COMMUNITY ECOLOGY - ORIGINAL RESEARCH

It's a predator–eat–parasite world: how characteristics of predator, parasite and environment affect consumption

**Sarah A. Orlofske · Robert C. Jadin ·
Pieter T. J. Johnson**

Abstract Understanding the effects of predation on disease dynamics is increasingly important in light of the role ecological communities can play in host–parasite interactions. Surprisingly, however, few studies have characterized direct predation of parasites. Here we used an experimental approach to show that consumption of free-living parasite stages is highly context dependent, with significant influences of parasite size, predator size and foraging mode, as well as environmental condition. Among the four species of larval trematodes and two types of predators (fish and larval damselflies) studied here, parasites with larger infective stages (size >1,000 μm) were most vulnerable to predation by fish, while small-bodied fish and damselflies (size <10 mm) consumed the most infectious stages. Small parasite species (size approx. 500 μm) were less frequently consumed by both fish and larval damselflies. However, these results depended strongly on light availability; trials conducted in the dark led to significantly fewer parasites consumed overall, especially those with a size of <1,000 μm, emphasizing the importance of circadian shedding times of parasite free-living stages for

predation risk. Intriguingly, active predation functioned to help limit fishes' infection by directly penetrating parasite species. Our results are consistent with established theory developed for predation on zooplankton that emphasizes the roles of body size, visibility and predation modes and further suggest that consumer–resource theory may provide a predictive framework for when predators should significantly influence parasite transmission. These results contribute to our understanding of transmission in natural systems, the role of predator–parasite links in food webs and the evolution of parasite morphology and behavior.

Introduction

In natural systems, host–pathogen interactions can be strongly influenced by the broader ecological community, including predators. Predation can be an important factor influencing infection across diverse host–pathogen systems (Ostfeld and Holt 2004; Borer et al. 2009; Duffy et al. 2011). The most well-documented effects of predation tend to be indirect, in which predation influences host population densities, leading to changes in transmission, or when selective predators remove infected individuals from the population, thereby reducing infection risk for other hosts (Packer et al. 2003; Ostfeld and Holt 2004; Borer et al. 2009). For example, loss of vertebrate mesopredators can increase the number of infected rodent prey (Ostfeld and Holt 2004), potentially altering the abundance of infected vectors and human disease risk. Predation can also induce changes in host traits, such as in behavior, susceptibility or morphology, leading indirectly to changes in transmission

S. A. Orlofske · R. C. Jadin · P. T. J. Johnson
Department of Ecology and Evolutionary Biology,
University of Colorado, Boulder, CO 80309, USA

Present Address:
S. A. Orlofske (✉) · R. C. Jadin
Department of Biology, Northeastern Illinois University,
Chicago, IL 60625, USA
e-mail: s.a.orlofske@gmail.com

or pathology (Johnson et al. 2006; Keesing et al. 2006; Belden and Wojdak 2011; Duffy et al. 2011).

Concurrently, however, predators can also affect pathogen transmission directly. On the one hand, predators can become infected by multi-host parasites acquired during foraging (i.e. trophic transmission; Lafferty 1999; Hall et al. 2007); on the other hand, predators can directly consume parasites and thereby reduce transmission or persistence. Examples range from the grooming of ectoparasites to the consumption of free-living infectious stages encountered in the environment (Hopper et al. 2008; Thieltges et al. 2008; Prinz et al. 2009; Johnson et al. 2010; Orlofske et al. 2012). A specific example is the predation of trematode parasites by aquatic invertebrates, which was found to reduce transmission to tadpole hosts by 50 % in laboratory trials (Orlofske et al. 2012). Thus far, however, limited attention has focused on the role of direct predation on parasites and its importance for mediating transmission.

Understanding the influence of predation on parasite transmission requires explicit examination of the host, parasite and environmental conditions that influence parasite vulnerability. One valuable strategy to guide such investigations is to draw upon previous ecological research examining predator–prey interactions, such as that between zooplankton prey and aquatic predators. Aquatic infective stages of certain parasites, specifically trematodes, are similar in size to zooplankton (0.2–2 mm) and face many of the same challenges while trying to find a suitable host (Morely 2012). As with free-living prey, predation risk for parasites may differ based on predator foraging mode. In the study of Orlofske et al. (2012) active predators, ambush foragers, and filter feeders were effective predators of trematode parasite stages in aquatic systems, while insect predators with piercing sucking mouthparts were not. Predation risk for aquatic organisms will also depend on prey size, which influences their visibility to predators (Griffiths 1975; O'Brien et al. 1976). In a study of trematode infectious stages in marine systems, Kaplan et al. (2009) found that estuarine fish consumed larger parasites more frequently than small parasites. Factors that influence prey visibility, including pigmentation, water clarity and amount and/or timing of activity (e.g., diurnal vs. nocturnal), are also well documented to affect the vulnerability of zooplankton prey (Zaret and Kerfoot 1975; O'Brien et al. 1976; Wright and O'Brien 1982). Interestingly, however, because some parasites can survive a predation event or even infect the predator (e.g. trophic transmission), in some cases it may be adaptively advantageous for a parasite to increase its own visibility or that of its host to predators (Lewis et al. 1989; Combes 1991). It is therefore important to consider the parasite life cycle (or transmission mode) along with species traits and environmental factors when the aim is to determine predation risk to parasites.

In the study reported here, we used an experimental approach to examine the effects of predator and parasite species traits on the consumption of parasite free-living stages under different environmental conditions. We tested the capacity of two common aquatic predator groups, fish and larval insects, to consume free-living infective stages of four trematode species, which are an important group of parasites in aquatic ecosystems (Lafferty et al. 2008; Preston et al. 2013). We investigated the following questions: (1) How does predator identity and size influence their consumption of parasites? (2) How does parasite (prey) identity and size influence vulnerability to predation? (3) How is predation mediated by parasite (prey) density and light availability?. By selecting parasites with a range of transmission strategies and carefully tracking the fate of individual parasites (successful infection, eaten or died), we also investigated the role of predation in regulating transmission directly to predators, which simultaneously serve as hosts for some of the parasites included in the study. Our primary aim was to provide an empirical exploration of how predation can lead to changes in disease risk, using the relative importance of species traits and environmental context on consumer–resource interactions.

Materials and methods

Study system

We used four species of trematodes for our experimental procedures: a distinct morphotype "Magnacauda", *Ribeiroia ondatrae*, *Echinostoma trivolvis* and *Cephalogonimus americanus* (Fig. 1a–d). Details on the collection of infected snails and morphological and molecular species identification are provided in Electronic Supplementary Material (ESM) Appendix A. All species have complex life cycles that involve sequential infection of freshwater snails (*Helisoma trivolvis*) as the first intermediate hosts followed by larval amphibians as the second intermediate hosts. However, "Magnacauda" and *E. trivolvis* can also use fish and snails, respectively, as second intermediate hosts. We specifically chose these parasite species to provide a range of sizes, behavior and transmission strategies (Table 1). For three taxa (*C. americanus*, *E. trivolvis*, *R. ondatrae*) transmission occurs when cercariae directly penetrate the second intermediate host. Alternatively, "Magnacauda" is trophically transmitted to a second intermediate host through consumption. Notably, "Magnacauda" cercariae were observed to aggregate in large clumps and engage in synchronous swimming, which may facilitate detection by fish hosts by appearing to be larger (Beaver 1939a; Kaplan et al. 2009). In contrast, both *E. trivolvis* and *R. ondatrae* are continuous swimmers with no aggregation behavior, while *C.*
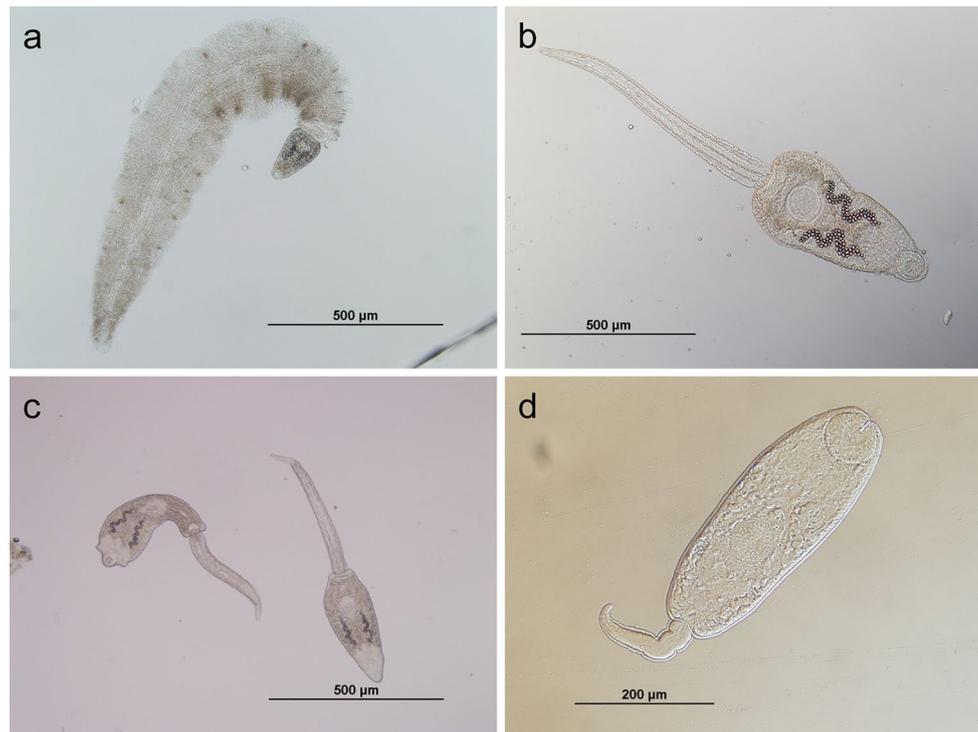
Fig. 1 Plate of trematode cercariae used in laboratory study [average total length (in microns ± standard error (SE)]. **a** "Magnacauda" (1,179 ± 117 μm), **b** *Ribeiroia ondatrae* (1,077 ± 57 μm), **c** *Echi-* *nostoma trivolvis* (771 ± 43 μm), **d** *Cephalogonimus americanus* (532 ± 74 μm). Note differences in the length of the *scale bars*

Table 1 Morphological data on the four trematode species used in the predation trials, including body, tail and total length and number of individuals measured

Parasite species[a]	Number of individuals measured (*n*)	Body length (μm)	Tail length (μm)	Total length (μm)
"Magnacauda"	5	113 ± 14	1,066 ± 104	1,179 ± 117
Ribeiroia ondatrae	9	427 ± 34	651 ± 28	1,078 ± 57
Echinostoma trivolvis	7	344 ± 19	428 ± 37	772 ± 43
Cephalogonimus americanus	3	374 ± 54	158 ± 20	533 ± 74

Data are presented as the average ± standard error (SE)

[a] Parasites are presented in the order of largest (top) to smallest (bottom) based on total length

americanus swim actively but intermittently with periods of resting (Beaver 1939b; Lang 1968; Haas 2003). In addition, these parasite species differ in their circadian emergence times, which may influence the risk of consumption by visual predators, such as fish and some invertebrates. *Cephalogonimus americanus* and *R. ondatrae* emerge at night, whereas *E. trivolvis* and "Magnacauda" emerge during the day or in response to light, which we determined based on life-history data gathered through literature searches and direct observation of infection and shedding behavior in the laboratory (Beaver 1939a, b; Lang 1968, 1969; Dronen and Lang 1974; Johnson et al., unpublished observations).

We examined predation by two types of aquatic organisms, namely, fish and invertebrates, which commonly co-occur with these parasites in freshwater ecosystems (Orlofske et al. 2012). Specifically, we used western mosquitofish (*Gambusia affinis*) and damselfly nymphs (*Enallagma* spp.), both of which have been observed to consume parasites in laboratory studies (Schotthoefer et al. 2007; Orlofske et al. 2012). Mosquitofish have an exceptionally wide diet range and have served in studies as a model system for investigating consumer–resource dynamics (Bence and Murdoch 1986; Pyke 2008). Vertebrate predators, such as fish, are generally active foragers that rely on visual cues

for prey detection, often leading to a positive relationship between prey size and consumption, particularly when visibility is high (Zaret 1980; Hanazato and Yasuno 1989). For our experiments, we selected two body sizes of mosquitofish that roughly correspond to juveniles (<10 mm body length) and adults (>25 mm body length) and therefore treated fish size as a categorical variable in all analyses. Gape size in mosquitofish is linearly related to standard length (Bence and Murdoch 1986). Based on evidence from studies with free-living prey (García-Berthou 1999; Blanco et al. 2004), we predicted that juvenile fish would consume higher numbers of parasites than adults due to the similarity in size of parasites to their typical zooplankton prey (Morely 2012).

Damselflies are largely opportunistic, ambush foragers, with their diet broadly reflecting the prey composition of the habitat (Johnson 1973; Thompson 1978). Invertebrate predators, including many aquatic insects, are size-dependent predators that exhibit a non-linear relationship between consumption and prey size, such that small prey are avoided and large prey are often too difficult to capture (Dodson 1974; Zaret 1980; Hanazato and Yasuno 1989). Because damselflies are tactile, ambush foragers, the amount of light in the environment may have less of an influence on prey detection (Peckarsky 1982). The methods for predator collection and maintenance are provided in ESM Appendix B and predator body sizes are provided in ESM Table B1.

Predation bioassay

Laboratory bioassays were used to determine how parasite, predator and environmental traits influenced the ability of predators to consume free-living cercariae (Orlofske et al. 2012). The experimental design consisted of an individual predator, naïve to parasite prey, provided with a single density of one parasite species under either light or dark conditions. Each individual predator was fasted for 24 h prior to the assay and used only once. We isolated cercariae from field-collected snails (*Helisoma trivolvis*) by placing them individually in 50-mL centrifuge tubes during their appropriate peak shedding time (day or night) and collecting cercariae within 2 h. For each assay, we placed a predator in 60 ml of water with 30 cercariae; after 30 min, we removed the predator and counted any remaining cercariae. Containers were 8 cm in diameter and 2.5 cm deep and were open to the air during the course of the experiment. To examine the effect of light condition on predation, half of the experimental containers were covered with a box sealed to the bench top to prevent all light from entering the box. Standard fluorescent room lights were the light source for all experiments.

Because mosquitofish also have the potential to become infected by some of the parasites used, we necropsied fish 48 h after the trials to quantify encysted parasites (see ESM Appendix C for necropsy procedures). We also dissected a subset of damselflies to confirm that they never became infected (*n* = 10/each parasite species) (see also Schotthoefer et al. 2007). To account for parasite loss in the absence of predators, we included control containers without predators, for which we successfully recovered all administered cercariae, verifying the effectiveness of our approach in tracking parasites. We directly observed mosquitofish and damselflies consuming cercariae and recovered a low number of cercariae tails [fish + *R. ondatrae* 2.9 ± 0.4 (mean ± SE) of 30 parasites administered], which is indicative of a low level of attempted infection (Orlofske et al. 2012).

Density of infectious stages

The design of the laboratory bioassay used to characterize consumption by both mosquitofish and damselfly nymphs to varying densities of two parasite species, *R. ondatrae* and *E. trivolvis*, was similar to that of the predation assay. For each assay, we placed a single individual of each predator species in 60 ml of water with one of three parasite treatments: 30, 60 or 90 cercariae. Different densities were selected in order to identify whether the proportion of parasites consumed was influenced by parasite density, and the specific densities chosen were based on previous data suggesting that fish could consume over 90 % of cercariae at the lowest density (Orlofske et al. 2012). Each trial lasted 30 min, after which we removed the predator and counted any remaining cercariae. We conducted all trials of parasite density in the light condition.

Analysis

We assessed (1) whether predator consumption differed among parasite (prey) species, (2) whether this relationship could be explained by parasite or predator size and (3) how light availability mediated these relationships. The fate of each parasite was recorded as "consumed" or "remaining" after a 30-min trial and analyzed using generalized linear mixed models (GLMMs) with a binomially distributed error and logit-link function in the R package lme4 (using the Laplace approximation method; R Development Core Team 2008; Zuur et al. 2009). Thus, in a trial involving one predator and 30 administered parasites, the fate of each parasite was recorded as 0 (persisting after 30 min) or 1 (consumed). Although "consumed" parasites could also have infected the predator, we conducted necropsies of sub-sets of predators to quantify successful infection, see following text). Predictor variables included parasite species (categorical), parasite total length (in millimeters), predator body length (in millimeters) and light condition (light or dark). Similarly, for

the parasite density trials conducted with *R. ondatrae* and *E. trivolvis*, we analyzed the relationship between consumption and parasite densities using GLMMs with the binomially distributed error and logit-link function and parasite species as a covariate. In all analyses, individual predator identity was included as a random effect to account for variation due to predator satiation or individual differences among predators. We included all possible interactions; if the interaction was found to be non-significant, the models were subsequently reduced and reassessed (Crawley 2007).

For both the bioassay and density trials, we analyzed data for each predator species (fish vs. dragonflies) separately for theoretical and empirical reasons: (1) consumption patterns were expected to differ between these predators; (2) our aim was to examine the effect of predator size, which would otherwise be confounded with predator identity (Zaret 1980). For the bioassay data, we used body length as a continuous measure of damselfly size; for fish, we treated body size as a categorical variable (juvenile vs. adult) because of the distinct size classes used (see ESM Appendix B). Parasite length (tail included) was included as both a linear and non-linear effect (polynomial regression) in statistical models based on theory derived from zooplankton systems suggesting that the relationship between predation risk and prey body size is often non-linear (Zaret 1980). We further examined the relationship of parasite length on predation through a series of non-linear regressions (ESM Appendix D).

Depending on the parasite-by-predator combination, several fates were possible. For damselflies, consumption was equivalent to the number of parasites removed during trials. However, for two of the parasite-by-fish combinations, infection was also possible. For *R. ondatrae*, infection of fish can be measured directly by quantifying metacercariae. For "Magnacauda", where consumption leads to infection (trophic transmission), we considered all the cercariae removed during the trial to be consumed (i.e. predation and infection were equivalent). To examine the effects of predator size and light condition on infection of *R. ondatrae* and "Magnacauda", we used GLMMs with individual predator identity included as a random effect. For this analysis, we used the number that successfully infected the host (based on necropsy) versus the number that did not (eaten or remaining at the end of 30 min). Mosquitofish used in the density trials were not evaluated for parasite infection because they were euthanized and necropsied within 20 min of the trial to evaluate cercariae digestion time (Orlofske et al., unpublished data). However, based on the results of the bioassay experiment (see Results) with fish of similar size and light conditions, we assumed that the contribution of infection to the number eaten was minimal for *R. ondatrae* and that the fish were refractory to infection with *E. trivolvis*.
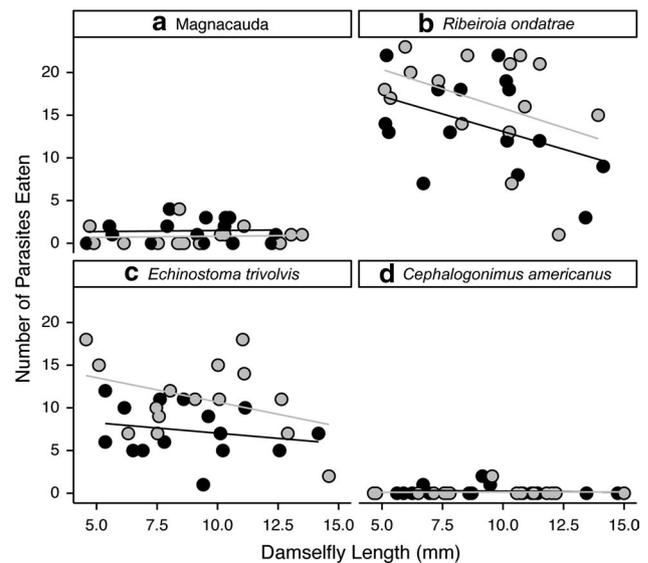

Fig. 2 Number of parasites eaten by damselfly nymphs under light (*gray circles*) or dark (*black circles*) conditions across a range of predator body length (range 4–15 mm; $n = 15$ per each parasite species; light treatment). Panels are arranged in descending order of parasite body size: **a** "Magnacauda" (1,179 ± 116 µm), **b** *R. ondatrae* (1,078 ± 57 µm), **c** *E. trivolvis* (772 ± 43 µm), **d** *C. americanus* (533 ± 74 µm)

Results

Predation bioassay

For damselflies, predation success depended strongly on the species of parasite (GLMM effect of parasite species $Z \leq 2.64$, $P \leq 0.008$; Fig. 2a). Damselflies consumed an average of 30 % of *E. trivolvis* and 50 % of *R. ondatrae* cercariae, but <1 % of *C. americanus* or "Magnacauda". Because cercariae of the smallest and largest parasite species were least vulnerable to predation (whereas the two intermediate-sized species were the most vulnerable; Table 1), the relationship between predation risk and parasite body size was non-linear (parasite size $Z = 11.26$, $P < 0.0001$; (parasite size)^2 $Z = -2.97$, $P = 0.003$; Table 2; Fig. 2b–c). Both damselfly size and the absence of ambient light (i.e. darkness) reduced parasite consumption (damselfly size $Z = -11.34$, $P < 0.0001$; light $Z = 3.43$, $P = 0.0006$; Fig. 2a). Even in the dark, however, there was still a strong non-linear relationship with parasite size (parasite size $Z = 14.62$, $P < 0.0001$; (parasite size)^2 $Z = -14.74$, $P < 0.0001$; ESM Appendix D) and a negative relationship with damselfly size ($Z = -3.73$, $P < 0.0001$; Fig. 2b). These results suggest a stronger effect of prey and predator traits relative to environmental condition for these tactile predators (Table 2).

Mosquitofish were highly effective predators of all parasite species, with an average consumption ranging from 38 to 75 % (GLMM of parasite species identity $Z \leq 0.73$,

Table 2 Results of generalized linear mixed models examining the consumption of parasite prey by damselfly nymphs and the role of parasite species, parasite total length, predator body size and light condition on those trophic interactions

Trials	Variable	Z value	P value	Bayesian Information Criterion[a]	Akaike Information Criterion[a]	N[b]
Light trials–parasite species[a]	Intercept	−2.73	0.006*	4.22	1,312	1,800
	"Magnacauda"	−7.69	0.000*	55.82		
	Ribeiroia ondatrae	2.64	0.008*	3.70		
	Cephalogonimus americanus	−6.08	0.000*	33.75		
Light trials–parasite size[b]	Intercept	−11.11	0.000*	120.18	1,347	1,800
	Parasite body size	11.26	0.000*	123.53		
	(Parasite body size)^2	−2.97	0.003*	5.57		
	Predator body size	−11.34	0.000*	125.34		
Light and dark trials–parasite size[c]	Intercept	−14.52	0.000*	207.22	2,699	3,600
	Parasite body size	14.62	0.000*	210.07		
	(Parasite body size)^2	−14.74	0.000*	213.65		
	Predator body size	−3.73	0.000*	10.36		
	Light condition	3.43	0.001*	8.19		

Results are arranged based on partitions of the dataset by light condition and whether parasites were analyzed by species (categorical) or body size (continuous)

* Significant at $P = 0.05$

[a] Bayesian Information Criterion (BIC) values indicate the relative strength of the relationship of the particular predictor variables to the response of numbers of cercariae consumed while the Akaike Information Criterion (AIC) indicates the relative fit of the overall model

[b] N = sample size based on number of cercariae

[a] Trials conducted in the light using parasite species identity

[b] Trials conducted in the light using parasite body size

[c] Both light and dark trials and parasite body size

$P \geq 0.223$). Consumption patterns again depended on predator size (fish size class × parasite size $Z = -3.4$, $P = 0.0006$; Fig. 3); for all parasites except "Magnacauda", juvenile fish consumed more parasites than adult fish ($Z \leq 7.37$, $P < 0.0001$; Fig. 3b–d; "Magnacauda" $Z = 1.12$, $P = 0.262$; Fig. 3a). For juvenile fish only, light availability increased consumption (light × fish size class $Z = 18.05$, $P < 0.0001$; Table 3). Parasite species also tended to be more vulnerable as a function of light availability, with the exception of "Magnacauda", which was consumed readily in both conditions (light × parasite size $Z = -2.23$, $P = 0.026$). Thus, consumption of parasites by fish was strongly influenced by the body size of both predator and prey and the environment in which they interacted, resulting in varying predation risk for the parasite species.

Infection patterns within fish differed based on parasite species and transmission mode. "Magnacauda" infection intensities were marginally higher in adult fish relative to juvenile fish ($Z = -1.87$, $P = 0.062$), but showed no relationship to light condition ($Z = 0.25$, $P = 0.801$). For *R. ondatrae*, which actively infects fish, light availability interacted with predator size to influence infection (light × predator size $Z = -3.22$, $P = 0.001$; ESM Appendix D; ESM Table E1; Fig. 4b). Under ambient light, juvenile fish
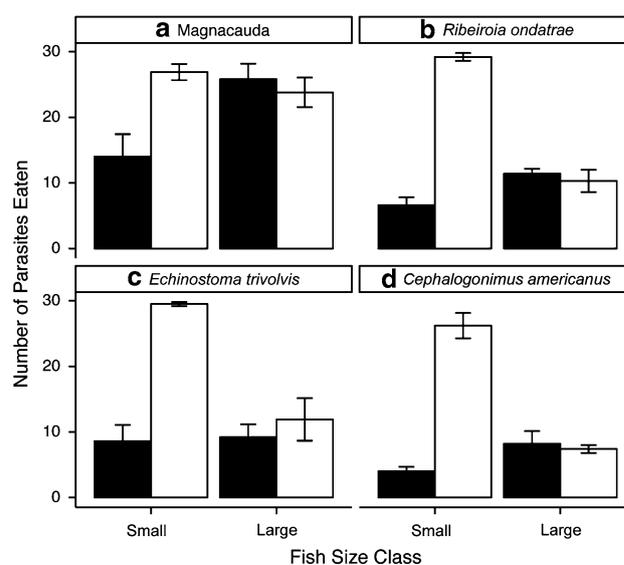

Fig. 3 Mean number of parasites eaten by mosquitofish predators under light (*open bars*) or dark (*filled bars*) conditions and between two size classes (*Large* >25 mm, *Small* <10 mm; $n = 5$ per each body size and light treatment for *C. americanus*, $n = 10$ for all others). Panels are arranged in descending order of parasite body size: **a** "Magnacauda" (1,179 ± 116 μm), **b** *R. ondatrae* (1,078 ± 57 μm), **c** *E. trivolvis* (772 ± 43 μm), **d** *C. americanus* (533 ± 74 μm)

Table 3 Results of generalized linear mixed models examining the consumption of parasite prey by mosquitofish and the role of parasite species, parasite total length, predator body size and light condition on those trophic interactions

Trials	Variable	Data[a]	Z value	P value	BIC[b]	AIC[b]	N[c]
Light trials–parasite species[d]	Intercept		3.12	0.002*	6.40	1,529	2,070
	"Magnacauda"		0.73	0.468	−2.79		
	Ribeiroia ondatrae		−0.27	0.786	−3.24		
	Cephalogonimus americanus		−1.22	0.223	−1.83		
Light trials–parasite size[e]	Intercept		−1.02	0.308	−2.28	1,691	2,070
	Parasite body size		−0.11	0.916	−3.30		
	(Parasite body size)^2		0.95	0.341	−2.41		
	Predator size class		7.60	0.000*	54.37		
	Predator size class × parasite body size		−3.40	0.001*	8.24		
Partition × parasite species[e]	Intercept	Mag	3.68	0.000*	10.75	402	570
	Predator size class		1.12	0.262	−1.50		
	Intercept	R. o.	−2.15	0.032*	1.83	439.2	600
	Predator size class		7.37	0.000*	51.52		
	Intercept	E. t.	−0.90	0.368	−1.97	337.9	600
	Predator size class		4.88	0.000*	21.00		
	Intercept	C. a.	−3.66	0.000*	10.94	284.2	300
	Predator size class		6.79	0.000*	43.57		
Light and dark trials–parasite size[f]	Intercept		0.31	0.755	−3.52	4,056	4,170
	Light condition		2.09	0.037*	0.73		
	Parasite body size		−2.60	0.009*	3.12		
	(Parasite body size)^2		4.31	0.000*	14.98		
	Predator body size		2.34	0.019*	1.85		
	Predator size class × parasite body size		−4.44	0.000*	16.12		
	Light condition × parasite body size		−2.23	0.026*	1.33		
	Light condition × predator size class		18.05	0.000*	322.04		
Dark trials[c]	Intercept		0.75	0.453	−2.76	2,161	2,100
	Parasite body size		−1.57	0.116	−0.86		
	(Parasite body size)^2		2.21	0.027*	1.55		
	Predator body size		1.08	0.278	−2.15		
	Predator size class × parasite body size		−1.98	0.047*	0.61		
Dark trials × parasite species[f]	Intercept	Mag	3.90	0.000*	12.46	454	600
	Predator size class		−3.20	0.001*	7.44		
	Intercept	R. o.	−3.26	0.001*	7.86	718.7	600
	Predator size class		−3.49	0.000*	9.42		
	Intercept	E. t.	−2.55	0.011*	3.71	650.6	600
	Predator size class		−0.36	0.720	−2.65		
	Intercept	C. a.	−4.03	0.000*	13.80	298	300
	Predator size class		−2.31	0.021*	2.84		

Results are arranged based on partitions of the dataset by light condition and whether parasites were analyzed by species (categorical) or body size (continuous)

* Significant at $P = 0.05$

[a] Parasite species are referred to in table by the first letter of genus and species names (*C. a.* = *Cephalogonimus americanus*, *E. t.* = *Echinostoma trivolvis*, *R. o.* = *Ribeiroia ondatrae*, Mag = "Magnacuada")

[b] BIC values indicate the relative strength of the relationship of the particular predictor variables to the response of numbers of cercariae consumed while AIC indicates the relative fit of the overall model

[c] N = sample size based on number of cercariae

[d] Trials conducted in the light using parasite species identity

[e] Trials conducted in the light using parasite body size and was further partitioned by parasite species to examine significant interactions

[f] Both light and dark trials and parasite body size and was further partitioned by light treatment and parasite species to examine significant interactions
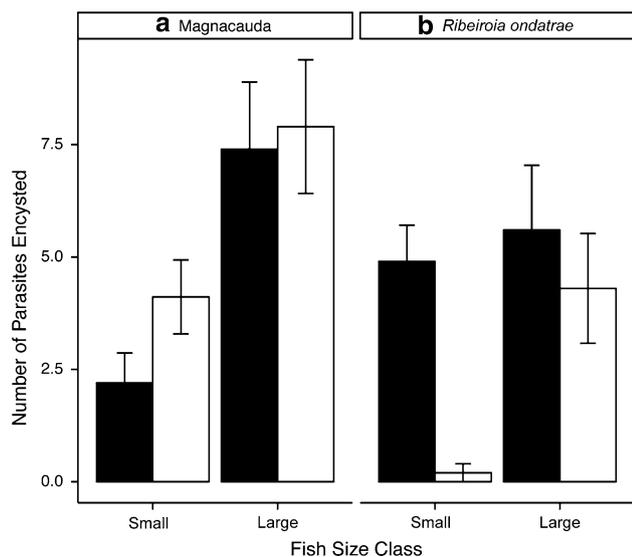
Fig. 4 Mean number of parasites encysted in mosquitofish predators/hosts under light (*open bars*) or dark (*filled bars*) conditions and between two size classes (*Large* >25 mm, *Small* <10 mm; *n* = 10 per each body size and light treatment). **a** "Magnacauda", **b** *R. ondatrae*. *Error bars*: ± 1 SE
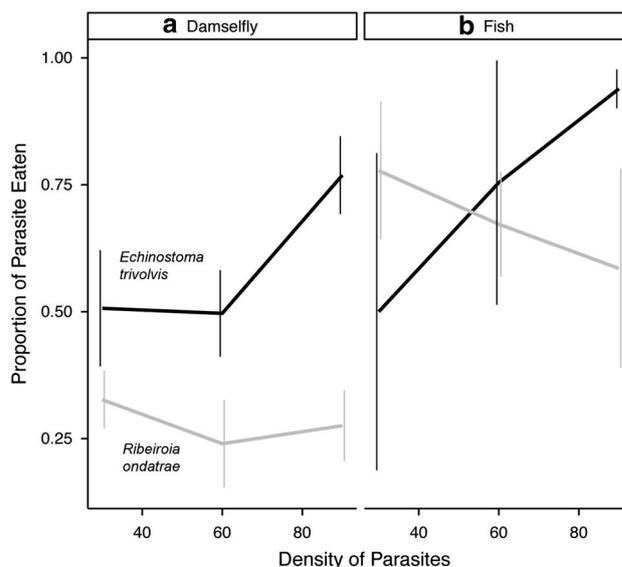

Fig. 5 Proportion of parasites *R. ondatrae* and *E. trivolvis* eaten across a range of densities for both Damselfy nymphs (**a**; *n* = 15) and Mosquitofish (**b**; *n* = 12 for *E. trivolvis* and *n* = 9 for *R. ondatrae*). *Error bars* ±1 SE

had lower infection intensities than adult fish ($Z = -3.37$, $P < 0.001$); in the dark, however, infection intensities in both groups increased and did not differ from each other ($Z = -0.27$, $P = 0.791$). The high number of *R. ondatrae* cercariae eaten by the juvenile fish in the light treatment (Fig. 3b) suggests that these fish limited their infection by consuming the parasites before they were able to infect.

Parasite density

For *E. trivolvis*, the proportion of parasites consumed by damselfly predators increased from 50 % to almost 80 % with increases in parasite density. For *R. ondatrae*, however, consumption remained level at approximately 30 % (parasite density × species $Z = -2.06$, $P = 0.039$; ESM Table E2; Fig. 5a). For mosquitofish, the proportion of parasites consumed showed a weakly positive relationship with parasite density (parasite density $Z = 2.01$, $P = 0.044$; ESM Table E2). However, the relationship appeared to be negatively related to density for *R. ondatrae* and positive for *E. trivolvis*, as indicated by a marginally significant interaction between density and parasite species (parasite density × species $Z = -1.83$, $P = 0.067$; ESM Table E2; Fig. 5b).

Discussion

Our results indicate that both the vertebrate and invertebrate predators studied here were highly effective at consuming

free-living trematode stages (consumption rate 50–75 %) in the laboratory. This relationship depended critically on predator traits, parasite (prey) traits and the environmental context in which predator and parasite interacted. Damselflies only consumed parasites of intermediate sizes (size range 0.7–1 mm), with consumption declining with increasing damselfly body size and in the absence of ambient light. Fish consumed all parasite species, particularly the largest parasite species (mean size 1.2 mm); juvenile fish were the most effective predators, but darkness strongly reduced their consumption rate. While current research on predation on parasites focuses on contributions to food web structure and function or on the consequences of predation on parasites for the transmission of individual parasite species (Orlofske et al. 2012; Thieltges et al. 2013), here we contribute to a trait-based foundation for predicting when predation on parasites is likely to occur, with consequences for transmission dynamics (Kaplan et al. 2009).

Consistent with results from earlier studies (Schotthoefer et al. 2007; Orlofske et al. 2012), we found that damselfly nymphs were effective predators of parasites; however, by testing a range of parasite species we identified specific traits that influence their efficiency. Specifically, predation by damselflies exhibited a strongly non-linear relationship with parasite total length, potentially due to a reduced detection of *C. americanus*, the smallest and least active parasite, and/or the avoidance of the largest parasite "Magnacauda", possibly due to handling limitations (Zaret 1980; Hanazato and Yasuno 1989). In addition to their large individual size, we propose that the unique clumping behavior

of "Magnacauda" cercariae may have further contributed to their low predation rate, assuming damselflies did not respond to a large mass (diameter of approx. 1 cm) that appears as a single prey item. Additional studies with other species of parasites across a wider range of body size with different activity patterns will be necessary to distinguish the importance of these various traits. Consumption of parasites also tended to decrease with progressive increases in damselfly body size, likely because of optimal foraging considerations where parasite prey may be too small to be useful energetically (Peckarsky 1982). Light availability had a significant, but much weaker effect on parasite consumption, which agrees well with predictions based on the primarily ambush foraging mode of these tactile predators (Peckarsky 1982). While the absence of light may have limited damselfly vision, it may also have reduced parasite activity, thereby reducing the consumption rate of the damselflies. Our study only examined a single invertebrate predator that used an ambush foraging strategy, so a wider range of invertebrates, including ambush foragers, need to be examined in order to distinguish between these factors.

For vertebrate fish predators, traits of both prey and predator species and light availability mediated the predation of parasites. Predation by fish was greatest on the largest parasite ("Magnacauda"), which approaches the size of large-bodied *Daphnia* prey (mean size 1.2 mm; Brooks and Dodson 1965). Larger bodied fish were less effective predators of cercariae, which has also been reported for estuarine fish and cercariae species (Kaplan et al. 2009). This is similar to our results for damselflies and could also be a reflection of optimal diet theory where fish select the most profitable prey items (Bence and Murdoch 1986). As fish grow and mature, the diet shifts towards selection for larger prey, as in mosquitofish with zooplankton (García-Berthou 1999). Because cercariae represent more closely the size range of zooplankton prey commonly used by juvenile fish, they may be more effective predators than adult fish which more commonly feed on macroinvertebrates (Blanco et al. 2004). Fish consumption of parasites also tended to decrease in the absence of light, likely because these visual predators rely on light for detecting prey (Aksnes et al. 2004). In general, these patterns of fish–parasite predation follow the expectations for how these visual predators interact with free-living prey based on prey and predator body size and visibility (Zaret 1980).

By examining the role of parasite density in relation to their consumption by damselfly and mosquitofish, we further explored how the experimental results on species traits and light conditions are applicable for a range of parasite densities. An individual infected snail can produce hundreds of cercariae in a single 24-h period, ranging from approximately 90 cercariae by *E. trivolvis* up to approximately 580 cercariae by *C. americanus* (Preston et al.

2013). Given the varying densities of infected snails in nature, we expect highly variable levels of parasite densities in nature. Our experimental results specifically suggest that damselflies continued to consume large proportions of parasites even at relatively high densities, especially the smaller parasite *E. trivolvis*. Based on the proportion consumed, the overall number of parasites eaten continued to increase with density, suggesting that in no case had satiation or handling limitation been reached. For *R. ondatrae*, however, the proportion of cercariae consumed by fish was relatively constant across increases in parasite density. This contrast between the two parasite species may be related to size differences between the two parasites, where predators were unable to consume more of the larger parasite (*Ribeiroia*) during the short (30 min) time trials. Investigating functional responses remains an important area for future research as it may help predict when predators will regulate parasite populations and thereby transmission (Murdoch and Oaten 1975). Previous experiments with both damselflies and mosquitofish demonstrate that parasites are consumed in the presence of alternative prey (Orlofske et al. 2012); however, this is another important influence on predator prey selection and subsequent effects on transmission.

Predation of free-living stages can reduce transmission by increasing parasite mortality, sometimes with substantial reductions in host pathology, making predicting when predation on parasites may be significant particularly relevant (Hopper et al. 2008; Thieltges et al. 2008; Prinz et al. 2009; Orlofske et al. 2012). Based on our experimental results, we suggest that information on species traits and environmental conditions can provide a useful framework for understanding when transmission may be affected. Parasites ranging in size from 0.5 to 1 mm were vulnerable to both predator types, while those ≥1 mm in size were particularly at risk of predation from fish. Communities with a high diversity and abundance of both vertebrate and invertebrate taxa have the potential to regulate transmission of the widest breadth of parasite species. Furthermore, predation risk may change seasonally based on the presence of small, early ontogenetic stages of predators, which may prefer parasite prey as well as correspond with peaks in availability of cercariae in the environment (Kaplan et al. 2009; Preston et al. 2013). Our results also highlight the role of visibility in determining predation risk to parasites. While light availability is a well-known factor which mediates predation on free-living species—even driving vertical migrations patterns in zooplankton (Zaret 1980)—precisely how predation risk varies among parasites that emerge at different times of day remains poorly understood. We propose that the transmission of parasite species with a diurnal shedding pattern is more likely to be influenced by predation. In contrast, parasites that exhibit a nocturnal circadian

release of free-living stages may decrease their visibility to non-host predators, thereby increasing potential contact with appropriate hosts (Lewis et al. 1989; Combes 1991). Intriguingly, predation may be an additional selective force on the circadian emergence behavior and body size of parasites (Orlofske et al. 2014). In a specific case, parasites frequently consumed may be selected to acquire predators as hosts through trophic transmission (Schmid-Hempel 2011).

Through their consumption of infectious stages, predators may even influence their own infection risk. For the trophically transmitted cercariae, "Magnacauda", consumption by fish led to infection. By tracking the fate of individual parasites, we observed that the predator body size and light treatments with the highest consumption also had the highest infection levels, suggesting that increased predation leads to higher transmission. Importantly, we found that cercariae behavior, including active, synchronous swimming and aggregation, may have prevented consumption by unsuitable hosts (invertebrates) while increasing predation by appropriate hosts (fish), even in conditions of low visibility. In contrast, juvenile mosquitofish exposed to the directly penetrating parasite, *R. ondatrae*, limited their own infection in conditions of high visibility from an average of approximately five in the dark treatment to 0.2 (Fig. 4). This process may contribute to the low infection levels of *R. ondatrae* in wild mosquitofish populations (Orlofske et al. 2012). Similarly, zebrafish consumption has been observed to limit infection by cercariae of *Trasversotrema patialense* (Anderson et al. 1978). By reducing their own infection levels, predation on cercariae parallels grooming behavior where organisms reduce their own ectoparasite infection intensities (Johnson et al. 2010).

Results from our experimental approach provide preliminary evidence that aquatic predators respond to parasite prey in a manner consistent with patterns based on consumer–resource theory developed for freshwater communities (Brooks and Dodson 1965; Zaret 1980), including the responses of different types of predators (ambush and active foragers), the role of prey and predator body sizes and the availability of light. This theory may therefore provide a useful bridge for linking predator–prey and disease ecology, both for addressing when predation on parasites may influence transmission and for food web dynamics natural systems. Studies of additional species of parasites and predators as well as processes such as functional response can lend further support to the generalization of this theory. Further extensions of this research include examining the role of predator–parasite relationships in the stability of aquatic food webs, the contribution of parasites to predator diets and the evolutionary selection of parasite traits, such as circadian shedding rhythms, body size and transmission mode in response to predation threats (Zaret 1980; Lafferty et al. 2008; Johnson et al. 2010).

References

Aksnes DL, Nejstgaard J, Sœdberg E, Sørnes T (2004) Optical control of fish and zooplankton populations. Limnol Oceanogr 49:223–238

Anderson RM, Whitfield PJ, Dobson AP, Keymer AE (1978) Concomitant predation and infection processes: an experimental study. J Anim Ecol 47:891–911

Beaver PC (1939a) The morphology and life history of *Petasiger nitidus* Linton (Trematoda: Echinostomatidae). J Parasitol 25:269–276

Beaver PC (1939b) The morphology and life history of *Psilostomum ondatrae* Price 1931 (Trematoda: psilostomidae). J Parasitol 25:383–393

Belden LK, Wojdak JM (2011) The combined influence of trematode parasites and predatory salamanders on wood frog (*Rana sylvatica*) tadpoles. Oecologia 166:1077–1086

Bence JR, Murdoch WW (1986) Prey size selection by the mosquitofish: relation to optimal diet theory. Ecology 67:324–336

Blanco S, Romo S, Villena M-J (2004) Experimental study on the diet of mosquitofish (*Gambusia holbrooki*) under different ecological conditions in a shallow lake. Int Rev Hydrobiol 89:250–262

Borer ET, Mitchell CE, Power AG, Seabloom EW (2009) Consumers indirectly increase infection risk in grassland food webs. Proc Natl Acad Sci USA 106:503–506

Brooks JL, Dodson SI (1965) Predation, body size and the composition of plankton. Science 150:28–35

Combes C (1991) Ethological aspects of parasite transmission. Am Nat 138:866–880

Crawley MJ (2007) The R book. Wiley, West Sussex

Dodson SI (1974) Zooplankton competition and predation: an experimental test of the size-efficiency hypothesis. Ecology 55:605–613

Dronen NO Jr, Lang BZ (1974) The life cycle of *Cephalogonimus salamandrus* sp. n. (Digenea: cephalogonimidae) from *Ambystoma tigrinum* (green) from Eastern Washington. J Parasitol 60:75–79

Duffy MA, Housley JM, Penczykowski RM, Cáceres CE, Hall SR (2011) Unhealthy herds: indirect effects of predators enhance two drivers of disease spread. Funct Ecol 25:945–953

García-Berthou E (1999) Food of introduced mosquitofish: ontogenetic diet shift and prey selection. J Fish Biol 55:135–147

Griffiths D (1975) Prey availability and the food of predators. Ecology 56:1209–1214

Haas W (2003) Parasitic worms: strategies of host finding, recognition and invasion. Zoology 106:349–364

Hall SR, Sivars-Becker L, Becker C, Duffy MA, Tessier AJ, Cáceres CE (2007) Eating yourself sick: transmission of disease as a function of foraging ecology. Ecol Lett 10:207–218

Hanazato T, Yasuno M (1989) Zooplankton community structure driven by vertebrate and invertebrate predators. Oecologia 81:450–458

Hopper JV, Poulin R, Thieltges DW (2008) Buffering role of the intertidal anemone *Anthopleura aureoradiata* in cercarial transmission from snails to crabs. J Exp Mar Biol Ecol 367:153–156

Johnson DM (1973) Predation by damselfly naiads on cladoceran populations: fluctuating intensity. Ecology 54:251–268

Johnson PTJ, Preu ER, Sutherland DR, Romansic J, Han B, Blaustein AR (2006) Adding infection to injury: synergistic effects of predation and parasitism on salamander limb malformations. Ecology 87:2227–2235

Johnson PTJ, Dobson A, Lafferty KD, Marcogliese DJ, Memmott J, Orlofske SA, Poulin R, Thieltges DW (2010) When parasites become prey: ecological and epidemiological significance of eating parasites. Trends Ecol Evol 25:362–371

Kaplan AT, Rebhal S, Lafferty KD, Kuris AM (2009) Small estuarine fishes feed on large trematode cercariae: lab and field investigations. J Parasitol 95:477–480

Keesing F, Holt RD, Ostfeld RS (2006) Effects of species diversity on disease risk. Ecol Lett 9:485–498

Lafferty KD (1999) The evolution of trophic transmission. Parasitol Today 15:111–115

Lafferty KD, Allesina S, Arim M, Briggs CJ, DeLeo G, Dobson AP, Dunne JA, Johnson PTJ, Kuris AM, Marcogliese DJ, Martinez ND, Memmott J, Marquet PA, McLaughlin JP, Mordecai EA, Pascual M, Poulin R, Thieltges DW (2008) Parasites in food webs: the ultimate missing links. Ecol Lett 11:533–546

Lang BZ (1968) The life cycle of *Cephalogonimus americanus* Stafford, 1902 (Trematoda: Cephalogonimidae). J Parasitol 54:945–949

Lang BZ (1969) Modes of infection of *Rana clamitans* with *Cephalogonimus americanus* (Trematoda). J Parasitol 55:832

Lewis MC, Welsford IG, Uglem GL (1989) Cercarial emergence of *Proterometra macrostoma* and *P. edneyi* (Digenea: azygiidae): contrasting responses to light: dark cycling. Parasitology 99:215–223

Morely NJ (2012) Cercariae (Platyhelminthes: trematoda) as neglected components of zooplankton communities in freshwater habitats. Hydrobiologia 691:7–19

Murdoch WW, Oaten A (1975) Predation and population stability. Adv Ecol Res 9:1–131

O'Brien WJ, Slade NA, Vinyard GL (1976) Apparent size as the determinant of prey selection by bluegill sunfish (*Lepomis macrochirus*). Ecology 57:1304–1310

Orlofske SA, Jadin RC, Preston DL, Johnson PTJ (2012) Parasite transmission in complex communities: predators and alternative hosts alter pathogenic infections in amphibians. Ecology 93:1247–1253

Orlofske SA, Jadin RC, Hoverman JT, Johnson PTJ (2014) Predation and disease: understanding the effects of predators at several trophic levels on pathogen transmission. Freshwater Biol. 59:1064–1075

Ostfeld RS, Holt RD (2004) Are predators good for your health? Evaluating evidence for top-down regulation of zoonotic disease reservoirs. Front Ecol Environ 2:13–20

Packer C, Holt RD, Hudson PJ, Lafferty KD, Dobson AP (2003) Keeping the herds healthy and alert: implications of predator control for infectious disease. Ecol Lett 6:797–802

Peckarsky BL (1982) Aquatic insect predator-prey relations. Bioscience 32:261–266

Preston DL, Orlofske SA, Lambden JP, Johnson PTJ (2013) Biomass and productivity of parasites in pond ecosystems. J Anim Ecol 82:509–517

Prinz K, Kelly TC, O'Riordan RM, Culloty SC (2009) Non-host organisms affect transmission processes in two common trematode parasites of rocky shores. Mar Biol 156:2303–2311

Pyke GH (2008) Plague minnow or mosquito fish? A review of the biology and impacts of introduced *Gambusia* species. Annu Rev Ecol Evol Syst 39:171–191

R Development Core Team (2008) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available at: http://www.R-project.org

Schmid-Hempel P (2011) Evolutionary parasitology: the integrated study of infections, immunology, ecology, and genetics. Oxford University Press, Oxford

Schotthoefer AM, Labak KM, Beasley VR (2007) *Ribeiroia ondatrae* cercariae are consumed by aquatic invertebrate predators. J Parasitol 93:1240–1243

Thieltges DW, Jensen KT, Poulin R (2008) The role of biotic factors in the transmission of free-living endohelminth stages. Parasitology 135:407–426

Thieltges DW, Amundsen P-A, Hechinger RF, Johnson PTJ, Lafferty KD, Mouritsen KN, Preston DL, Reise K, Dieter Zander C, Poulin R (2013) Parasites as prey in aquatic food webs: implications for predator infection and parasite transmission. Oikos 122:1473–1482

Thompson DJ (1978) Prey size selection by larvae of the damselfly, *Ischnura elegans* (Odonata). J Anim Ecol 47:769–785

Wright DI, O'Brien WJ (1982) Differential location of *Chaoborus* larvae and *Daphnia* by fish: the importance of motion and visible size. Am Midl Nat 108:68–73

Zaret TM (1980) Predation and freshwater communities. Yale University Press, New Haven

Zaret TM, Kerfoot WC (1975) Fish predation on *Bosmina longirostris*: body-size selection versus visibility selection. Ecology 56:232–237

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer, New York