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Intermediate host availability masks the strength of experimentally-derived colonisation patterns in echinostome trematodes

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ABSTRACT

A fundamental goal of parasite evolutionary ecology is to elucidate patterns of host use and determine the underlying mechanisms of parasite colonisation. In order to distinguish the relative contributions of host encounter rates and host compatibility to infection outcomes, we compared host use in both field and experimental laboratory settings. Two years of bi-weekly snail sampling at a freshwater pond demonstrated fluctuating availability among three potential second intermediate snail host species and suggested that two trematode species (*Echinostoma revolutum* and *Echinoparyphium* sp.) did not colonise the three potential snail host species, *Lymnaea elodes, Physa gyrina* and *Helisoma trivolvis,* differentially. However, a series of experimental infections demonstrated that both parasites colonised *H. trivolvis* more so than the other two host species. Thus, more echinostome parasites utilised snail hosts that cannot serve as their first intermediate host. In experimental infections, host size and vagility were not strong determinants of infection. By utilising field and laboratory approaches, we were able to compare the strength of host compatibility under controlled conditions with patterns of infection in nature. Based on the results from these studies, it appears that host encounter is the primary mechanism dictating infection outcomes in the field.

1. Introduction

Ecological factors mediate parasite colonisation, ultimately leading to patterns of parasite specialisation. The most common measurement of parasite specialisation is host specificity, which is the number of host species colonised by a particular parasite stage. However, the range of infected host species may underestimate the degree of specialisation. Parasites often exhibit greater infection success for particular host species which is evident by the greater intensity of parasite infection (number of parasites per host species) on or in the host species (Lymbery, 1989; Poulin, 2005). Specialisation can vary across a continuum from parasites that infect a large number of host species but achieve greater infection success in a few of those host species to parasites that colonise a few host species but achieve approximately equal infection success among all host species (Brooks and McLennan, 2002; Poulin and Keeney, 2008). Particular ecological factors may explain more or less of the variation in specificity depending upon where a parasite falls along the specialisation continuum.

The ecological mechanisms responsible for infection patterns may be placed into two main categories, host encounter and compatibility (Combes, 2001). First, parasite colonisation may be the result of encountering a particular host. Host-related factors such as species distributions and host behaviour will influence the probability of contact between host and parasite. Once a parasite encounters a host, the second category, degree of host-parasite compatibility, becomes important. Host factors that influence compatibility include the available resource (size of colonisation site within host) and the host immune system. Overall, patterns of host specificity result from how open or closed the encounter and compatibility filters are (Euzet and Combes, 1980).

The degree of openness of the encounter filter depends ultimately on host availability. Many parasites and their developmental stages have very limited ability to move, especially in the abiotic environment. Thus, host distribution and movement can be significant factors in determining host availability in nature (McCoy et al., 2003). In addition, host size can be viewed as a proxy for availability as larger hosts may contain greater numbers of parasites (Sandland et al., 2001). Some parasites colonise a wider range of host species when experimentally exposed to hosts they do not encounter naturally, suggesting that high host specificity can be the product of low encounter probabilities (Perlman and Jaenike, 2003; Detwiler and Janovy, 2008) rather than compatibility issues. Therefore, host ecology often constrains the range of potential host specificity, so that the 'realized' specificity observed in nature is only a fraction of the 'fundamental' specificity that exists. To understand parasite specialisation, both field and experimental

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approaches are necessary. Patterns of parasite infection observed under natural conditions may reflect a wide array of factors operating either singly, synergistically, or antagonistically, making it difficult to distinguish the roles of encounter and compatibility in host-parasite interactions. Experimental approaches can minimise differences in encounter probabilities and thus identify compatibility differences or preferences for particular host species. Using comparative approaches for host specificity-related patterns is particularly important considering that contributing factors could be parasite and/or host driven (Schalk and Forbes, 1997; Scott, 2006).

Compared with free-living organisms, many parasite species face specificity-related constraints at a number of points during development where specialisation can vary from a single host to many potential host species. For example, most trematode parasites are fairly specialised in their early larval stages, often colonizing a single host species (first intermediate host). Later larval stages tend to exhibit less specialisation for particular host species, infecting a wider array of host species (second intermediate hosts). In some parasite species, the same host species is utilised as the first and second intermediate host. In these instances, negative consequences may arise for both host and parasite as larval parasites generate greater negative effects on host survival, growth and reproduction in first intermediate hosts compared with second intermediate hosts (Kuris and Warren, 1980; Sorensen and Minchella, 2001).

To isolate the factors underlying infection intensity, we utilised trematode-snail systems where infection success could be assessed at different points of a complex life cycle using both experimental and field approaches. Echinostomes are tractable experimental models that utilise more than one host at different stages in their life cycle. Laboratory experiments and field observations have shown that the two echinostome trematodes utilised in this study develop in a single first intermediate host species (*Lymnaea elodes*) (Najarian, 1953, 1954; Sorensen et al., 1997). The cercarial stage is the product of asexual reproduction within the first intermediate host. Once they emerge from the first intermediate host, cercariae have low dispersal potential and remain infective for only a few hours in the abiotic environment (Lo and Cross, 1975; Fried and Bennett, 1979). If a second intermediate host is present, the cercariae penetrate it and encyst, remaining viable for weeks to years in the metacercarial stage. Several snail species (*L. elodes, Physa gyrina* and *Helisoma trivolvis*) are potential second intermediate hosts in freshwater habitats of northern Indiana, USA. The echinostome life cycle is completed when the metacercarial stage is ingested by the third successive host (definitive host) as it consumes the second intermediate host. Within the definitive host, the parasite matures into an adult and sexually reproduces. In general, species of *Echinostoma* and *Echinoparyphium* utilise birds and mammals as natural hosts (Fried, 2001).

The goal of this study was to determine whether echinostomes demonstrate differences in infection success among potential second intermediate snail species. In a natural setting, we assessed the infection patterns of two co-occurring echinostome species among three host species from April to August during two consecutive years. Experimentally, infection compatibility was determined by offering parasites a 'choice' between pairs of host species in three combinations. In this setting, the potential effects of host ecology on infection intensity were evaluated by examining host species, host size and host movement. In our study, we predicted that increasing host abundance would correlate with increasing parasite infection. By experimentally isolating the potential effects of host movement and host species, we expected that more vagile hosts with the least potential fitness costs (i.e., species that cannot serve as first intermediate hosts) would have the highest parasite intensities.

2. Materials and methods

2.1. Field collections

To assess the prevalence of cercarial infections in co-occurring second intermediate hosts, bi-weekly field collections of *L. elodes*, *P. gyrina* and *H. trivolvis* were conducted at a freshwater pond (Pond A) in northeastern Indiana, USA (85° 28′W, 41° 18′N). The entire pond was divided into 10 m × 10 m quadrats then seven and eight sites (randomly selected quadrat) were chosen for collections in 2006 and 2007, respectively. Due to temporal fluctuations in snail densities, from 0 to 30 snails were collected per site per bi-weekly sampling for two consecutive years. Snails were collected haphazardly with respect to host species and snail size, resulting in an unequal number of individuals per host species. Although unequal, this collection method reflected the relative abundance of the three host species within the pond during each bi-weekly sampling period. For all collected snails, parasite prevalence was assessed by placing snails in spring water and then under lights for 2 h to induce cercarial shedding. Parasites were identified as echinostomes or other species using a taxonomic key for cercariae (Schell, 1985).

Densities of the three snail species were assessed bi-weekly from May 2006 to October 2006 and from April 2007 to August 2007 using the methods of Sandland and Minchella (2004). Briefly, snail densities for each site were estimated from four replicate samples of all snails within a bottomless plastic garbage can, which standardised the sampling area (0.14 m^2). Densities of young snails (<11 mm) were reported in this study.

After determining snail densities at each site, a subset of hosts was returned to the laboratory for further infection assessment. For each host species, ~10% of the total number of individuals found at each site (n = 0–5, 2006 sampling), or up to 10 individuals (2007 sampling) were haphazardly selected to determine the intensity of metacercarial infection. Snail length (mm) and parasite infection status were assessed in the laboratory for each individual. In addition to determining the prevalence of cercarial infection (methods described above), the intensity of metacercarial infection was assessed by crushing snails and counting the larval cysts. For this study we used data from small snails (<11 mm) because they have accumulated parasites over the same general time period (<1 year). By using this young cohort, we were able to control for potential temporal differences in parasite accumulation across host species. In 2006, a total of 40 snails were assessed for metacercarial infection (29 *P. gyrina*, 11 *L. elodes*). A total of 287 individuals were assessed in 2007 (173 *P. gyrina*, 32 *L. elodes*, and 82 *H. trivolvis*).

2.2. Experimental exposures

For *Echinostoma revolutum* and *Echinoparyphium* sp., the snail *Lymnaea elodes* (LE) serves as the first intermediate host, while it and the snails *Physa gyrina* (PG) and *Helisoma trivolvis* (HT) serve as potential second intermediate hosts. Parasites for experimental infections were obtained from sampled snails and from an additional 30 *L. elodes* (>20 mm) collected at each site during each sampling period. Snails were placed in well plates with spring water and kept under lights for 2 h to induce cercarial shedding. Cercariae were identified as *Echinostoma revolutum, Echinoparyphium* sp., or non-echinostome trematode species. Representative specimens have been deposited in the H.W. Manter Museum, University of Nebraska, 48977 (*Echinoparyphium* paratype), 48979 (*E. revolutum* paratype), 48981 (*Echinoparyphium* voucher), 48983 (*E. revolutum* voucher). The cercariae of *E. revolutum* were identified by the presence of collar spines and a posterior tail flap (Olsen, 1962). *Echinoparyphium* cercariae also possess collar spines, however the pos-

terior tail flap is absent (Olsen, 1962). Randomly selected *L. elodes* with patent infections served as parasite sources of *E. revolutum* (24 snails) or *Echinoparyphium* sp. (20 snails) for experimental exposures.

The experimental design for infections with either *E. revolutum* or *Echinoparphium* sp. was identical. Uninfected, laboratory-reared F1 snails from field-collected adults were size-matched (<11 mm) and used in a total of three host species combinations: LE versus PG, PG versus HT, and LE versus HT. Each exposure trial was replicated using two pairs of host species. A total of 96 and 80 hosts was exposed to *E. revolutum* or *Echinoparyphium* sp. for each of the host combinations (24 and 20 host pairs per two jars), respectively (Fig. 1). Host pairs were placed into single 110-mL glass jars filled with spring water. After giving the snails 5 min of acclimation to the jar, 50 cercariae from an infected first intermediate host were pipetted into each jar. Snail movements were recorded for 2 min by tracing the snail paths with a permanent marker and then determining the total distance traveled with a piece of string. The water in each jar was changed after 3 h of exposure to cercariae. After 7 days of ad libitum feeding with lettuce, snails were dissected and the number of echinostome metacercariae was counted in each snail host to determine intensity.

2.3. Statistical analyses

2.3.1. Field infections

An ANCOVA was used to determine the importance of host species, collection month and host size (covariate) on metacercarial intensity (SAS 9.1.3). The dependent variable (infection intensity) best fit a negative binomial distribution (Wilson et al., 1996). All possible interactions were included in the model (PROC GLIMMIX) and then non-significant interaction terms were removed. The relationship between host size and parasite intensity ($\log(x+1)$ transformed) among host species was examined with Spearman Rank Correlations (SPSS 15.0). An ANOVA was used to determine the effects of host species and month of collection on the relative host abundance of the naïve snails (SAS 9.1.3). A Gaussian distribution was utilised because assumptions of normality and homogeneity of variance were met and the dependent factor was a proportion (number of individuals of a species/total number of individuals for all three species). Least squares means (LS-means) of relative abundance were generated for each host species (Littel et al., 2006). The means of relative abundance between host species within each month were also compared using the DIFF = ANOM option.

2.3.2. Experimental infections

For each exposure trial, two host pairs (two jars) were exposed to parasites collected from a single snail. Analysis consisted of repeated-measures ANCOVA with a compound symmetry variance/covariance matrix (Littel et al., 2006). A negative binomial distribution was utilised because it generated the best fit model (Wilson et al., 1996). Host species and host movement were fixed factors, initial host size (snail length) was a covariate and parasite source was treated as the within-subjects factor. All possible interaction terms were initially included in the model (PROC GLIMMIX), then non-significant interactions were dropped from the model in order of decreasing order of complexity and in order of increasing F value. Degrees of freedom were calculated according to Kenward and Rogers (1997) to account for inflated Type I error rates. As in the previous analysis, LS-means of parasite infection were generated for each host species and differences between the means were compared. A similar model was fitted with PROC GLM to generate Type III sums of squares (SS). Partial R^2 ($SS_{\text{parasite source}}/SS_{\text{total}}$) was calculated to determine the amount of variation explained by parasite source (Neter et al., 1996).

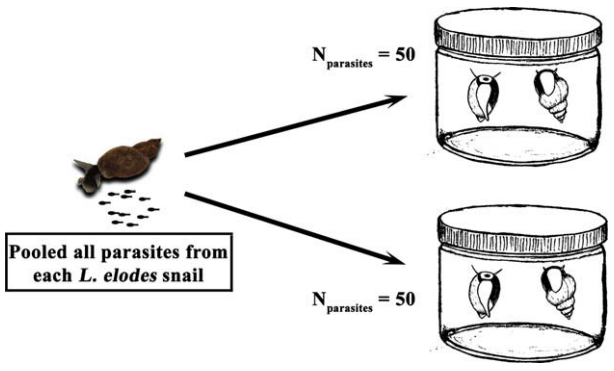

Fig. 1. Experimental design for trials with *Echinostoma revolutum* and *Echinoparyphium* sp. Each experimental trial (two jars) was exposed to the same source of parasites (cercariae from a single *Lymnaea elodes* snail).

3. Results

3.1. Field infections

A total of 2,695 snails and 1,098 snails were assessed for prevalence of cercarial infection (primary infection) in 2006 and 2007, respectively. Overall trematode prevalence in the pond was 18.3% (694/3,793). In both years, the majority of primary infections within the three species of snails were echinostome species, 67.4% (289/429, 2006) and 77.7% (206/265, 2007). In both years, maximum echinostome prevalence occurred May to July (2006: 13.2%–36.4%; 2007: 33.9%–32.0%).

Snail density was sampled 16 times from May to October 2006 and April to October 2007, during which a total 2,672 small snails (<11 mm) were counted in the pond. The overall number of hosts available to trematodes was similar in all months, but the availability of different species fluctuated by month (Fig. 2). The variation in relative abundance of snails was explained by host species ($F_{2,30} = 0.87$, $P < 0.0001$) and host species × month interaction ($F_{10,30} = 5.42$, $P = 0.0001$), while month alone was not significant ($F_{5,30} = 0.09$, $P = 0.9925$). *Physa gyrina* was the most abundant species in July 2006 (PG versus LE: $t_{30} = 3.14$, $P = 0.0101$; PG versus HT: $t_{30} = 4.34$, $P = 0.0004$), May 2007 (PG versus LE: $t_{30} = 3.14$, $P = 0.0103$; PG versus HT: $t_{30} = 2.91$, $P = 0.0179$) and June 2007 (PG versus LE: $t_{30} = 5.69$, $P < 0.0001$; PG versus HT: $t_{30} = 4.40$, $P = 0.0004$). In July and August, *L. elodes* was the least abundant snail (PG versus LE: $t_{30} = 3.65$, $P = 0.0028$; LE versus HT: $t_{30} = -4.52$, $P = 0.0003$).

To determine the intensity of infection with metacercariae among the three host species, a subset of the total snails was dissected ($n_{PG} = 202$, $n_{LE} = 43$, $n_{HT} = 82$) which yielded 0–1859, 0–273, and 0–1055 metacercariae, respectively. The mean infection intensity (LS-means ± standard error) for each host species was PG = 4.56 ± 0.11; LE = 3.04 ± 0.32; HT = 4.47 ± 0.19. In the field, variation in metacercarial intensity was best explained by month of collection and not by an overall effect of host species or a host species × month interaction (Table 1). The months with the highest intensities of infection were August, September and October (5.71 ± 0.21; 5.17 ± 0.19; 5.08 ± 0.23, respectively). Each species acquired infections differently with respect to host size (Fig. 3). The size × host species interaction was significant due to the positive relationship of host size and parasite intensity for *H. trivolvis* ($r_s = 0.430$, $P < 0.0001$).

3.2. Experimental results – Echinostoma revolutum

For each of the three host species combinations (A = PG versus LE, B = PG versus HT, and C = LE versus HT), a total of 96 snails

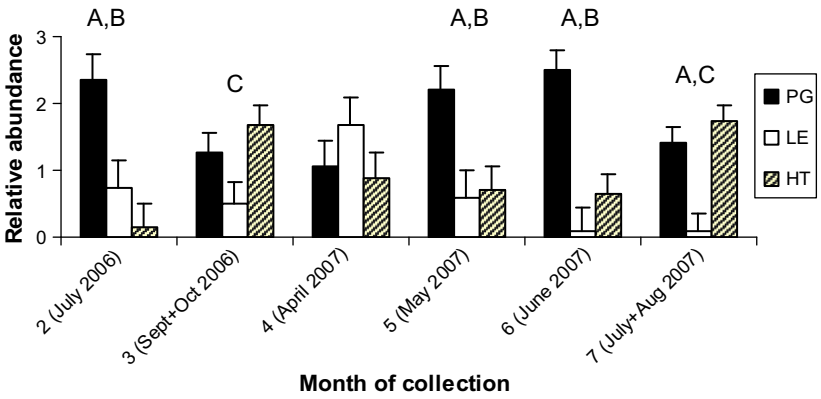

Fig. 2. Relative abundance of snail species less than (■ = Physa gyrina PG), □ = *Lymnaea elodes* (LE), ▨ = *Helisoma trivolvis* (HT)) from months 2–7. Each value is the least squares mean (±standard error) based on the ANOVA (Gaussian distribution) reported in the text. Relative abundance is the proportion (number of individuals of a species/total number of individuals for all three species) that was transformed (2 ∗ arcsin(sqrt(relative abundance))). Letters denote which host combinations have significantly different means ($P < 0.05$). A = PG versus LE, B = PG versus HT, C = LE versus HT.

Table 1
ANCOVA results (negative binomial distribution) for the effect of host species, month of collection, host size (snail length) and the significant interaction on parasite intensity from the field.

Factors	ndf,[a] ddf[b]	*F*	*P*
Host species	2, 315	2.61	0.0750
Month of collection	6, 315	25.08	<0.0001[c]
Host size	1, 315	3.69	0.0555
Host size ∗ host species	2, 315	4.28	0.0147[c]

[a] ndf, numerator degrees of freedom.
[b] ddf, denominator degrees of freedom.
[c] $P < 0.05$.

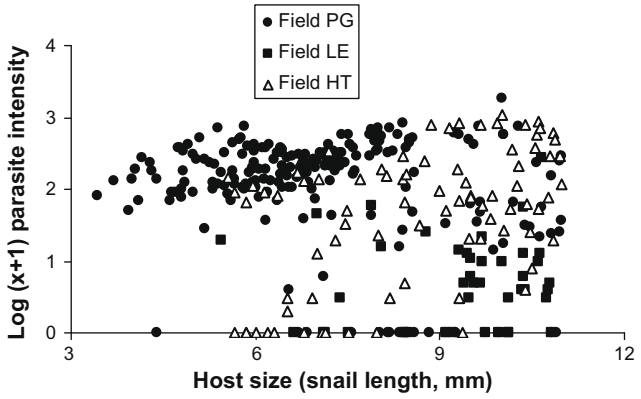

Fig. 3. Relationship between the size and parasite infection for each host species. Each point represents an individual snail. *Physa gryina* (PG), *Lymnaea elodes* (LE), *Helisoma trivolvis* (HT).

(24 host pairs × two jars) were exposed to field-collected *E. revolutum* cercariae from 24 *L. elodes* (parasite sources). All hosts survived exposure and the subsequent infection period except for three hosts in combination A ($n = 93$). For all the host combinations, there was a significant difference in parasite infection between the host species (Fig. 4). The effect of parasite source on infection was not significant in host combinations A–C ($P > 0.05$) and explained 33%, 26%, and 23% of infection variation, respectively. *Physa gyrina* were significantly more infected than *L. elodes* (host combination A: $t_{75} = 2.94$, $P = 0.0043$). *Helisoma trivolvis* were significantly more infected than *P. gyrina* and *L. elodes* (host combination B: $t_{61} = 3.07$, $P = 0.0032$; host combination C: $t_{70} = 4.55$, $P = <0.0001$).

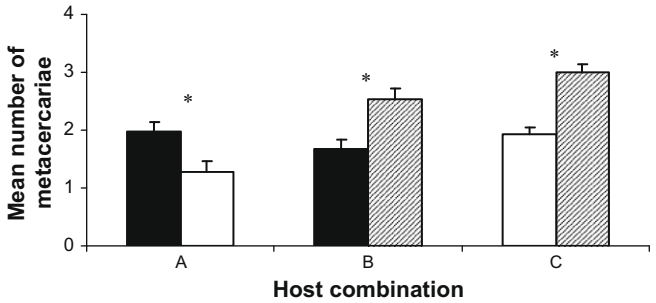

Fig. 4. Mean intensity of *Echinostoma revolutum* metacercarial infection for each host species combination (■ = *Physa gyrina* (PG), □ = *Lymnaea elodes* (LE), ▨ = *Helisoma trivolvis* (HT)). Each value is the least squares mean (±standard error) based on the statistical model from Table 2. Units for mean number of metacercariae are natural log-scaled. An asterisk denotes significance ($P < 0.05$). Letters denote which host combinations which have significantly different means ($P < 0.05$). A = PG versus LE, B = PG versus HT, C = LE versus HT.

Size and movement were significant factors in some of the host species combinations, but not universally so among the host species combinations (Table 2). Furthermore, a significant interaction between host size and movement occurred in host species combination A.

3.3. Experimental results – Echinoparyphium sp.

For each of the three host species combinations (A = LE versus PG, B = PG versus HT, and C = LE versus HT), a total of 80 snails (20 host

Table 2
Repeated-measures ANCOVA results (negative binomial distribution) for the effect of host species, host movement and host size (initial snail length), and any significant interactions on *Echinostoma revolutum* metacercarial intensity.

Host combination	Factors	ndf,[a] ddf[b]	*F*	*P*
A (PG versus LE, $n = 93$)	Host species	1, 75	8.67	0.0043[c]
	Host movement	1, 84	4.68	0.0334[c]
	Host size	1, 67	6.83	0.0110[c]
	Host size × movement	1, 84	5.30	0.0238[c]
B (PG versus HT, $n = 96$)	Host species	1, 92	9.41	0.0032[c]
	Host movement	1, 57	0.56	0.4579
	Host size	1, 61	0.84	0.3618
C (LE versus HT, $n = 96$)	Host species	1, 22	20.72	>0.0001[c]
	Host movement	1, 76	0.01	0.9207
	Host size	1, 70	5.29	0.0314[c]

[a] ndf, numerator degrees of freedom.
[b] ddf, denominator degrees of freedom.
[c] $P < 0.05$. PG, *Physa gryina*; LE, *Lymnaea elodes*; HT, *Helisoma trivolvi*.

pairs × two jars) survived exposure to parasites (*Echinoparyphium* sp. cercariae) from 20 field-collected *L. elodes* (parasite sources). For two host species combinations, none of the factors explained the variation in metacercarial intensity (Table 3). In contrast, snail movement and host species affected infection intensity in host combination C (Table 3). For host combinations A–C, parasite source was not significant ($P > 0.05$) and explained 9.4%, 28%, and 8.1% of the variation in infection intensity, respectively. For *Echinoparyphium* sp., infection intensities differed in only host combination C ($t_{58} = 5.64$, $P < 0.0001$; Fig. 5), where *H. trivolvis* was significantly more infected. In all host combinations, none of the interactions among the independent variables were significant.

4. Discussion

By comparing patterns of colonisation from field and experimental settings, the relative importance of the encounter and compatibility filters can be assessed in this host-parasite system. In our study, there was a significant temporal pattern in infection intensity that varied by month suggesting that cercaria-snail encounter rates increase as the prevalence of primary infections increases. In nature, metacercarial infection levels among the three host species were not significantly different which may be explained in part by the monthly variation in host availability. In contrast, the effect of host species was almost always significant in the experimental exposures. Both echinostome species demonstrated the greatest compatibility with *H. trivolvis*. Overall, experimental infection patterns revealed the extent of parasite-host compatibilities, but natural infection patterns indicated that host availability, and thus encounter rates, are stronger determinants of infection success.

Table 3
Repeated-measures ANCOVA results (negative binomial distribution) for the effect of host species, host movement, and host size (initial snail length) on *Echinoparyphium* sp. metacercarial intensity.

Host combination ($n = 80$)	Factors	ndf,[a] ddf[b]	F	P
A (LE versus PG)	Host species	1, 73	0.03	0.8735
	Host movement	1, 65	1.65	0.2038
	Host size	1, 19	0.00	0.9452
B (PG versus HT)	Host species	1, 70	0.11	0.7432
	Host movement	1, 72	2.91	0.0923
	Host size	1, 19	0.51	0.4856
C (LE versus HT)	Host species	1, 58	31.82	<0.0001[c]
	Host movement	1, 73	8.61	0.0045[c]
	Host size	1, 20	3.11	0.0927

[a] ndf, numerator degrees of freedom.
[b] ddf, denominator degrees of freedom.
[c] ($P < 0.05$). PG, *Physa gryina*; LE, *Lymnaea elodes*; HT, *Helisoma trivolvis*.

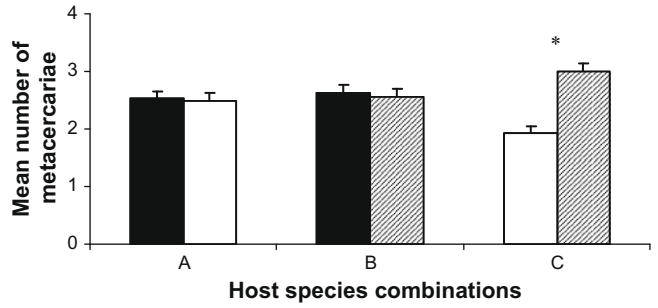

Fig. 5. Mean intensity of *Echinoparyphium* sp. metacercarial infection for each host species combination (■ = *Physa gryina* (PG), □ = *Lymnaea elodes* (LE), ▨ = *Helisoma trivolvis* (HT)). Each value is the least squares mean (±standard error) based on the statistical model from Table 3. Units for mean number of metacercariae are natural log-scaled. An asterisk denotes significance ($P < 0.05$).

Infection intensity in second intermediate hosts was low early in the season, increased between May and July, and then decreased between August and October. This seasonal trend has been shown in other trematode-host systems in first intermediate hosts, which produce the infective stages that penetrate second intermediate hosts (Lemly and Esch, 1984). No time lag in infection pattern was expected because the larval stages must penetrate a second intermediate host within a few hours of being released into the water. As in previous studies, we found that host availability corresponded to seasonal parasite infection patterns. Naive *P. gyrina* were the most abundant during the peak months of infection, however, they were not significantly more infected than other species during those months. In our pond, *L. elodes* and *P. gyrina* use similar microhabitats, while *H. trivolvis* exhibits less niche overlap for both species (Brown, 1982). Whereas microhabitat use might influence encounter rates with parasites, this effect does not appear to be a strong determinant given that infection levels were not significantly different among snail species.

Although there were no overall differences in infection levels among potential host species in the field, the effect of host species was almost always significant in the experimental exposures. Both echinostome species infected *H. trivolvis* significantly more than the other species, suggesting that compatibility is greatest in these host-parasite combinations. However, the fact that this general pattern was not reflected in the field results suggests that ecological factors and host availability can mask compatibility differences in a natural setting.

Younger snails are predicted to accumulate infection in a linear manner (Wetzel and Shreve, 2003). In our study, only one species, *H. trivolvis*, accumulated parasites in a linear manner. This species was also most abundant during months when overall parasite levels in the pond were low. When the probability of encountering a host is lower, parasite colonisation is dependent upon host size/age or the length of time spent in the pond. *Physa gyrina* tended to be highly infected no matter the shell length suggesting that host size did not influence infection. Instead, *P. gyrina* were most abundant during months of peak infection and thus were more likely to encounter parasites and become infected earlier than the other host species. Young *L. elodes* were the least infected and also the least abundant at our freshwater pond. However, an additional factor may help explain infection patterns in this species.

Given that *L. elodes* acts as first intermediate host, parasites may prefer to colonise another host species as a second intermediate host (McCarthy and Kanev, 1990). Larval parasites within the first intermediate host often have negative fitness effects on the host such as reduced survival and reproduction (Minchella and Scott, 1991). The severity of parasite infection appears to differ among host species. As first intermediate hosts, lymnaeid snails appear to suffer greater mortality during parasite infections compared with planorbid snails. In a review of trematode-snail interaction studies, Sorensen and Minchella (2001) found that mortality during prepatent (not yet producing cercariae) and patent (producing cercariae) trematode infections was 50% and 81%, while planorbid (including *H. trivolvis*) mortality was 61% and 60%, respectively. In our study, there were significantly fewer young *L. elodes* than the other host species in most months. However, the role of the parasite-induced mortality was not directly examined. Although cercarial infections may have negative consequences, host mortality is not likely to be due to metacercarial infections unless hosts are exposed to hundreds of cercariae simultaneously. A series of experimental exposures demonstrated that the fitness consequences of encysted metacercariae were confined to the early stages of infection with most hosts surviving and eventually accumulating several hundred encysted parasites over a few days (Kuris and Warren, 1980). Utilising hosts without cercarial infections is beneficial to the parasite because the host may live longer, thereby

increasing the probability of transmission to the final host in the life cycle.

The natural definitive hosts in North America are unknown for the two species in our study. However, *E. revolutum* and *Echinoparyphium* spp. have been identified from a wide array of bird species throughout the world (Fried, 2001; Fried and Graczyk, 2004). For parasites with a wide host range and geographic distribution, it is difficult to predict how intermediate host infection patterns affect the probability of transmission (see Leung and Poulin, 2008). Some duck hosts of echinostomes, especially in shallow water habitats (as in our study) have been shown to eat a wide variety of second intermediate host molluscs (Evans et al., 1981). With generalist consumers, snail abundance could be an important determinant of prey and infection dynamics.

Peak bird activity at our site would correspond to April and October with some resident birds occupying the freshwater ephemeral pond during the breeding season (May to August). If most *L. elodes* die due to their patent infections in June and July, the metacercariae utilising these hosts will not be transmitted to birds. Furthermore, snail death prevents overwintering and thus infected snails will not be available for host consumption in April. Utilising alternative host species such as *P. gyrina* and *H. trivolvis* as second intermediate hosts increases the likelihood of parasite transmission to bird definitive hosts. Interestingly, the most abundant snail species early and late in the season were *P. gyrina* and *H. trivolvis*, respectively.

The differential infection patterns seen in our experimental study demonstrates that parasites can specialise on particular host species. By comparing field and experimental infection levels among three host species, we find that two main factors mediate parasite specialisation. Field studies indicated that infection patterns were best described by temporal factors such as month of collection and monthly fluctuations in host abundance. By contrast, experimental exposures demonstrated that the two species of echinostomes did indeed colonise potential hosts differentially. Comparing field and experimental evidence has yielded similar conclusions using an echinostome-mollusc system (Evans et al., 1981; Evans and Gordan, 1983). In our study, comparisons infer that echinostome parasites preferentially enter snail hosts that cannot serve as first intermediate hosts, but will enter all three species. Observed infection levels in natural populations suggest that host availability (encounter filter) can mask differences that may exist in the compatibility filter. The maintenance of broader host specificity may be particularly important given the temporal variation in habitat availability, which for a parasite includes both host and the abiotic environment in these ephemeral ponds.

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