

Metacercarial utilization of a naturally infected single species (*Lymnaea peregra*) snail community by *Echinoparyphium recurvatum*

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Abstract

Utilization of a single-species molluscan community of *Lymnaea peregra* by metacercariae of *Echinoparyphium recurvatum* over a summer (July–September) period in south-east England showed an increase in the mean number of cysts per host with host size and time of exposure. Aggregation resulting from host and habitat-related factors increased with host size and time of exposure. Encystment within the host was restricted to the peripheral organs in smaller juvenile snails but as snails increased in size, metacercariae were distributed throughout the tissues.

Introduction

Echinostomes, at the metacercarial stage, can utilize a wide variety of invertebrates and vertebrates as second intermediate hosts. In particular, many molluscan species are utilized as hosts but with differing degrees of susceptibility (Huffman & Fried, 1990). Metacercarial utilization of the second intermediate host is under the control of a range of biotic and abiotic factors. Evans *et al.* (1981) considered that in the case of *Echinoparyphium recurvatum* the presence of primary infections in the first intermediate host, temperature, cercarial chemotaxis, cercarial density, second intermediate host size and resistance to infection, and the time of exposure to cercariae are all important factors in transmission. However, the importance of metacercarial dispersion patterns within the second intermediate host in relation to cercarial transmission has rarely been investigated. Indeed Evans *et al.* (1981) indicated that although the chance of *E. recurvatum* cercariae encountering a molluscan host does largely depend on the time of exposure, spatial overlap between host and parasite in the environment is also an important factor. This has been experimentally demonstrated by McCarthy (1990) who concluded that higher levels of cercarial transmission occurred in contagiously distributed *Lymnaea peregra* than

in evenly or randomly distributed snails and that transmission was related to host population size.

Echinoparyphium recurvatum has proved to be a popular model in studying the transmission of echinostome cercariae under experimental conditions (Evans, 1982; Evans & Gordon, 1983a,b; McCarthy, 1990, 1999; Adam & Lewis, 1992; Morley *et al.*, 2002) with high levels of compatibility between *L. peregra* and *E. recurvatum*, but little is known about the distribution of metacercariae under field conditions. Evans *et al.* (1981) found that a multi-species molluscan community showed a variable susceptibility to *E. recurvatum*, with the clam *Pisidium subtruncatum* and the prosobranch *Valvata piscinalis* being the most utilized species. However, experimental studies have shown that mixed populations of molluscs can influence the recruitment of metacercariae into the most susceptible species with certain species acting as 'decoy' hosts (Evans & Gordon, 1983a; McCarthy & Kanev, 1990). In the present study, a field site in south-east England containing a single-species snail community of *L. peregra* was studied over a continuous three month period during the summer of 1985 mainly because previous studies by Adam & Lewis (1993) had shown that snail abundance, the prevalence of metacercarial infections and temperature were optimal during this period. The present paper therefore considers whether or not the metacercarial cyst load and encystment sites of *E. recurvatum* within the *L. peregra* community are related to snail size and the time of exposure.

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Materials and methods

Field site

Snails were collected from a stream in Bushy Park, Hampton, Middlesex (National Grid Reference TQ 160694) during July to September 1985. This stream, which connects two leisure ponds (Heron lake and Diana fountain), is slow flowing and the surface of the water is covered by floating vegetation especially during the summer months. The stream is shallow with a maximum depth of 30 cm and forms an L-shape course about 200 m × 2 m. The bottom is composed of soft mud and sand, matted with dense filamentous algae. Little vegetation grows on the banks because of regular trimming. The invertebrate fauna is rich, mainly comprising *L. peregra*, insect larvae and crustaceans. The most prominent fish species is the three-spined stickleback, *Gasterosteus aculeatus*, and the two ponds connected by the stream attract large numbers of resident mallards. Only half the length of the stream (inflowing into the Diana fountain via a culvert under a road) was sampled. As the relative abundance of *L. peregra* in the sampling area varied between 93–97% of the molluscan population, the community is considered a single-species one.

Sampling and data analysis

Snails were collected using a 30 cm² quadrat which was randomly dropped into the stream. Snails and, in some cases, lumps of algae in which the snails were entangled were transferred to polythene bags and fixed immediately in 4% formaldehyde to prevent cross infection *en route* to the laboratory. The number and size of snails in each sample were measured to the nearest 0.1 mm. Following snail dissection, the number of infected snails with cercarial and metacercarial infections and the distribution and number of metacercarial cysts in the snail tissues were recorded. The occurrence of cysts within the soft tissues of the host were divided into two categories: (i) cysts restricted to peripheral organs such as the mantle lobe and foot, and (ii) cysts occurring throughout the entire soft tissues. The distribution of metacercariae within the host population was analysed from each monthly sample by calculating the mean intensity of infection, standard deviation, variance/mean ratio, aggregation (*k*) and the goodness of fit of data to the negative binomial distribution (*P*) according to Elliott (1977) and Fowler *et al.* (1998). A conservative *P* value of 0.001 was taken to indicate significance because of the increased possibility of Type I errors at the standard *P* value of 0.05 according to Shaw *et al.* (1998).

Results

The density of the *L. peregra* population increased from July to September with an aggregated distribution on the stream bottom. Young snails were relatively rare and were mostly found near the bank. The size structure of *L. peregra* demonstrated a rapid change during the summer months (fig. 1). In July and August there was a wide range in snail size indicating both juvenile and adult generations. This suggested that breeding continued

during these months, although the absence of juveniles in September showed that breeding had now ceased. Nevertheless, by September the rapid growth of young snail generations and the death of parental generations resulted in a normal distribution of size classes of *L. peregra*. Snails harbouring primary cercarial infections increased from 20.4% in July to a peak of 69.9% in August, with about 30% of infections being prepatent, before declining to 56.7% in September. This coincided with an increase in the mortality of adult snails.

The smallest size class of snails harbouring a metacercarial infection in July was 10–10.9 mm with a peak in prevalence of 100% occurring in the size range 15–17.9 mm (table 1, fig. 1). During August, the size classes of snails containing metacercariae extended to a range of 7–17.9 mm with 96–100% prevalences occurring in the 10–17.9 mm size categories (table 2, fig. 1). During September, all size classes of snails contained metacercarial infections, with the largest snail category of 10–14.9 mm showing 97–100% prevalences (table 3, fig. 1).

The mean intensity and standard deviation of metacercarial cysts increased with a corresponding increase in snail size from July through to September (tables 1–3). The variance/mean ratios for individual

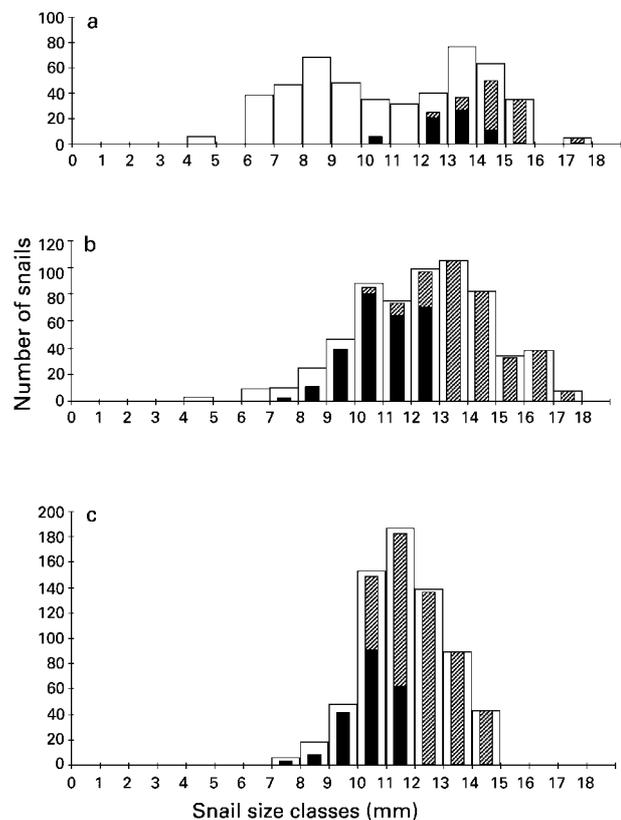


Fig. 1. Size frequency distribution of *Lymnaea peregra* and sites of encystment of *Echinoparyphium recurvatum* during (a) July 1985, (b) August 1985 and (c) September 1985. ■, Number of snails in which cysts were found in the mantle lobe and foot only; ▨, number of snails in which cysts were found in the entire tissues; □, total number of snails.

Table 1. Metacercarial utilization of *Lymnaea peregra* by *Echinoparyphium recurvatum* during July 1985 relative to snail size.

Snail size class (mm)	No. examined	Prevalence (%)	Mean intensity	Standard deviation	Variance/mean ratio	<i>k</i>	<i>P</i>
4-4.9	6	0	0	0	-	-	-
5-5.9	0	0	0	0	-	-	-
6-6.9	12	0	0	0	-	-	-
7-7.9	15	0	0	0	-	-	-
8-8.9	14	0	0	0	-	-	-
9-9.9	14	0	0	0	-	-	-
10-10.9	14	17.0	4.0	6.40	10.24	0.43	0.367
11-11.9	0	0	0	0	-	-	-
12-12.9	21	62.5	5.8	5.39	5.00	1.45	0.097
13-13.9	26	48.0	20.0	28.41	40.35	0.51	0.153
14-14.9	31	79.3	45.9	48.74	51.78	0.90	0.028
15-15.9	23	100.0	160.7	276.19	474.70	0.34	0.124
16-16.9	0	0	0	0	-	-	-
17-17.9	5	100.0	612.0	902.99	1332.30	0.46	0.037
Total	181	31.9	49.0	199.10	808.99	0.06	0.666

Table 2. Metacercarial utilization of *Lymnaea peregra* by *Echinoparyphium recurvatum* during August 1985 relative to snail size.

Snail size class (mm)	No. examined	Prevalence (%)	Mean intensity	Standard deviation	Variance/mean ratio	<i>k</i>	<i>P</i>
4-4.9	3	0	0	0	-	-	-
5-5.9	0	0	0	0	-	-	-
6-6.9	9	0	0	0	-	-	-
7-7.9	10	20.0	0.5	1.27	3.22	0.23	0.768
8-8.9	11	44.0	2.5	3.53	5.08	0.59	0.377
9-9.9	18	82.9	7.3	10.97	16.41	0.48	0.264
10-10.9	20	96.6	28.8	34.76	41.95	0.70	0.072
11-11.9	22	97.3	64.0	72.30	81.67	0.79	0.030
12-12.9	22	97.8	200.0	314.50	494.55	0.41	0.080
13-13.9	24	100.0	207.0	467.0	1053.57	0.19	0.255
14-14.9	24	100.0	181.2	331.70	607.20	0.29	0.147
15-15.9	25	97.0	467.0	602.0	776.02	0.60	0.018
16-16.9	18	100.0	714.0	705.0	696.11	1.03	0.001
17-17.9	8	100.0	853.0	675.0	534.14	1.59	<0.001
Total	214	91.9	220.7	448.89	913.05	0.24	0.192

Table 3. Metacercarial utilization of *Lymnaea peregra* by *Echinoparyphium recurvatum* during September 1985 relative to snail size.

Snail size class (mm)	No. examined	Prevalence (%)	Mean intensity	Standard deviation	Variance/mean ratio	<i>k</i>	<i>P</i>
4-4.9	0	0	0	0	-	-	-
5-5.9	0	0	0	0	-	-	-
6-6.9	0	0	0	0	-	-	-
7-7.9	6	50.0	0.83	1.60	3.08	0.48	0.616
8-8.9	12	44.4	2.3	3.55	5.60	0.49	0.431
9-9.9	13	85.4	7.1	7.98	8.99	0.89	0.143
10-10.9	19	97.3	39.2	37.25	35.38	1.14	0.017
11-11.9	18	98.3	103.1	85.80	71.40	1.46	0.002
12-12.9	20	97.8	213.6	159.80	119.55	1.80	<0.001
13-13.9	23	100.0	416.6	346.60	288.36	1.45	<0.001
14-14.9	26	100.0	807.0	543.0	365.36	2.21	<0.001
15-15.9	0	0	0	0	-	-	-
16-16.9	0	0	0	0	-	-	-
17-17.9	0	0	0	0	-	-	-
Total	137	95.6	274.1	406.10	601.66	0.46	0.054

monthly snail size classes and total monthly samples were always greater than unity indicating an aggregated or overdispersed distribution of cysts in the snail population. The variance/mean ratios for individual size classes of snails also increased with snail size in July and September (tables 1 and 3). However, in August (table 2) this ratio increased abruptly in the snail range of 7–8 mm to a maximum at 13–13.9 mm and then unevenly declined in the 17–17.9 mm size categories. Therefore, the aggregation of metacercarial cysts in the snail population decreased at either side of the dominant size class. The variance/mean ratio also increased from July to August but declined in September. This suggested that with the continuous exposure of snails to cercarial invasion, metacercarial encystment increased from July to August with an increase in the growth and size of the snail population followed by a decrease in September with a reduction in the range of size classes of an even larger snail population. Consequently, the degree of aggregation in September decreased indicating less variation in the abundance of cysts per host. Parasite dispersion (k) for most snail size classes throughout the sampling period was less than 1 indicating significant aggregation. However, in the larger snails sampled in August and September, although the metacercariae were only marginally overdispersed, the negative binomial provided a better fit than the Poisson distribution. Over the three month sampling period, an increase in mean cyst intensity and prevalence of infection was also closely associated with an increase in empirical values for k (fig. 2).

The sites of metacercarial encystment within the tissues of *L. peregra* varied with host size and the time of sampling (fig. 1). Cysts restricted to peripheral organs such as the mantle lobe were only observed in small to medium-sized snails, whereas cysts from larger snails were observed in all soft tissues especially the pericardial and kidney cavity. All snails with

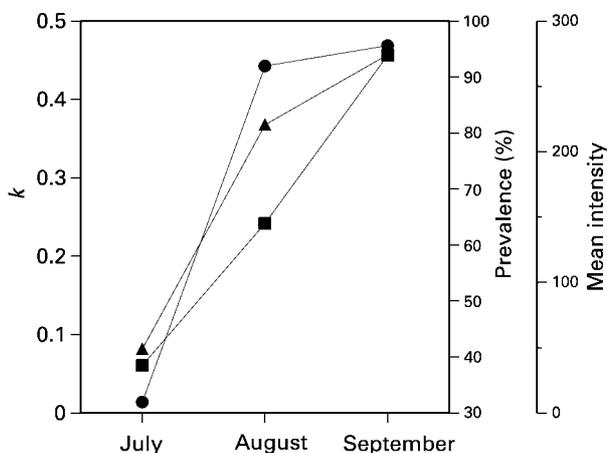


Fig. 2. Relationship between the negative binomial parameter estimate, k (■), the arithmetic mean intensity (●), and the prevalence of infection (▲) of *Lymnaea peregra* infected with metacercariae of *Echinoparyphium recurvatum* during July–September 1985.

metacercariae in the visceral mass also harboured patent primary infections.

In July, the occurrence of cysts in the entire tissues of *L. peregra* increased with snail size, commencing with the 12–12.9 mm category, and with all snails being infected in the size range 15–17.9 mm. By August, a wider range of hosts contained cysts in all tissues, commencing with some in the size range 10–10.9 mm, with 100% of snails containing cysts throughout their tissues in the range 13–17.9 mm. In September, the pattern in sites of encystment changed even further due to the mortality of larger snails, hence the occurrence of cysts in the peripheral organs was restricted to a small number of snails in the 7–9.9 mm category. The proportion of snails with cysts throughout their soft tissues increased from 37% in snail size 10–10.9 mm to a 100% in the 12–14.9 mm category.

Discussion

The present study has demonstrated that the infection parameters of *E. recurvatum* metacercariae within a single snail host community increase through July and August and peak in September when the majority of the host population are heavily infected. This appears to be the result of several factors. Both the prevalence of patent cercarial infections and the frequency of a minimum susceptible host size significantly increase in August and September. Metacercarial recruitment is dependent upon cercarial transmission, especially when cercarial production is high. In addition, the exposure time of snails to cercariae increases as the season advances and also in the more confined habitat of a narrow, shallow stream where snails are more readily in contact with cercariae. In addition, during these summer months the diurnal range of stream water temperatures falls within the optimum range for cercarial transmission (McCarthy, 1999).

The distribution of metacercarial cysts in the snail population, whether in monthly samples or individual size classes, is aggregated or overdispersed, with estimated k values being less than 1 indicating a negative binomial probability. Differences between k values over the three month sampling period were associated with changes in both the prevalence and mean intensity of infection. Parasites are generally overdispersed amongst hosts, with few hosts harbouring the highest number of parasites, resulting in a low prevalence of infection (Anderson & Gordon, 1982; Shaw & Dobson, 1995; Shaw *et al.*, 1998). In the present study, despite the high prevalence of metacercarial infections, an aggregation of metacercariae in the snail population is evident and this is similar to other studies on metacercarial distribution within invertebrate hosts (e.g. Stromberg *et al.*, 1978; Spelling & Young, 1986).

A number of reasons exist for the aggregated distribution of *E. recurvatum* metacercariae in the *L. peregra* population. There is a marked variation in the abundance of cysts per host, and this abundance generally increases with host size. A significant factor is the increasing length of time of exposure of the snail host to cercarial invasion with the larger and older snails therefore harbouring more metacercariae. In the present

study, metacercarial encystment is a continuous process during the summer period, resulting in an increase in the prevalence and abundance of metacercarial cysts in the snail population which continues as long as cercarial production is sustained. However, the degree of aggregation of *E. recurvatum* in *L. peregra* increases throughout July and August but declines in September indicating that variability in the number of metacercariae has reduced by this time. This is the result of the continuous accumulation of metacercarial cysts in the snail population from July, with a marked increase in mean individual snail size thereby making more snail hosts available for infection. Similar metacercarial distributions of *E. recurvatum* were reported for the snail hosts *P. subtruncatum* and *V. piscinalis* in a single sample study on a multi-snail species community (Evans *et al.*, 1981). Therefore, utilization of invertebrate second intermediate hosts by larval digeneans increases linearly with host age.

An increase in host population size over the sampling period is another factor leading to the aggregation of *E. recurvatum* in *L. peregra* as the prevalence and probability of cercariae contacting suitable snail hosts increases with increasing snail density. The pattern of snail distribution on the stream bottom is also important, because juvenile snails take refuge under dead leaves and objects on the bottom floor near the water edge whereas the more frequently occurring larger/older snails become aggregated in the mainstream zone. As cercarial emergence mainly occurs from larger snails, more than 10 mm in size, this results in higher cercarial densities and in turn an aggregated distribution of cercariae in these snails. Consequently, the highest rates of cercarial transmission will occur in the mainstream zone, inhabited by larger snails. In slow moving water there is a downstream dispersal of cercariae leading to high metacercarial recruitment being confined to a small area. Indeed, large aggregations of *L. peregra* are known to provide a target stimulus for attracting large numbers of *E. recurvatum* cercariae (McCarthy, 1990).

The presence of metacercariae in specific encystment sites is in agreement with the experimental studies of Adam & Lewis (1992). The present study has confirmed that the location of metacercariae within the snail host is related to host size, with juveniles harbouring cysts on the peripheral organs and older snails with cysts present in all internal organs, especially towards the end of the summer period. Variability in metacercarial abundance in individual snail hosts might be due to variation in cercarial accessibility to the host. *Echinoparyphium recurvatum* cercariae lack penetration glands and enter their target host through natural openings (Adam & Lewis, 1992). Vanoverschelde (1978) and Vanoverschelde & Vaes (1980) considered that reduced transmission of *Himasthla militaris* cercariae into the polychaete *Nereis* sp. was due to a reduction in the size of natural openings which produced insufficient chemical stimuli. It is possible that the same difficulty exists for cercariae of *E. recurvatum* gaining access to young *L. peregra*. Compared with the larger snails, the low distribution of cysts in juvenile snails is likely to be explained by dispersion factors tending towards randomness rather than aggregation.

Host-induced mortality by echinostome metacercariae has been experimentally demonstrated by Kuris & Warren (1980) and Fried *et al.* (1995) with mortality being particularly high amongst juvenile snails and where cercarial densities are high such snail mortalities may also occur under field conditions. The disproportionately small number of juveniles collected in the present study may be due to mortalities induced by heavy cercarial penetration in the lower snail size classes. The presence of juveniles near the banks of the stream therefore may have been due to an avoidance behaviour by these snails to higher cercarial densities in the mainstream zone.

The chances of metacercarial transmission to the definitive bird host is dependent upon the ingestion of the snail host so that any widespread metacercarial-induced host mortality is disadvantageous to the long term maintenance of the echinostome life cycle. In this case an aggregation of metacercarial infections over the largest part of the *L. peregra* population may have prevented accumulation of lethal numbers of metacercariae in smaller numbers of individual hosts. However, host behaviour may change with increasing infection. Large and heavily infected snails are more frequently observed on the floating vegetation in the stream. In laboratory cultures, large and heavily infected snails migrate out of water on to the tank wall of aquaria or onto lettuce leaves without undergoing any feeding. Heavy metacercarial infections in the pericardial cavity may possibly squeeze the heart, thereby weakening the circulation of the haemolymph through the snail tissues and in turn impairing snail respiration. Moreover, encystment in the mantle cavity not only reduces the capacity of snails to store air but also decreases gaseous exchange on the mantle surface. Under natural conditions, *L. peregra* occasionally floats to the water surface to take in air. With a reduced respiratory capacity, there is a tendency for *L. peregra* to stay out of the water longer to secure sufficient air supplies, thereby making the snails more vulnerable to predation but nevertheless allowing successful completion of the echinostome life cycle. The interactions between metacercarial cyst load, snail behaviour and vulnerability to predation by the definitive host would therefore warrant further investigation.

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