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The infection of nymphal *Baetis bicaudatus* by the mermithid nematode *Gasteromermis* sp.

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Abstract. 1. This study reports the infection in nymphs of a bivoltine mayfly host (*Baetis bicaudatus*) in a high-elevation watershed by the mermithid nematode *Gasteromermis* sp. Infection by *Gasteromermis* causes mortality in two ways. Fifty per cent of the infections do not successfully develop beyond the initial stage of penetration and result in the early death of both host and parasite.

2. Infected hosts that survive this initial stage are rendered completely sterile by the infection (reproductively dead). In addition to complete sterility, the emergence size of parasitized nymphs is reduced and development time lengthened compared with unparasitized nymphs.

3. Parasite infection levels are stable from year to year at one site, but with a higher incidence of infection in the mayfly summer generation. Size differences between the generations at the time of infection may account for their different susceptibilities.

4. Within a year infection levels vary seasonally and spatially from 1 to 71%. Seasonally, there is a condensation of parasitized hosts towards the end of development as unparasitized nymphs emerge earlier. Spatially, infection levels show a downstream decline that may result from upstream dispersal by infected hosts or differential parasite survivorship at different elevations.

Key words. Mermithid, mayflies, parasitism, growth

Introduction

Mermithid nematodes have an obligate endoparasitic larval stage in a variety of invertebrates, particularly aquatic insects. As a group they have received considerable attention as potential biological control agents (Petersen, 1973; Nickle, 1984; Elsey, 1991). Because of their conspicuous size, mermithids have also often been reported in the ecological literature (Söderström & Johannsson, 1988; Peckarsky *et al*., 1993; Pritchard & Zloty, 1994), but few studies have considered their ecology in any detail (Hominick & Welch, 1980; Popiel & Hominick, 1992). This lack of attention is unfortunate, because the scant literature available suggests that mermithids can have significant ecological impacts not only on host regulation (Garris & Noblet, 1975; Hominick & Tingley, 1984; Anderson & Shemanchuk, 1987; Harkrider, 1988), but also on individual growth and behaviour (Benton & Pritchard, 1990; Andersen & Skorping, 1991).

The purpose of this study was to investigate the infection of the mayfly nymph, *Baetis bicaudatus* Dodds (Ephemeroptera: Baetidae) by the mermithid nematode *Gasteromermis* sp. Micoletzky (Nematoda: Mermithidae) from a high-elevation watershed over a 4-year period. Data are reported on: (i) the seasonal infection level of the parasite; (ii) the impact of the parasite on host growth, fecundity and development and (iii) the year-to-year and spatial infection levels of the parasite within the river.

Materials and Methods

Baetis bicaudatus nymphs were collected from a 12-km stretch of the headwaters of the East River, Gunnison County, Colorado (Fig. 1). The East River is a cold-water, high-elevation trout stream fed by a number of permanent and temporary tributaries. It originates from snow-melt at Emerald Lake (3485 m) and flows south through a gradient of subalpine spruce–fir–aspen forests to montane meadow landscape (3010 m) (Peckarsky, 1979). Much of the river has an open canopy and a coarse, cobble substrate.

The mayfly host is bivoltine. Nymphs of the winter generation

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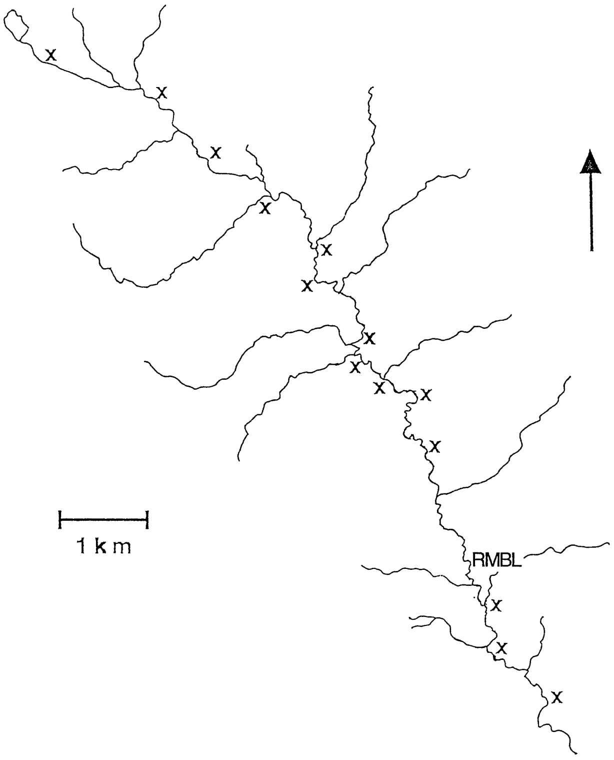

Fig. 1. Map of sample sites (x), including the site of the Rocky Mountain Biological Laboratory (RMBL), on the East River, Gunnison County, Colorado from the river source at lake outlet (3485 m) to an altitude of 3100 m, 12 km downstream.

overwinter from October with little development until the following spring when they emerge in late June and early July. Nymphs of the summer generation hatch and grow rapidly from early July and emerge in September (Cowan & Peckarsky, 1994).

Free-living stages of the mermithid *Gasteromermis* sp. (Poinar, 1991) penetrate the integument of the host and then grow within the abdomen of the nymph absorbing nutrients from the haemocoel. Upon completion of its development the mermithid, which now fills the host abdomen, ruptures the host cuticle. The emergence of the parasite kills the host. Once free of the host, the mermithid matures to adulthood where mating and oviposition complete the life cycle. Emergence may occur from immature or adult stages of the host [see Hominick & Welch (1980) for more details of life cycle]. In *B. bicaudatus* the parasite does not interfere with the emergence of its host (Flecker & Allen, 1988; Vance, 1996).

Seasonal infection levels, host growth and fecundity

Samples to document the annual infection levels were taken from the East River at the site of the Rocky Mountain Biological Laboratory (RMBL), Gothic (3225 m). Monthly samples of 300–400 *B. bicaudatus* nymphs were taken from June 1992 to June 1993 by disturbing the substrate upstream of an aquatic D-frame net (mesh size = 100 µm). All samples were immediately preserved in 70% ethyl alcohol. The two generations were separated based on cerci morphology and wing pad development (Cowan & Peckarsky, 1994). Because nymphal stages of mayflies cannot be determined (Clifford *et al*., 1979), samples from each generation were divided into four developmental classes – stage I (no wing pads), stage II (wing pads shorter than wide), stage III (wing pads longer than wide) and stage IV (wing pads darkened – 24 h prior to emergence) (Delucchi & Peckarsky, 1989). All nymphs were dissected to determine their parasitized state. All stage IV nymphs were examined for testes and eggs. The head capsule widths of a random subset of parasitized and unparasitized nymphs from each stage were measured.

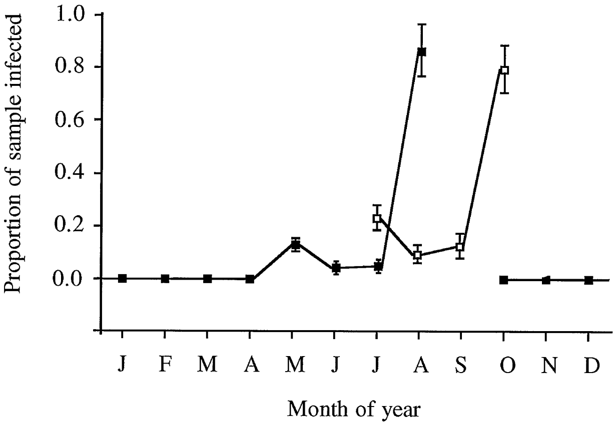

Fig. 2. Seasonal infection rate of *Baetis bicaudatus* nymphs by *Gasteromermis* for summer (□) and winter (■) host generations from 1992 to 1993 at the RMBL site. Summer generation nymphs are only present from July until October. Winter generation nymphs occur from October through to the following August. Data are shown as the proportion of a single sample infected ± 1 SE. Standard errors were calculated by (proportion/√*n*) (Snedecor & Cochran, 1989).

Year-to-year and spatial variation in infection levels

Single samples of stage IV *B. bicaudatus* nymphs (*n* = 200–300) from the winter and summer generations were collected from the RMBL site from 1991 to 1994 to document yearly fluctuations in infection levels.

Single samples of stage IV nymphs (*n* = 100–150) from the winter (1993) and summer (1994) generations were also taken to document spatial variation in infection levels. Fifteen sites along a 12-km stretch of the East River valley from its outlet at Emerald lake (3485 m) to where the river enters lower gradient valley (3010 m) were sampled (Fig. 1). All samples were immediately preserved in 70% ethyl alcohol and later dissected to determine parasitized state.

Results

Seasonal infection level

Both summer and winter generations of *B. bicaudatus* were infected by *Gasteromermis* at the RMBL site in 1993–94

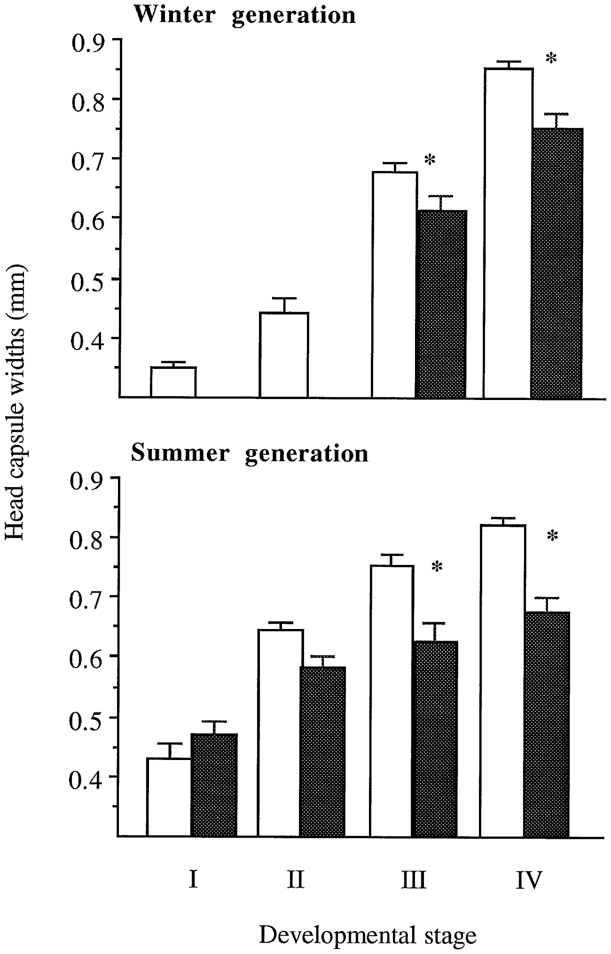

Fig. 3. Head capsule widths of unparasitized (□) and parasitized (■) *Baetis bicaudatus* nymphs from the RMBL site for each developmental stage (I–IV). Data are shown as mean ± 1 SE. * indicates $P < 0.05$.

Table 1. Year to year variation in the percentage of *Baetis bicaudatus* nymphs infected by *Gasteromermis*. Data are shown as the percentage of individuals infected each year from a single sample of stage IV nymphs from the RMBL site. Both generations were sampled each year and the size of each sample is shown (n).

	Percentage infected	
Year	Winter generation	Summer generation
1991	5.1%, $n = 61$	9.3% , $n = 195$
1992	3.8%, $n = 277$	8.4%, $n = 375$
1993	2.2%, $n = 392$	8.9%, $n = 416$
1994	3.6%, $n = 209$	7.9%, $n = 298$

(Fig. 2). All infected individuals contained a single mermithid parasite. Other parasites infecting the mayfly nymphs were extremely rare (sixteen unidentified fungal infections out of ≈ 5000 individuals spread over 4 years). The summer generation was already infected with *Gasteromermis* when *B. bicaudatus* first appeared in the samples as stage I nymphs (July, proportion of the sample infected (P) = 0.22, $n = 330$, SE = 0.012). Standard errors were calculated by (proportion/$\sqrt{n}$) (Snedecor & Cochran, 1989). The nematode was in a crescent shape in this sample, indicating that the host had recently become infected (Nickle, 1984). Infection levels dropped by half in stage II and III nymphs (August, $P = 0.11$, $n = 386$, SE = 0.01) but then rose rapidly in late stage IV nymphs (October, $P = 0.79$, $n = 213$, SE = 0.05). No summer generation nymphs were found after this date until the following June (stage I nymphs). The infection pattern was different in the winter generation. Parasitism was not detected in the overwintering *B. bicaudatus* instars until the host was at stage III in May ($P = 0.13$, $n = 332$, SE = 0.01). Mermithids in this sample were in a crescent shape, suggesting recent infection (see above). Infection levels dropped in June to $P = 0.08$, $n = 216$, SE = 0.01 (still stage III nymphs) but then rose rapidly through August (late stage IV nymphs, $P = 0.83$, $n = 183$, SE = 0.06). No further winter generation nymphs were found after this date until October (stage I nymphs).

Host growth and fecundity

Parasitized individuals had head capsule widths that were indistinguishable from their unparasitized counterparts early in development – stage I and II (Fig. 3). By stages III and IV, however, parasitized individuals were significantly smaller than unparasitized nymphs; *t*-test of unparasitized vs. parasitized head capsule width for stage III winter $P = 0.023$, summer $P = 0.015$; for stage IV winter $P = 0.007$, summer $P = 0.002$ (unparasitized sample size = 18, parasitized sample size = 14 for each comparison). Unparasitized stage IV nymphs had well developed eggs and testes that were clearly visible upon dissection ($n = 211$). Parasitized nymphs at the same stage of development had no developing eggs or testes ($n = 181$).

Year to year and spatial variation in infection levels

Infection levels at the RMBL site varied little annually from 1991 to 1994 (Table 1). ANOVA of transformed data ($\arcsin\sqrt{\text{proportions}}$) for summer generation $P = 0.225$; winter generation $P = 0.326$.

All fifteen sites within the East River valley had detectable infections of mermithids. The infection rates ranged from 4 to 71% for the summer generation, 1 to 55% for the winter generation. For both generations there is a significant downstream decline in the proportion of the *B. bicaudatus* sample parasitized (Fig. 4). Regression of $\arcsin\sqrt{}$transformed data for summer $P = 0.002$, for winter $P = 0.016$.

Discussion

Both generations of the *B. bicaudatus* nymphs are parasitized by mermithid nematodes. Nymphs were only ever found infected with a single mermithid worm. This suggests that the parasite population is under-dispersed throughout its host population. In parasite systems in which more than one parasite

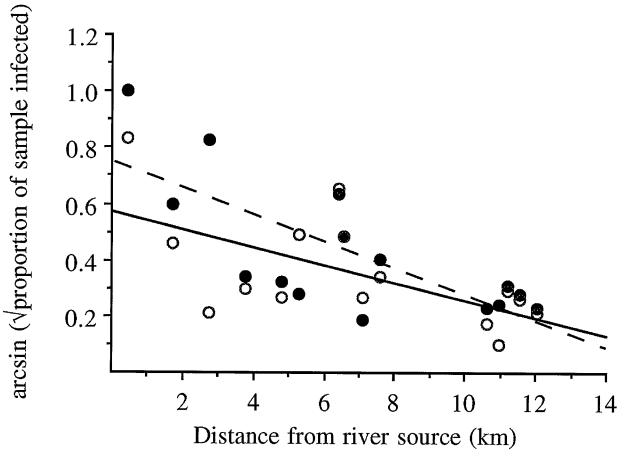

Fig. 4. Downstream decline in proportion of *B. bicaudatus* stage IV nymphs infected by *Gasteromermis* for winter (○, solid line) and summer (●, dashed line) host generations. The fifteen sites (see Fig. 1) are plotted successively as distance from river source. Both regressions are significant ($P < 0.05$). Untransformed infection levels range from 1 to 71%.

results in the death of the host, and/or in systems where sex determination is strongly influenced by parasite crowding [both of which occur in mermithids (Hominick & Tingley, 1984)], low degrees of aggregation of the parasite within the natural host population will be favoured (Tingley & Anderson, 1986).

The summer and winter *B. bicaudatus* generations are differentially impacted by the parasite. The parasite does not infect its host in the winter months (October–April). When parasitism first appears (May) the winter generation *B. bicaudatus* nymphs are much larger than the summer generation. Attempted parasitism by mermithids decreases with host size (Finney & Mokry, 1980; Petersen, 1981) and this may explain the lower infection level found in the winter generation.

For both generations infection levels are highest in the early instars. This could be explained by hatching of uninfected individuals into the population diluting the percentage of parasitized nymphs. This seems unlikely, however, because the *B. bicaudatus* cohorts are synchronous (Cowan & Peckarsky, 1994). It is more likely that the early drop in infection levels is due to infections that do not successfully develop beyond the initial stage of penetration, thus resulting in the death of both the parasite and the host. Such early mortality is found in many other mermithid–insect systems (Petersen *et al.*, 1967; Finney & Mokry, 1980). For *B. bicaudatus* this is therefore an important (and easily missed) source of early nymphal mortality that has not previously been documented (Cummins & Wilzbach, 1988). In the summer generation 22% of the population is initially infected, of which half dies to leave an infection rate of 11%. *Gasteromermis* infection therefore appears to kill 11% of the summer generation as stage I nymphs. For the winter generation the initial infection rate is 15%, 40% of which die soon after infection resulting in 9% of the mayfly population being killed as stage III nymphs (nymphs are older than summer generation when infection occurs).

The impact of mermithid infection on growth and development is similar for both generations. All infected individuals contained a single mermithid nematode and by emergence (stage IV) were castrated by the infection. Because castrated individuals are reproductively dead, this is an additional mortality event caused by parasitism affecting around 11% of the summer mayfly generation and 6% of the winter generation. During the early stages of infection, parasitized individuals are the same size as their unparasitized counterparts. The final size of stage IV nymphs, however, is considerably smaller for parasitized individuals. Reduction in host size has been reported from other mermithid infections (Schmidt & Platzer, 1980; Galloway & Brust, 1985) but not previously for mayfly hosts.

The rapid increase in the infection level towards the end of each host generation is misleading. Mermithids slow the development of their hosts and this results in a condensation of parasitized hosts as non-parasitized individuals emerge (Welch, 1965; Nickle, 1974; Flecker & Allan, 1988). Parasitized *B. bicaudatus* nymphs were the last to complete nymphal development and emerged up to 4 weeks later than unparasitized nymphs.

When repeatedly sampled at the same developmental stage (stage IV), infections of *B. bicaudatus* nymphs by *Gasteromermis* appeared at consistent levels from year to year in both host generations. This is the first study to document the natural year-to-year infection dynamics of mermithids in mayflies and shows that mermithid parasites are capable of maintaining stable and persistent infection levels in this host species.

Infections of *B. bicaudatus* nymphs by *Gasteromermis* are widespread in the East River valley. Overall there was a downstream decrease in infection levels. An upstream concentration in infection levels has been suggested to be caused by the upstream migration of winged adult infected hosts (Colbo & Porter, 1980). *Gasteromermis* does not interfere with mayfly emergence and infected *B. bicaudatus* adults have been reported (Flecker & Allen, 1988; Vance, 1996). Alternatively, different sites may affect the survival of the free-living mermithid due to their physical attributes (Colbo, 1990; Andersen & Skorping, 1991) and this could also influence infection levels.

Infection by the mermithid nematode *Gasteromermis* was found wherever *B. bicaudatus* was present and appears to be an important component of *B. bicaudatus* nymphal ecology. Infection rates of the mermithid showed a complex seasonal and spatial phenology with drastic impacts on individual survival, growth and fecundity. Infections kill or castrate a significant proportion of the mayfly population and infection rates vary little from year to year. The data presented underscore the potential importance of parasitic interactions in the ecology of stream systems.

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