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Host community composition and defensive symbionts determine trematode parasite abundance in host communities

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Abstract. Host species vary in their propensity to become infected by and transmit parasites, and this variation in host competency can influence parasite transmission within host communities. Host competency is often attributed to morphological, physiological, and behavioral defenses of hosts, but hosts commonly have an additional, lesser studied form of protection: defensive symbionts. For instance, snails are facultatively defended by ectosymbiotic oligochaete worms (*Chaetogaster limnaei*) that consume free-living trematode parasites, bacteria protect amphibians from the fungus that causes chytridiomycosis, and ants protect plants from herbivores. In addition to reducing infection on their hosts, defensive symbionts may influence parasite transmission to other hosts by redirecting parasites toward other hosts and/or removing parasites from the system. We explored these possibilities by examining the relative roles of community composition and the presence of defensive symbionts (*C. limnaei*) in determining trematode infection intensity among second intermediate host communities composed of snails (*Helisoma trivolvis*) and tadpoles (*Rana catesbeiana*). Parasites were dramatically more successful at infecting snails than tadpoles, which led to more total parasites in host communities where snails were present. In addition, defensive symbionts substantially reduced snail infection intensity and thus reduced the total number of parasites in communities containing symbiont-defended snail hosts. Neither host community composition nor the presence of defensive symbionts on snails influenced individual tadpole infection in our experiments. Therefore, in our experiments, second intermediate host community structure did not influence individual host tadpole infection risk, but did influence total parasite transmission.

Key words: *Echinoparyphium*; *Echinostoma*; *Physa gyrina*; symbiosis; Virginia.

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Introduction

From a parasite's perspective, not all host species are created equal. Host species vary in competency (Keesing et al. 2010, Gervasi et al. 2015, Johnson et al. 2015), which is a measure of the host's propensity to become infected by, maintain, and transmit a given parasite. Variation in competency has important implications for parasite transmission within ecological communities, because communities dominated by highly competent hosts may amplify focal host infection risk in comparison to communities dominated by hosts with low competency (Ostfeld and Keesing 2000, Keesing et al. 2006, 2010). Therefore, recent research has focused on elucidating the relationship between community diversity and the relative abundance of highly competent hosts (e.g.,

Johnson et al. 2013, Lacroix et al. 2014) and on identifying characteristics of host species that explain why some hosts are more competent than others (e.g., Johnson et al. 2012*b*, Ostfeld et al. 2014). In particular, morphological, physiological, and behavioral defenses of hosts—such as investment in immune responses and fastidious grooming behaviors—may explain much of the variation in host competency (e.g., Keesing et al. 2009, Johnson et al. 2012*b*).

However, hosts commonly have another, lesser studied form of defense: symbiotic organisms. For example, bacteria protect amphibians from the fungus that causes chytridiomycosis (Harris et al. 2009), guard crabs protect urchins from parasitic snails (Sonnenholzner et al. 2011), and ants protect plants from herbivores (Janzen 1966). Defensive symbionts may substantially reduce enemy attack success and survival (e.g., Heil et al. 2001, Katayama and Suzuki 2002, Okabe and Makino 2008, Oliver et al. 2008, Costopoulos et al. 2014) and influence natural enemy ecology and evolution (Glynn 1987, Goheen and Palmer 2010, Dion et al. 2011). However, despite the ubiquity of defensive symbionts, we know little about their role in the transmission of parasites within multihost communities (Johnson et al. 2015). Therefore, we explored how defensive symbionts can influence parasite distributions in a system where two host species vary in their defenses to echinostome parasites.

Echinostomes (Trematoda, Digenea) are flatworm parasites that are common in freshwater and have complex life cycles that rely on bird or mammal definitive hosts, snail first intermediate hosts, and snail or amphibian second intermediate hosts (Huffman and Fried 1990). In our experiments, we focused on the portion of the life cycle where first intermediate host snails (*Physa gyrina* and *Helisoma trivolvis*) release short-lived, free-living larvae called cercariae into the aquatic environment, and these cercariae search for and infect second intermediate host snails (*H. trivolvis* in our study) and tadpoles (*Rana catesbeiana*—bullfrogs in our study). The cercariae encyst within the kidneys of the second intermediate hosts as metacercariae, and the parasite life cycle is completed when the second intermediate hosts are consumed by a definitive host.

Amphibians have well-known behavioral (Koprivnikar et al. 2006, 2014, Daly and Johnson 2011) and immunological (Belden and Kiesecker 2005, Holland 2009, LaFonte and Johnson 2013) defenses when confronted with cercariae. However, amphibian species vary in their behavioral and immunological responses and thus their susceptibility to echinostome infection (e.g., Koprivnikar et al. 2014). Snail species also vary in their susceptibility to infection by echinostome cercariae (Christensen et al. 1980, Evans et al. 1981, Anderson and Fried 1987, Detwiler and Minchella 2009). Snail behavioral responses and immune responses may affect trematode infection success (e.g., Adema and Loker 2015), but the relative importance of these defenses is unclear. In addition, snails in this system, and in many other trematode systems (Smythe et al. 2015), are facultatively defended by ectosymbiotic oligochaete worms (*Chaetogaster limnaei limnaei*, hereafter *Chaetogaster*) that live on the snail headfoot, while tadpoles never harbor these symbionts.

Chaetogaster directly influence snail susceptibility to echinostome infection by consuming cercariae, and higher *Chaetogaster* infestation intensities cause greater reductions in cercariae attack success (Sankurathri and Holmes 1976, Hopkins et al. 2013). Also, observational evidence suggests that cercariae may abandon attempts to penetrate snails with *Chaetogaster*, causing cercariae to exhaust their energy stores and die before successfully infecting symbiont-defended snails (Sankurathri and Holmes 1976). Therefore, when oligochaetes are present on snails, echinostome cercariae might avoid symbiont-defended snails, leading to increased infection rates in the other available hosts (e.g., tadpoles).

To better understand the role of defensive symbionts in parasite transmission in multihost communities, we examined the relative roles of community composition and the presence of defensive symbionts in determining infection intensity among individual tadpole hosts and total parasite success within host communities. To do this, we exposed tadpoles or tadpoles together with snails to echinostome trematode parasites, where snails did or did not harbor *Chaetogaster* (Table 1). We performed this experiment at the population-scale, in a mesocosm experiment, and at the pairwise interaction-scale, in a laboratory experiment.

Table 1. Model formulation for the negative binomial generalized linear mixed models used for the mesocosm (shown here) and laboratory experiments.

Treatment	Abbreviation	Indicator variable in GLM			Inference
		High density	Snail presence	Chaetogaster presence	
10 tadpoles	10T	0	0	0	Intercept: comparison group
20 tadpoles	20T	1	0	0	Density effect
10 tadpoles, 10 snails	10T10S	1	1	0	Community composition effect
10 tadpoles, 10 snails, Chaetogaster	10T10SCh	1	1	1	Defensive symbiont effect

Methods

Mesocosm experiment

Twenty-four 375 L tanks were each randomly assigned to one of four treatment groups (six tanks/treatment): 10 tadpoles (10T), 20 tadpoles (20T), 10 tadpoles and 10 snails without *Chaetogaster* (10T10S), and 10 tadpoles and 10 snails with *Chaetogaster* (10T10SCh). Manipulating tadpole density and the presence of the snail hosts in this way allowed us to determine whether host community composition (i.e., the presence/absence of snail hosts) influenced tadpole infection or the total number of parasites in the host community when controlling for any effect of host density (Table 1). Comparisons of the latter two treatment groups allowed us to quantify the effects of defensive symbionts on individual infection risk and total parasite transmission in host communities (Table 1).

Mesocosms were filled with well water that was sieved through 75 μm mesh to prevent colonization by larger invertebrates, including *Chaetogaster*. Then 125 g of dry oak and maple leaf litter was added to each mesocosm, and they were covered with 1 mm screen to provide shade and to prevent predators from colonizing the mesocosms.

Parasite-free and *Chaetogaster*-free *Helisoma* snails (n = 120, average ± SD diameter = 12.91 mm ± 1.19) raised from eggs in stock mesocosms were taken to the laboratory prior to starting the experiment (Day −3). These snails were randomly assigned to the treatments and tanks containing snails (10T10S or 10T10SCh), and grouped in 1 L plastic cups containing dechloraminated tap water.

For the snail plus *Chaetogaster* treatment (10T10SCh), 50 *Chaetogaster* (~5 *Chaetogaster*/snail) removed from field-collected *H. trivolvis* snails were pipetted into each of the six 1 L cups containing the snails for that treatment (Day −2). Non-*Chaetogaster* snails (10T10S treatment) were exposed to the same volume of pipetted water from the collected *Chaetogaster* container as a sham exposure. Host snails were added to the appropriate mesocosms the next day (Day −1).

Rana tadpoles (n = 300, Gosner stage 25) raised from field-collected egg masses in separate stock mesocosms were pooled in 1 L plastic cups, and were then added to the appropriate mesocosms the day after the host snails were added (Day 0).

Two naturally infected *Physa* snails shedding echinostome cercariae from the genus *Echinoparyphium* (*sensu* Fried et al. 1998) were randomly assigned and added to each mesocosm after tadpole additions (Day 0; n = 48, average ± SD end snail diameter = 10.61 mm ± 1.72). Infected snails were collected over several weeks from ponds in Montgomery County, Virginia and maintained in the lab at 11 °C. Because these ponds have *Chaetogaster* populations, the *Physa* may have introduced a few *Chaetogaster* into all of the mesocosms. After infected snails were added, the mesocosms were left for 7 days while the infected snails shed cercariae (Days 0–6).

After 7 days (on Day 7), all snails and tadpoles (living or dead) were collected from the mesocosms. Snails were placed in individual 50 ml centrifuge tubes with dehcloraminated tapwater to prevent further movement of *Chaetogaster* among snail hosts, and tadpoles were pooled by mesocosm in 1 L containers. First intermediate host *Physa* were dissected the same day (Day 7) to quantify metacercariae and *Chaetogaster*. Also

Table 2. Summaries of percent mortality and infection prevalence in the mesocosm and laboratory experiments.

Treatment group	Percent tadpole mortality	Percent infected Physa mortality	Prevalence of snail infection	Prevalence of tadpole infection
Mesocosm experiment				
10T	60 (47–71)	42 (19–68)		66 (48–80)
10T10S	50 (38–62)	8 (0–35)	98 (91–100)	75 (60–86)
10T10SCh	62 (49–73)	17 (5–45)	82 (70–89)	89 (72–96)
20T	67 (58–74)	17 (5–45)		49 (37–62)
Laboratory experiment				
1T	15 (5–36)			88 (66–97)
1T1S	15 (5–36)		100 (84–100)	59 (36–78)
1T1SCh	5 (0–24)		95 (76–100)	53 (42–73)
2T	20 (10–35)			79 (60–91)

Note: The 95% Wilson confidence intervals for binomial point estimates are in parentheses.

on that day, dead tadpoles (n = 45; Table 2) were weighed, developmental stage was recorded (Gosner 1960), and then each tadpole was dissected to quantify metacercariae. Live tadpoles were euthanized in buffered MS-222, weighed and staged, and were then placed in individual tubes and frozen. *Helisoma* snails were kept in the laboratory at 11 °C over night. The next day (Day 8), shell diameter was recorded for all experimental snails. Then, those snails and the frozen tadpoles were dissected to quantify *Chaetogaster* numbers for snail hosts and trematode infection for all hosts (e.g., Belden and Wojdak 2011, Hopkins et al. 2013).

Laboratory experiment

In the laboratory experiment, animals were housed in 500 mL plastic containers filled with 100 mL of mesocosm water filtered through 250 μm mesh. The laboratory experiment also had four treatment groups: 1 tadpole (1T), 2 tadpoles (2T), 1 tadpole and 1 snail without *Chaetogaster* (1T1S), and 1 tadpole and 1 snail with *Chaetogaster* (1T1SCh). These treatment groups were analogous to the treatment groups in the mesocosm experiment (Table 1). There were 23 replicates of each treatment; twenty replicates of each treatment were exposed to cercariae and three were trematode-free controls.

Parasite-free, *Chaetogaster*-free *Helisoma* snails (n = 46, average ± SD end diameter = 7.16 mm ± 1.02) were raised from egg masses in an aquarium in the laboratory in dechloraminated tapwater. *Rana* tadpoles (n = 115, Gosner stage 25) were reared from eggs in a stock mesocosm. The day before parasite exposure (Day −1), snails and tadpoles were placed in individual 150 mL plastic cups containing 40 mL of the filtered mesocosm water. Snails and tadpoles were randomly assigned to treatments, and then *Chaetogaster* pooled from field-collected *H. trivolvis* snails were added to each snail in the *Chaetogaster* treatment group (10 *Chaetogaster*/snail). As with the mesocosm experiment, snails in the no *Chaetogaster* control cups were sham-exposed. Snails and tadpoles were fed fish flakes and left overnight.

The next day (Day 0), snails and tadpoles were transferred into the 500 mL experimental cups. For the subsequent 5 days (Day 0–4), 20 echinostome cercariae from the genus *Echinostoma* (*sensu* Fried et al. 1998) from field-collected *H. trivolvis* snails (n = 36) were added to each of the 80 experimental cups (excluding trematode-free controls) each day. Trematode-free controls received dechloraminated water. To obtain cercariae, the shedding snails were placed in individual 150 mL plastic cups for 1–2.5 hours each day, and all cercariae were pooled prior to additions.

If a tadpole died (Table 2) or had severe edema, it was removed from the cup, euthanized with buffered MS-222 if necessary, weighed, staged, and dissected to quantify metacercariae. Replicates with edema and tadpole mortality were removed from the final analysis of metacercariae counts, leaving 17, 12, 17, and 19 replicates in the 1T, 2T, 1T1S, and 1T1SCh treatment groups. Two days after the final cercariae addition, all tadpoles were euthanized and all snails and tadpoles were

Table 3. Parameter estimates and P-values for the models of per capita and total metacercariae infection.

Model	Parameter	Estimate	Standard error	P-value
Mesocosm tadpole metacercariae NB GLMM	Intercept (10T)	1.030	0.371	0.005
	Density effect	−1.000	0.514	0.052
	Snail effect	0.814	0.507	0.108
	Chaetogaster effect	0.214	0.525	0.684
	Variation among tanks	0.607		
Mesocosm snail metacercariae NB GLMM	Intercept (10T10S)	4.042	0.329	<0.001
	Chaetogaster effect	−1.025	0.466	0.023
	Variation among tanks	0.544		
Mesocosm total metacercariae NB GLM	Intercept (10T)	3.143	0.343	<0.001
	Density effect	−0.658	0.492	0.181
	Snail effect	4.036	0.485	<0.001
	Chaetogaster effect	−0.592	0.471	0.209
Laboratory tadpole metacercariae NB GLMM	Intercept (1T)	1.815	0.278	<0.001
	Density effect	−0.598	0.430	0.163
	Snail effect	−1.309	0.493	0.008
	Chaetogaster effect	0.010	0.506	0.985
	Variation among cups	1.080		
Laboratory snail metacercariae NB GLM	Intercept (1T1S)	3.772	0.136	<0.001
	Chaetogaster effect	−0.819	0.191	<0.001
Laboratory total metacercariae NB GLM	Intercept (1T)	2.184	0.174	<0.001
	Density effect	0.041	0.269	0.880
	Snail effect	1.581	0.259	<0.001
	Chaetogaster effect	−0.779	0.220	<0.001

Note: Parameter estimates are on the log scale.

dissected to quantify *Chaetogaster* and trematode infection (Day 6).

Statistical analyses

We used generalized linear mixed models (GLMMs) to determine whether the number of metacercariae per tadpole and/or per snail varied by treatment group (Table 1), with cup or mesocosm treated as a random effect to account for the correlated responses of individuals within the same cup or mesocosm. However, for snails in the laboratory experiment, a random effect was unnecessary because there was only ever one snail per cup, thus we used a generalized linear model (GLM). In addition to considering the number of metacercariae per animal, we also used a GLM to consider whether the total metacercariae (among all tadpoles and *H. trivolvis* snails) per mesocosm or cup varied by treatment group. For all models, we used negative binomial error distributions (NB GLM or NB GLMM) with log link functions to account for overdispersion in metacercariae counts. Visual assessment of Pearson residual plots and model predictions confirmed that these approaches were appropriate for our data. All analyses were conducted in R version 2.15.3 using packages MASS and lme4(1.0–4) and functions glm.nb and glmer.nb (R Development Core Team 2014). Parameter estimates from all models can be found in Table 3.

Results

Mesocosm experiment

At the end of the experiment, snails in the *Chaetogaster* treatment group (10T10SCh) had an average of ~7 *Chaetogaster* per snail, with a range of 2–17 *Chaetogaster* per snail. Snails in the *Chaetogaster* treatment group had only 20 metacercariae, on average, while snails in the treatment group without *Chaetogaster* had 57 metacercariae, on average (10T10SCh vs. 10T10S; $P = 0.023$; Fig. 1).

Contrary to our predictions, tadpole infection intensity did not depend on the presence of *Chaetogaster* on snails (*Chaetogaster* effect; $P = 0.685$; Table 3; Fig. 1). There was a trend toward a reduction in per capita tadpole cysts with tadpole density (density effect; $P = 0.052$). The presence

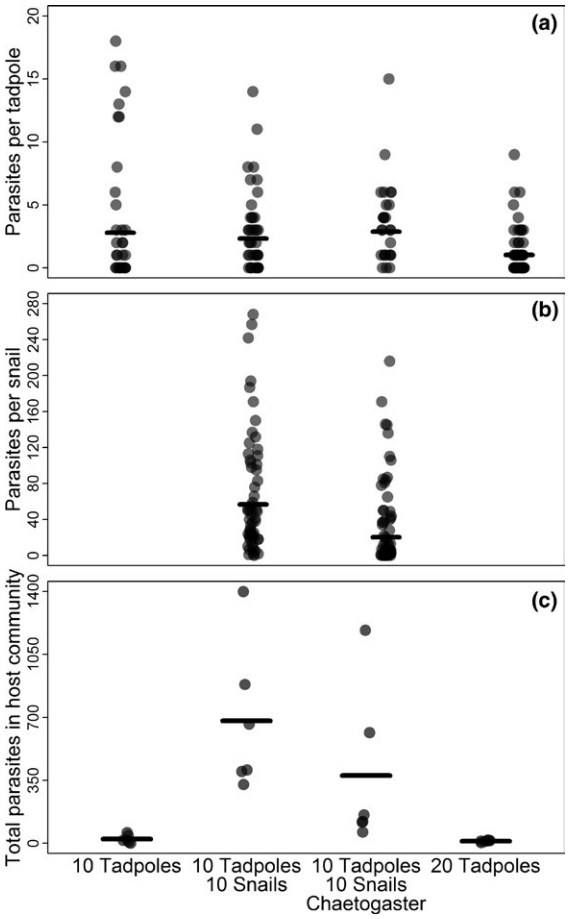

Fig. 1. In the mesocosm experiment, per capita tadpole metacercarial infection did not vary among treatment groups, though there was a trend toward fewer metacercariae in tadpoles in the 20T treatment group (panel [a]). Snails with *Chaetogaster* had 64% fewer metacercariae than snails without *Chaetogaster* (panel [b]). Mesocosms with tadpoles alone had 97% fewer total metacercariae than mesocosms with both snails and tadpoles (panel [c]), because snails had substantially higher infection intensity than tadpoles (panels [a] and [b]). In panels (a) and (b), each point represents the number of metacercariae in an individual host, and hosts from all mesocosms are shown together in each treatment group. Note the different scales on the *Y* axes. In panel (c), each point represents the total metacercariae in both tadpoles and *H. trivolvis* snails in one mesocosm. Points are jittered slightly on the *X*-axis to aid visualization. The black bars are the predicted means from the appropriate model.

of snails did not influence per capita tadpole cysts (community composition effect; $P = 0.108$).

Tadpoles acquired 94% fewer metacercariae than snails when considering the two snail treatment groups (10T10SCh and 10T10S; Fig. 1). Because of the large differences in per capita metacercariae, there were 97% fewer total metacercariae in the mesocosms with only tadpoles in comparison to mesocosms that had both snails and tadpoles when controlling for host density (community composition effect; $P < 0.001$; Fig. 1).

Laboratory experiment

Snails in the *Chaetogaster* treatment group (1T1SCh) had an average of 7 *Chaetogaster* per snail (range 0–11 *Chaetogaster* per snail) at the end of the experiment, which was similar to the *Chaetogaster* densities in the mesocosm experiment. As in the mesocosm experiment, *Chaetogaster* reduced the average number of metacercariae per snail by 56% (1T1S vs. 1T1SCh; 43 vs. 19 average per capita metacercariae; $P < 0.001$; Table 3; Fig. 2).

As in the mesocosm experiment, per capita tadpole infection in the laboratory experiment was not affected by the presence of *Chaetogaster* on snails (*Chaetogaster* effect; $P = 0.985$; Table 3; Fig. 2), nor on host density alone (density effect; $P = 0.163$). However, in contrast to the mesocosm experiment, when host density was controlled for, tadpoles in cups with snails had significantly fewer per capita metacercariae than tadpoles in the tadpole only treatment group (community composition effect; $P = 0.008$).

As tadpoles again acquired substantially fewer metacercariae than snails (Fig. 2), the tadpole only treatment had fewer total metacercariae per replicate than the replicates in the treatments with snails when controlling for host density (community composition effect; $P < 0.001$; Fig. 2; Table 1). In addition, *Chaetogaster* reduced total metacercariae per replicate in the laboratory experiment by 54% percent (*Chaetogaster* effect; $P < 0.001$). The total number of metacercariae per replicate was not affected by host density alone (density effect; $P = 0.88$).

Discussion

Host density, community composition, and competency may all influence parasite transmission,

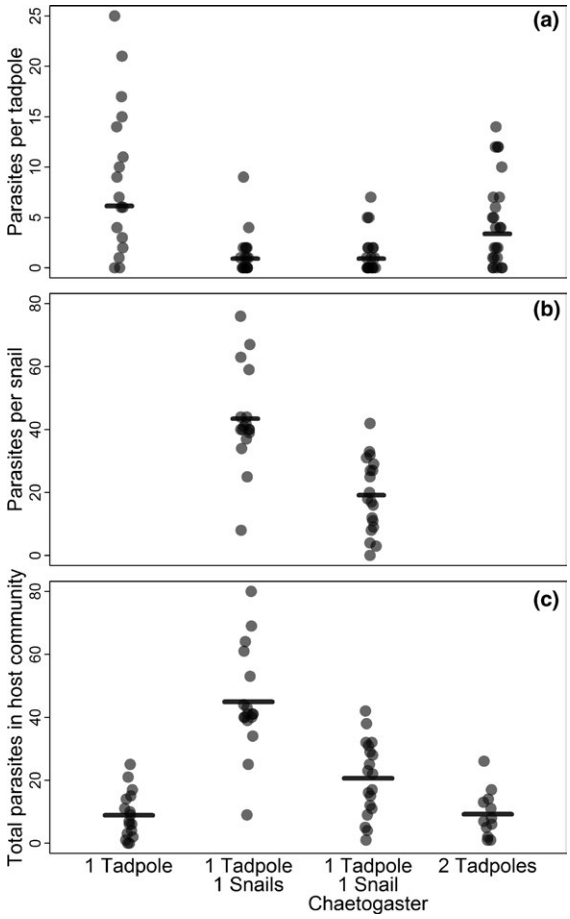

Fig. 2. In the laboratory experiment, per capita tadpole metacercarial infection did not vary among the two treatment groups with snails, indicating that *Chaetogaster* did not alter tadpole infection (panel [a]). However, tadpoles paired with snails had fewer metacercariae than tadpoles that were not paired with snails. There was also a trend toward fewer metacercariae in tadpoles in the 2T group than in the 1T group. Snails with *Chaetogaster* had 56% fewer metacercariae than snails without *Chaetogaster* (panel [b]). Cups with tadpoles alone had 80% fewer total metacercariae than cups with snails and tadpoles (panel [c]). In addition, there were 54% fewer total metacercariae when *Chaetogaster* were present on snails (1T1SCh) than when they were absent (1T1S). In panels (a) and (b), each point represents the number of metacercariae in an individual host, and hosts from all cups are shown together in each treatment group. Note the different scales on the *Y* axes. In panel (c), each point represents the total metacercariae in tadpoles and *H. trivolvis* snails in one cup. Points are jittered slightly on the *X*-axis to aid visualization. The black bars are the predicted means from the appropriate model.

and understanding the relative roles of these factors is necessary to understand the relationship between biodiversity and disease risk (e.g., Wojdak et al. 2014, Johnson et al. 2015). In our experiments, the total number of parasites that successfully encysted in second intermediate hosts was strongly influenced by both community composition and the presence of defensive symbionts. In particular, communities containing the highly competent snail hosts had 30 times more parasites than the tadpole only treatments. When snails had defensive symbionts, individual snail infection intensities were reduced by 65% and 56% in the mesocosm and laboratory experiments, respectively, which also reduced total parasite success. However, even with this symbiont-induced reduction, communities with symbiont-defended snails still contained more parasites than the tadpole only treatments. The presence of snail hosts led to a small reduction in infection among tadpoles in our laboratory experiment—though not in our mesocosm experiment—and the presence of defensive symbionts did not influenced tadpole infection. Therefore, in our experiments, second intermediate host community structure did not strongly influence individual tadpole infection risk, but played an important role in total parasite transmission. Therefore, second intermediate host community structure might influence infection risk to downstream hosts (=definitive hosts).

Host competency played the largest role in determining total parasite success in our experiments. And intriguingly, our results contrast those of a previous experiment that considered second intermediate host echinostome infection in host communities with varying densities and compositions (Wojdak et al. 2014). In that case, substituting snails for tadpoles—and thus changing host community composition and diversity while maintaining a constant host density—led to either no change in total parasite success or a slight decrease in total parasite success. In that experiment, all host species had roughly equal competency, so host species were effectively substitutable. In our experiments, individual snails acquired substantially more cercariae than tadpoles, so substituting snails for tadpoles caused a large increase in total parasite success.

It is unclear why cercariae were so much more successful at infecting snails than tadpoles in our

experiments. *R. catesbeiana* is a common pond-dwelling species that should be frequently encountered by echinostome cercariae. In addition, many species of Ranid frogs—including *R. catesbeiana*—can become infected by echinostome cercariae (e.g., McAlpine and Burt 1998). However, the behavioral responses (Koprivnikar et al. 2006, 2014, Daly and Johnson 2011) and immune responses (Holland 2009, LaFonte and Johnson 2013) of tadpoles to echinostome cercariae are known to vary among species. It could be that *R. catesbeiana* tadpoles are particularly good at avoiding cercarial infection (e.g., via high activity levels or microhabitat use) or clearing metacercariae that successfully penetrate the host. Regardless of the mechanism, the *R. catesbeiana* tadpoles used here may just be very poor hosts for echinostome metacercariae in comparison to the *Rana clamitans* tadpoles that had similar competency to snails (Wojdak et al. 2014).

Adding a host species that can absorb many parasites from the environment can reduce infection in other host species by reducing the probability of encounters between hosts and parasites (Mooring and Hart 1992, Keesing et al. 2006, Orlofske et al. 2012, Strauss et al. 2015). However, we found that adding a highly competent host species (*H. trivolvis* snails) while controlling for host density did not influence tadpole infection in our mesocosm experiment and only weakly reduced tadpole infection in our laboratory experiment. Other studies have also reported that adding alternative snail host species does not reduce focal host trematode infection risk (e.g., Johnson et al. 2012*a*, Wojdak et al. 2014), suggesting that encounter reduction may not necessarily occur when many parasites are being absorbed from the environment.

The small reduction in tadpole infection risk in the presence of snails that we observed in our laboratory experiment may indicate a cercariae host preference for snails over tadpoles. Cercariae use environmental stimuli and host chemical cues to actively seek out their next host (e.g., Haas 1994), so it is plausible that cercariae may preferentially target highly competent hosts (e.g., Evans et al. 1981, Detwiler and Minchella 2009, Wojdak et al. 2013). However, when the search time required for a parasite to encounter a host approaches the total time available for searching (i.e., the parasite's lifespan), the parasite should become less selective (i.e., increase host generality). And intriguingly, we found no effect of snail presence on tadpole infection intensity in our mesocosm experiment, where search times should have been relatively large, but we found evidence of a weak effect of snails on tadpole infection intensity in our laboratory experiment, where search times should have been relatively small. However, even in the small containers used in our laboratory experiment, the effect size of the possible preference for snails over tadpoles was weak and unlikely to substantially change parasite distributions among hosts in the field.

The large differences in parasite success among second intermediate host communities that we observed could have important implications for transmission to definitive hosts. However, several key pieces of information will be required before we can connect variation in second intermediate host competency to ultimate effects on transmission in this complex, three-host life cycle. Echinostome cercariae from tadpole and snail second intermediate hosts are equally infective to definitive hosts via trophic transmission (McCarthy 1999), but it is unclear which definitive hosts are being used in these systems and how frequently snails versus tadpoles are consumed by definitive hosts. If transmission via snail hosts is an important route, high prevalence and abundance of *Chaetogaster* may substantially reduce transmission of parasites to definitive hosts. Untangling how myriad host species and their parasites and other symbionts affect parasite transmission remains a tantalizing challenge for future ecological parasitology.

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