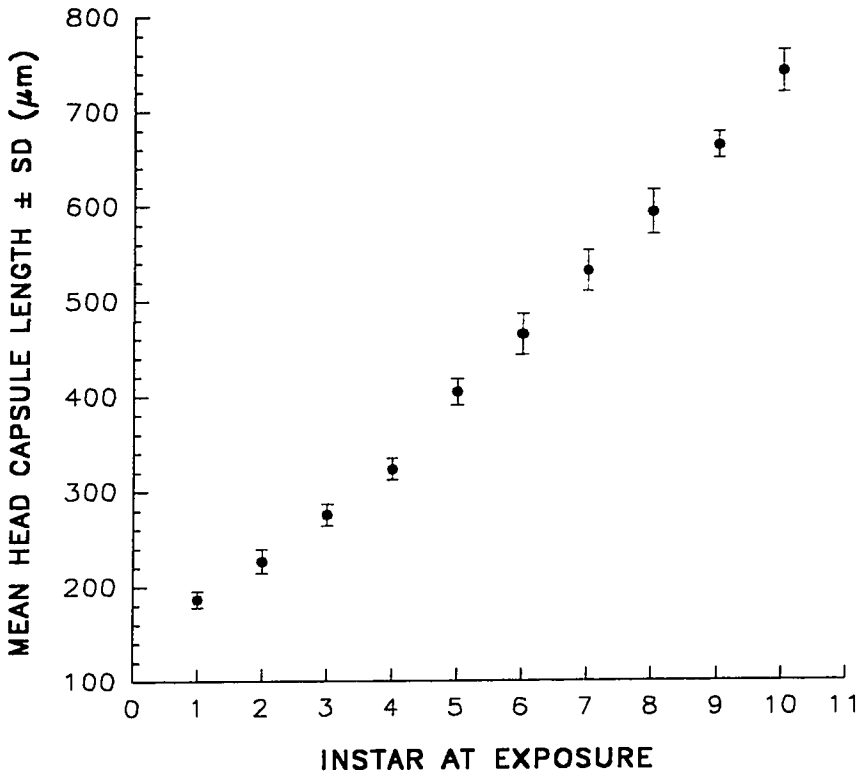

Figure 3: Mean head capsule lengths (± SD) of *H. azteca* in Instars 1 through 10.

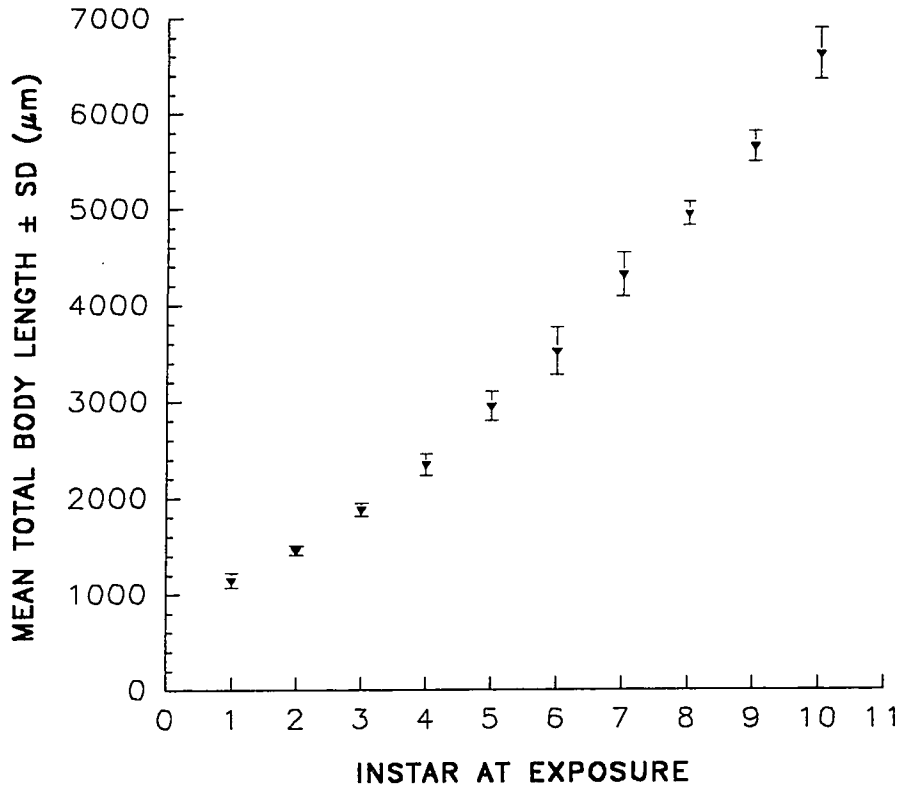

Figure 4: Mean body lengths (± SD) of *H. azteca* in Instars 1 through 10.

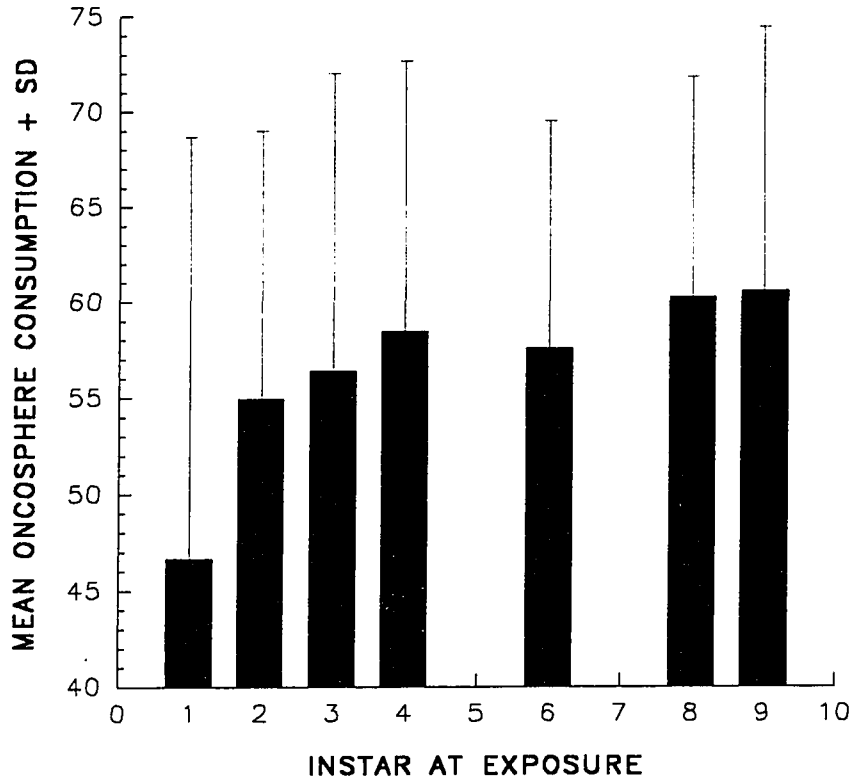

Figure 5: Mean number of oncospheres (+ SD) eaten by *H. azteca* when exposed during different instars.
Sample sizes: Instars 1–4, n=72; Instars 6, 8, and 9, n=144 (pooled data from males and females).

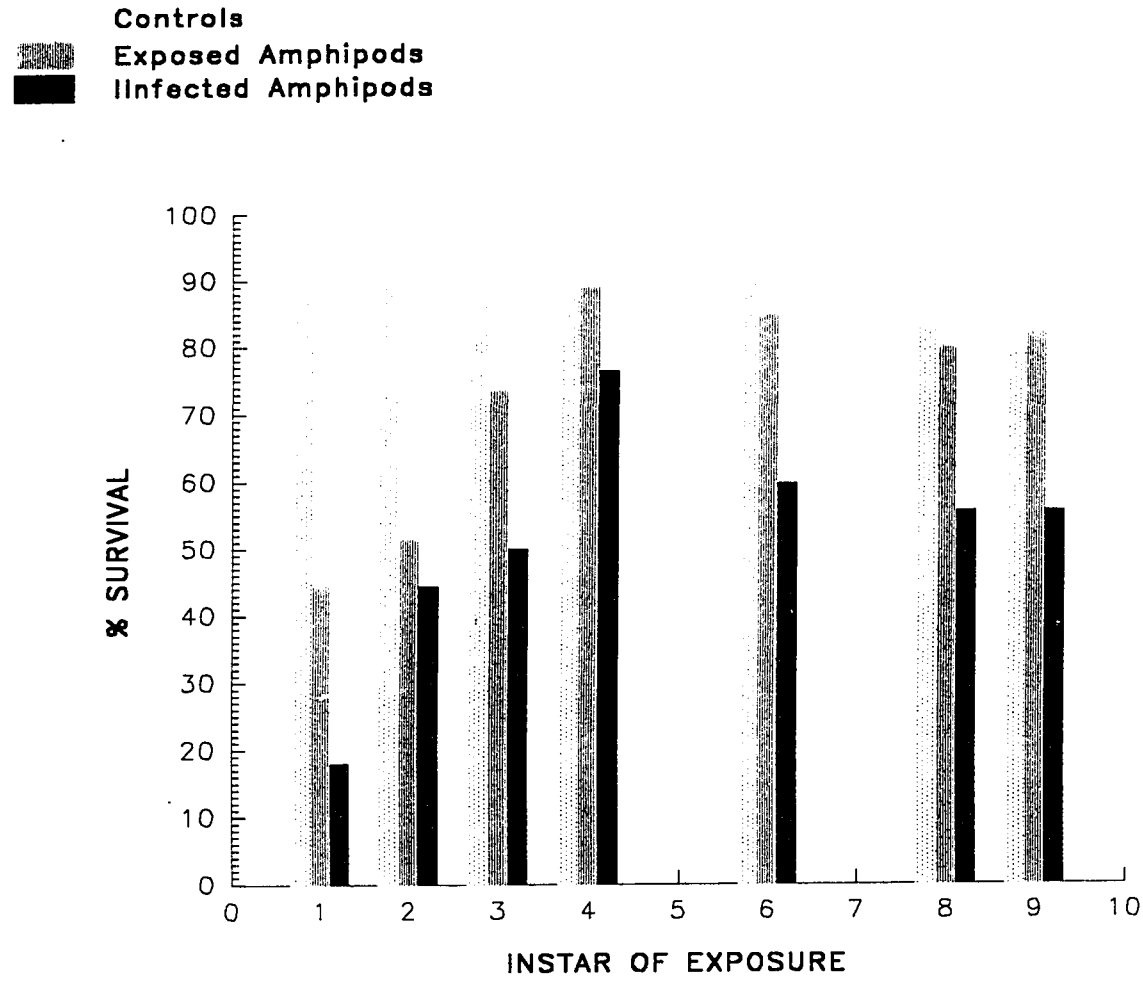

Figure 6: Overall percentage of control, exposed, and infected *H. azteca* that survived the 14 day experimental period. Sample sizes: Instars 1–4, n=72; Instars 6, 8, and 9, n=144 (pooled data from males and females).

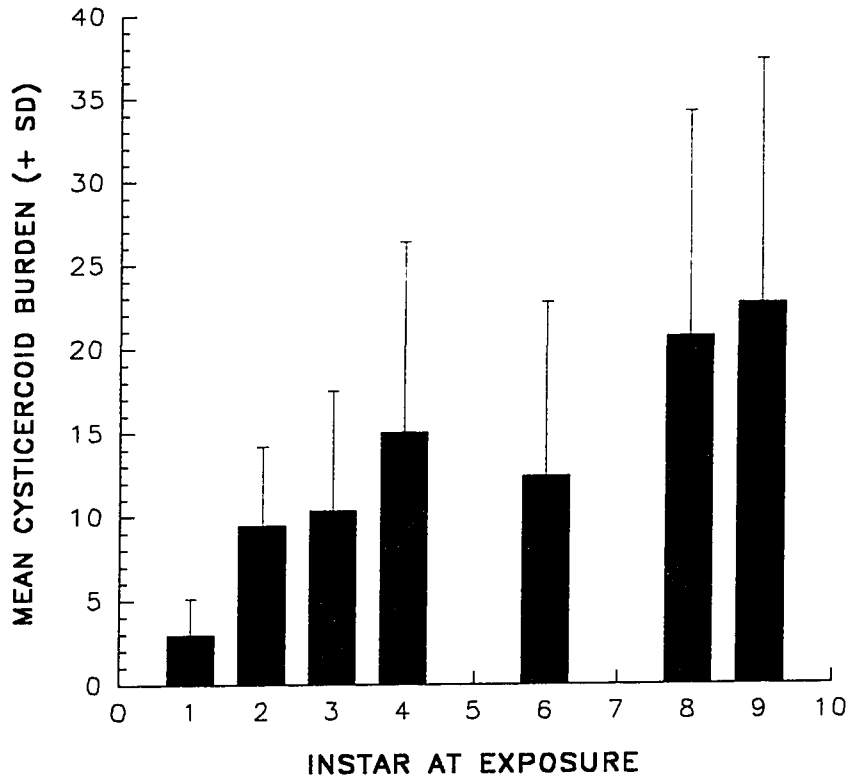

Figure 7: Mean number of cysticercoids (+ SD) at 14 days postexposure following infection of *H. azteca* at different instars.

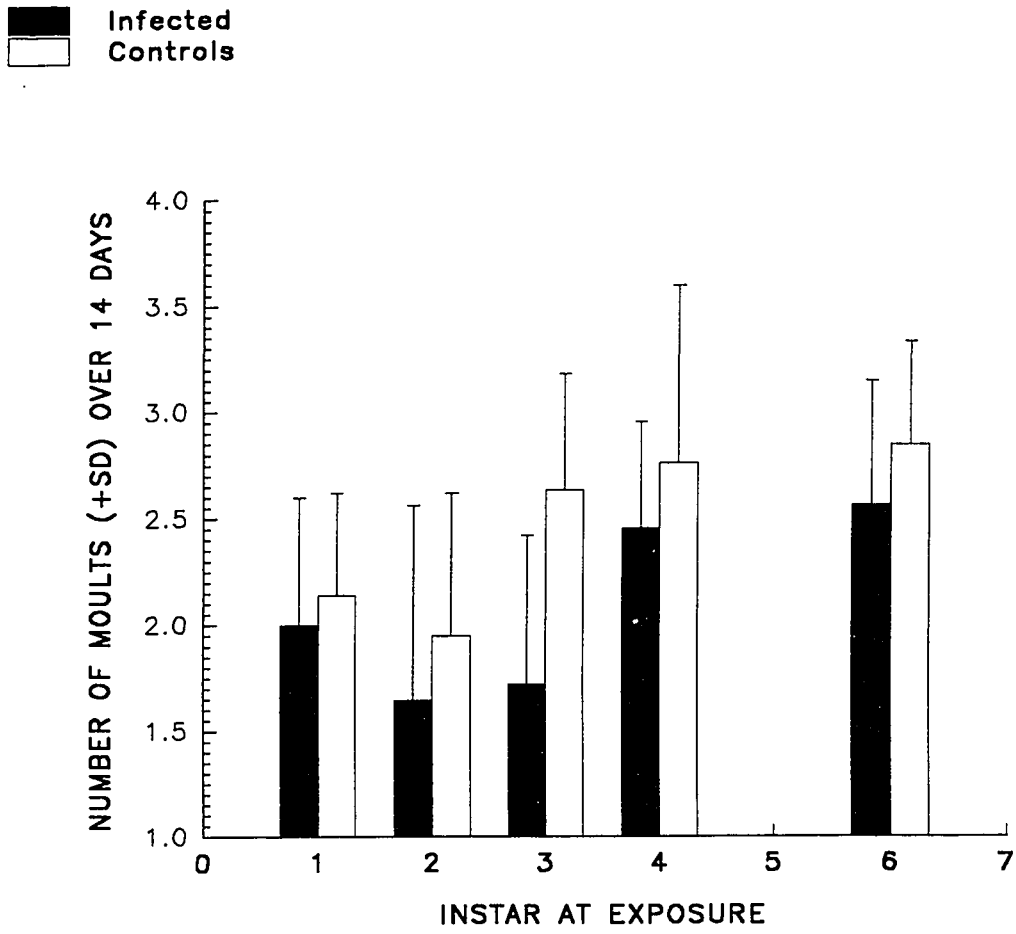

Figure 8: Comparison of the mean number of moults (+SD) occurring in infected and control *H. azteca* during the 14 days following exposure.

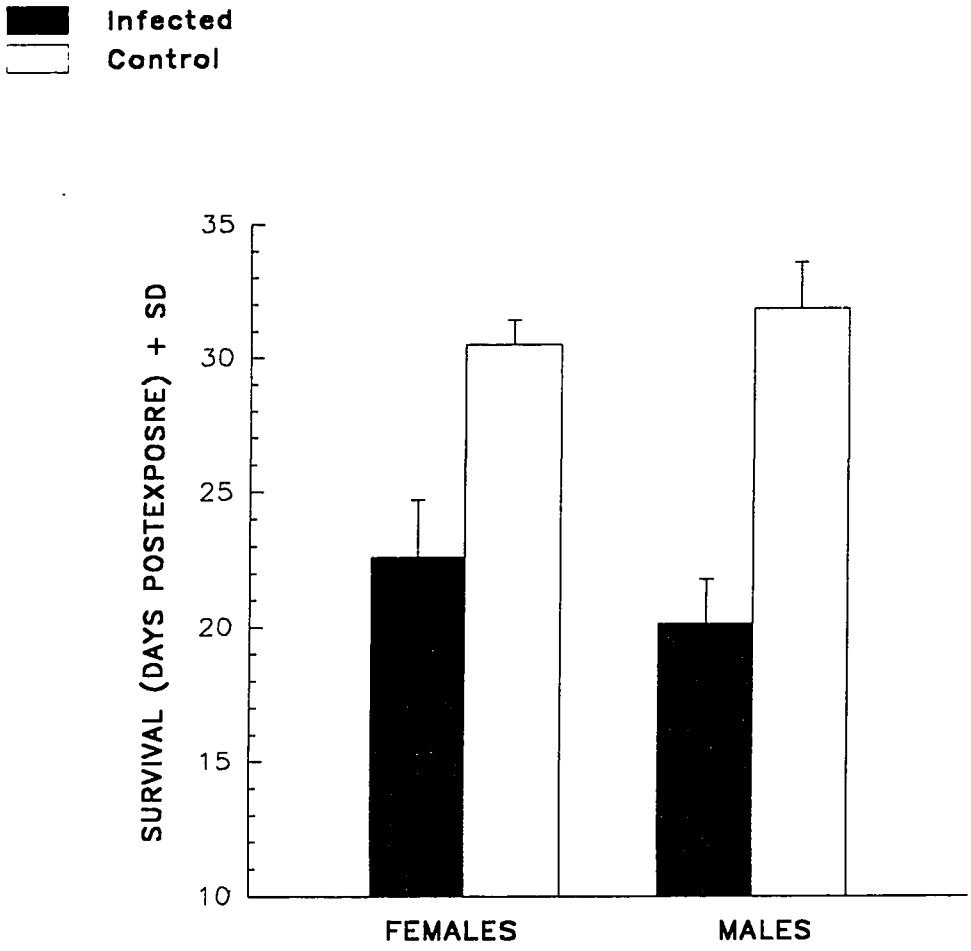

Figure 9: Comparison of the mean survival times (+SD) of female and male *H. azteca* infected during the fourth instar, with that of controls.

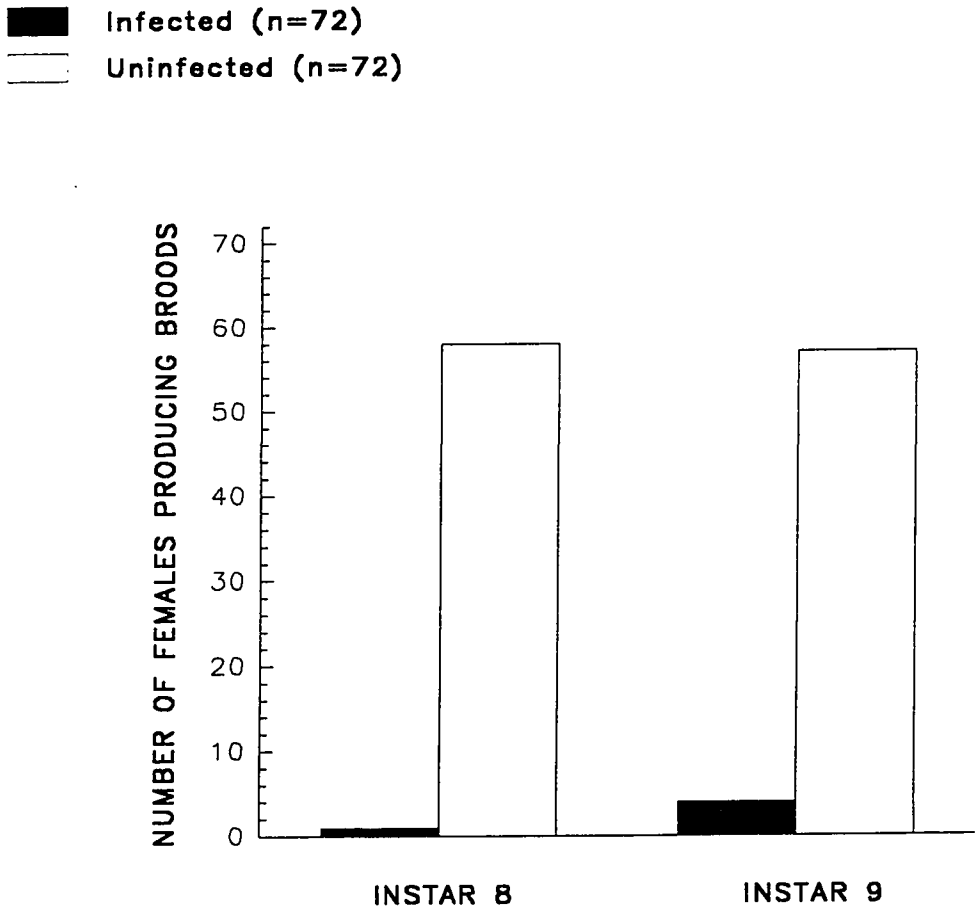

Figure 10: Comparison of the number of female *H. azteca* infected during the fourth instar, that produced broods in the eighth and ninth instar, with that of controls.

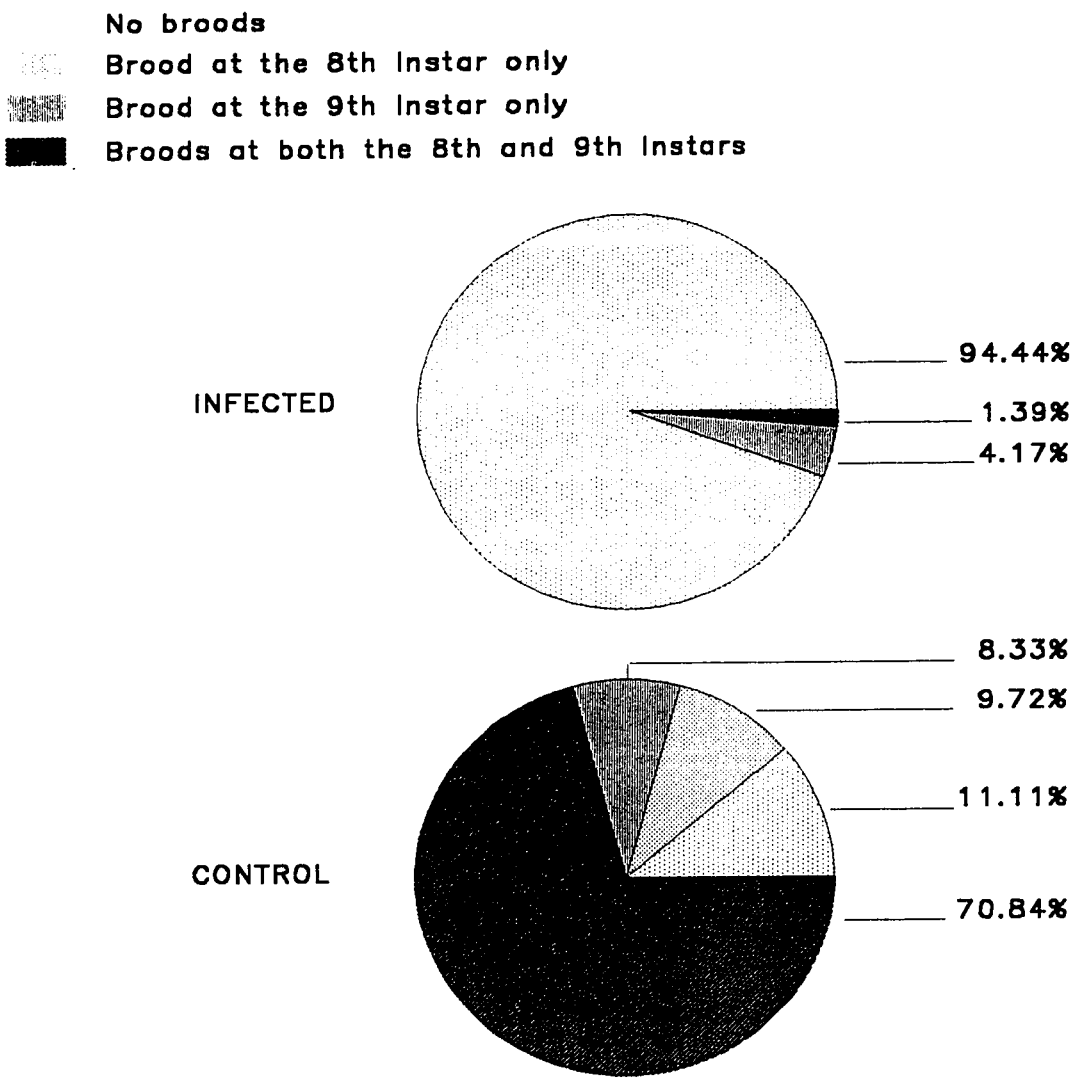

Figure 11: Comparison of the proportion of female *H. azteca*, infected during the fourth instar, that produced no broods, one brood during the eighth instar, one brood during the ninth instar, or broods in both instars, with that of controls.

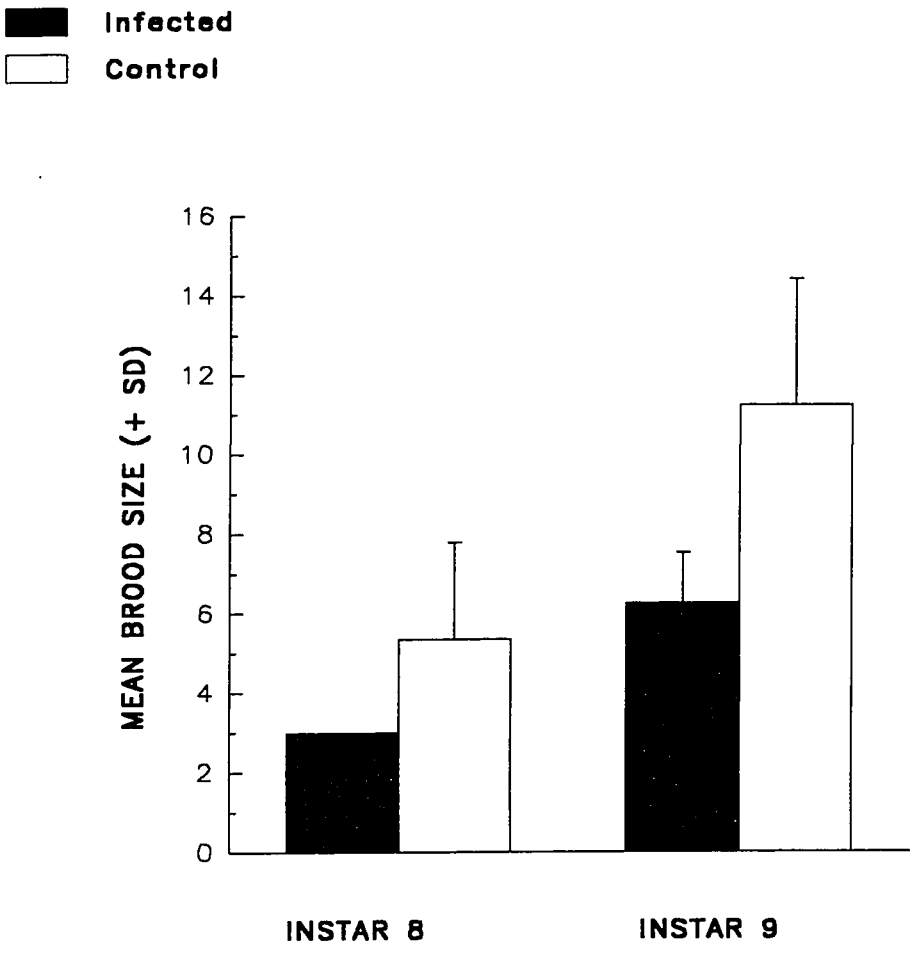

Figure 12: Comparison of the mean size of broods (+SD) produced during the eighth or ninth instar by female *H. azteca* infected during the fourth instar, with that of controls.