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Competition and host size mediate larval anuran interactions with trematode parasites

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Running Head: Competition, body size, and parasitism of tadpoles

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Summary

1. How parasites influence individual host traits and survival often depend on the ecological context of the host-parasite interaction, such as the presence of competitors or predators and trait variation among hosts.
2. We examined the effects of three key components of ecological context - host density, size structure and predator cue - on interactions between larval frogs and trematode parasites (Digenea: Echinostomatidae) in mesocosms.
3. We found that effects of parasites on host growth ranged could be either negative or positive, depending on host size and overall growth rate, but not on predator presence. A surprising positive effect of parasites on host growth under some conditions could represent an adaptive host life history response, whereby enhanced growth allows escape from a smaller, less tolerant size class that experiences more negative fitness effects of infection.
4. Notably, only host size class was a strong predictor of infection intensity, but not host density or predator cue.
5. Overall, these results suggest that parasitism, competition and host size interact to influence host fitness. Ecological context thus mediates the interactions between parasites and their hosts, with implications for parasite effects in nature.

Introduction

Parasites are well known to affect the performance or traits of individual hosts (e.g. Scott 1988). Such effects are frequently documented in small-scale experiments that examine pairwise host-parasite interactions. However, relating these effects to interactions in nature requires understanding of (or at least functional relationships regarding) how these impacts change with the ecological context of the individual host. For example, species density (Steinhaus 1958; Begon 2008), the intensity of competition (Barnes & Siva-Jothy 2000; Bedhomme *et al.* 2005), predator presence (Duffy *et al.* 2011) and the size of the organisms (McDonald *et al.* 2006; Hechinger 2013) can all have important effects on interactions with parasites. Context may thus mediate the influence of parasites on both host traits and survival, with consequences for other interactions, such as trait- and density-mediated indirect interactions (Werner and Peacor 2003).

Host density merits special attention because of its commonly central role in mediating parasite transmission (McCallum, Barlow & Hone 2001; Begon 2008). However, the direct effects of density on host-parasite interactions cannot easily be examined in isolation, because

the strength of competition also depends on density, and competition can affect interactions with parasites, for example through reduced nutrition, which may increase or decrease parasitism (Coop & Kyriazakis 1999; Smith, Ii & Smith 2005). Thus an increase in density may simultaneously increase competition for food resources while reducing the ratio of parasite infective stages to hosts (i.e. encounter-dilution; Cote & Poulin 1995; Rifkin, Nunn & Garamszegi 2012). Furthermore, the separate and joint effects of parasites and competition on individual hosts are unlikely to be uniform within populations, due to trait variation. For instance, host size structure can influence and be influenced by interactions with parasites and competitors (Persson 1983; Morin & Johnson 1988). Finally, the presence of predators can further modify the interaction between predators and parasites (Duffy *et al.* 2011, Ramirez & Snyder 2009), potentially interactively with competition or mediated by size-dependent differences in defenses to parasites and predators.

We evaluated the influence of parasitism, host density and predator presence on a size-structured assemblage of larval frogs. We focused on the interactions of trematode parasites (Digenea: Echinostomatidae) with two size classes of larval anurans (large and small green frogs [*Rana clamitans*]) that differ in the fitness effects of parasites, as larger tadpoles experience lower mortality post-infection, although also potentially higher infection due to increased contact rates associated with larger body surface area (Holland *et al.* 2007). We tested three main hypotheses regarding the context-dependence of the host-parasite interaction. 1) Increased small tadpole density should reduce per capita infection rates in both size classes of tadpoles, due to encounter dilution and higher total removal of infective stages from the water at high densities. We expected a density increase to reduce the ratio of infective stages to hosts because echinostomes are indirectly transmitted (in contrast to a directly transmitted parasite with a short generation time, for which transmission might be expected to increase with density), so that the number of cercariae present does not increase with local tadpole host density, at least over short timescales. 2) Parasites should indirectly benefit larger tadpoles in competitive interactions, because increased tolerance of infection (i.e. ability to limit harm at a particular parasite burden, Raberg, Graham & Read 2009) is conferred by greater size (Holland et al. 2007), resulting in density- and trait-mediated indirect effects of parasites. 3) Larger tadpoles should benefit from the presence of predators through competitive ability and parasite fitness effects, because smaller tadpoles respond more strongly to predator presence (Fraker 2008) and predator cues and

parasites can have interactive effects on tadpoles (Thiemann & Wassersug 2000; Szuroczki & Richardson 2009; Marino, Holland & Middlemis Maher 2014).

To test our hypotheses, we performed two new mesocosm experiments, which were then compared to two experiments from a previously published study (Marino and Werner 2013). In the first experiment, we manipulated parasite presence, host density and the presence of predator cue. We then performed a second experiment to further investigate the joint effects of density and parasites across a broader density gradient. Finally, we coupled the results of these experiments with findings from the two similar previous experiments to examine more generally the context-dependence of observed effects.

Methods

Study system

Echinostomes have a complex life cycle involving a snail first intermediate host, an amphibian, fish or mollusc second intermediate host, and a bird or mammal definitive host (Kanev, Sterner & Fried 2000). Within the snail first intermediate host, the parasite undergoes multiple rounds of asexual reproduction during sporocyst and redia stages before producing high numbers of a free-swimming infective stage, the cercaria, which then enters the second intermediate host. In larval amphibians, cercariae contact the host body, crawl toward and enter the cloaca, and migrate to the kidneys, where they encyst, forming metacercariae (Beaver 1937). If an appropriate definitive host consumes the amphibian host, the parasite completes its development to the adult stage in the host digestive tract and sexual reproduction occurs. Eggs pass in the faeces and hatch releasing free-swimming miracidia that infect the snail host, beginning the cycle anew.

Echinostomes can have a range of effects on amphibian hosts, such as reduced growth rates, impaired kidney function and death at high infection intensities (Fried, Pane & Reddy 1997; Holland *et al.* 2007), although such effects may be dose- and scale- dependent (Marino & Werner 2013; Marino *et al.* 2014). Larger tadpoles at later developmental stages tend to be more tolerant of infection (Schotthoefer, Cole & Beasley 2003; Holland *et al.* 2007). Green frogs, the species used here, have a long (~3 month) breeding season and often overwinter as tadpoles, so that tadpoles of different size classes frequently co-occur in natural ponds.

Animal collection and care

Green frog egg masses were collected from the experimental ponds on the Edwin S. George Reserve (ESGR) in Livingston County, MI, and placed in 300 L wading pools filled with aged well water. After hatching, tadpoles were fed Purina® (St. Louis, MO) Rabbit Chow *ad libitum* until the beginning of experiments. Mesocosms used in experiments and to culture large green frog tadpoles were 1,300 L cattle tanks (150 cm diameter x 75 cm depth) filled with aged well water, covered with 60% shade cloth and located in an open field. To each tank, ~300 g leaf litter (mostly *Quercus*) was added as a substratum, as well as zooplankton and phytoplankton inocula (the latter as a resource for tadpoles) and 25 g of Purina ® Rabbit Chow to provide an initial source of food and nutrients. This research was performed in accordance with University of Michigan UCUCA Protocol #07765.

Planorbella trivolvis snails (~1 g) were collected from three ponds in Livingston County, MI. Snails were screened for trematode infection by placing them in 60 mL water in cups under a 60 W light. After 4 h, all cups were examined under a dissecting microscope for the presence of trematode cercariae. A few cercariae from each snail were then placed in 70% ethanol and identified as echinostomes after Schell (1985). Snails included in the experiment produced >100 cercariae during the initial screening. Echinostomes in snails from these ponds were previously identified as *Echinostoma revolutum* using molecular methods (ponds referred to as Duck Pond [42.481308, -83.983442], Kaiser South Pond [42.430299, -84.036582], and East Marsh [42.45679, -83.996748] in Marino and Werner 2013). While we expect that we used the same species here, it is possible that we used a mixture of morphologically indistinguishable echinostome species (Detwiler, Bos & Minchella 2010).

Experiment 1: Parasitism in two size classes across a host density gradient

An experiment was performed in mesocosms to test the effects of parasites on two size classes of hosts across a density gradient. The experiment followed a 3 x 2 x 2 factorial, randomized block design with five replicates. Each mesocosm contained five large green frog tadpoles (LG) and 0, 50, or 100 small green frog tadpoles (SG), three uninfected or infected *P. trivolvis* snails, and two empty cages or two caged odonate predators. The densities and parasite exposure levels used fall well within the ranges observed in natural populations (Skelly *et al.* 2006). Predators were late-instar larval *Anax junius* or *A. longipes*, common odonate predators of

larval frogs in eastern North America, collected from the ESGR experimental ponds. Predator cages were constructed from a 10 x 10 cm piece of slotted drainpipe enclosed by window screening fixed with rubber bands. To generate chemical cue, caged predators were fed ~300 mg green frog tadpoles three times per week for the duration of the experiment.

LG were reared from eight egg masses collected on 8 and 10 June 2011. After three weeks, 600 tadpoles from these masses were moved from 300 L culture pools and divided equally among three 1,300 L mesocosms. Two additional mesocosms were set up after an additional two weeks, each containing 150 tadpoles, to ensure that enough LG would be available for the experiment. To encourage growth, an extra 25 g of rabbit chow was added to all tanks on 18 July. SG were reared from nine egg masses collected from 12-15 July.

Experimental mesocosms were filled with water on 20-22 July and set up with plankton inocula on 24 July. Treatments were assigned to mesocosms randomly within spatial blocks. To initiate the experiment, LG (400-450 mg each) and SG (10-15 mg each) were added on 1 and 2 August, and predators and snails were added to appropriate containers after all tadpoles were added on 2 August. The three snails in each container were put into a single cage. Dead snails and predators or predators that did not eat (identified by the presence of live tadpoles in cages during the subsequent feeding) were replaced throughout the experiment. After four weeks, the experiment was terminated, all tadpoles were collected, and all five LG from each container and a subsample of ten randomly-selected SG from the 50 and 100 SG containers were weighed. All tadpoles were then euthanized and preserved in 70% ethanol, and all LG and a subset of 10 SG were staged (Gosner 1960). To measure infection, 3 LG were dissected from all containers and ten SG were dissected from each container in the parasite treatments. The mesonephri and pronephri were removed and the number of echinostome metacercariae present in each kidney and nephric duct counted after Holland et al. (2007). LG from "uninfected snail" containers were examined to ensure that the field-collected uninfected snails used in the experiment did not harbour latent infection and produce cercariae during the experiment.

Experiment 2 – Effects across a broader density gradient

As the first experiment revealed evidence for an interactive effect of parasites and competition on growth (see Results), a second experiment was performed to examine the joint effects across a broader range of tadpole densities. The experiment followed a 3 x 2 factorial,

randomized block design with five replicates in which tadpole density (25, 100, or 200 SG) and the presence or absence of infected snails was manipulated. Mesocosms again contained five LG, but predators were not included as a factor. LG (250-300 mg each, from six egg masses collected on 24 May 2012) were reared throughout the summer in 300 L pools and fed rabbit chow *ad libitum*. LG in this experiment were smaller than in Experiment 1, because larger unexposed tadpoles were unavailable. SG (10 -15 mg each) were reared from seven egg masses collected on 25 and 30 July 2012. Cattle tanks were filled and leaf litter was added on 25 July. Tanks were inoculated with zooplankton and phytoplankton and Purina® rabbit chow was added on 30 July. Tadpoles and three caged uninfected or infected *P. trivolvis* snails were added on 10 August. The experiment was terminated after four weeks, at which point all tadpoles were collected and all LG and a subsample of ten randomly-selected SG were weighed. All tadpoles were then euthanized and preserved in 70% ethanol, and all LG and a subset of ten SG were staged (Gosner 1960). Three LG from all containers and ten SG from parasite treatment containers were later dissected to measure infection.

Statistical analyses

All analyses were performed in the R statistical package v.2.15 (http://www.r-project.org/). Log-transformed final mass and Gosner developmental stage were analysed using linear models. Final survival (proportion alive after 28 days) was analysed using generalized linear models with a quasi-binomial distribution. Final mass, stage, and survival analyses tested for effects of parasites, density, predator presence (for Experiment 1), all interactions among treatments, and block. Infection intensity (number of metacercariae) was analysed using generalized linear mixed effects models with a negative binomial distribution. In the infection analysis, fixed effects included density, block, and (for Experiment 1) predator presence and the predator x density interaction, with tank as a random effect.

Comparison with previous experiments

To further corroborate the experimental findings, the results of the above experiments were compared with results from two additional mesocosm experiments included in a previous study. The previous experiments were conducted for different purposes but used a similar design (see Table 4 and Appendix S1). Analyses were performed to examine how the effects of

parasites on SG growth and survival depended on absolute growth rate and initial density across experiments (details in Results and Appendix S1).

Results

Experiment 1: Parasitism in two size classes across a host density gradient

LG in one tank in the "uninfected snail" treatment (100 SG, predator absent) were infected with low numbers of metacercariae. A snail in that tank thus had latent infection and produced cercariae, so that tank was excluded from analysis. In the analysis of tank mean final mass, LG final mass decreased with greater SG density and the parasite x density interaction was significant for both size classes (Figure 1, Table 1), while other treatment effects and interactions were not significant. The parasite x density interactions occurred because parasite presence had no or negative effects on SG and LG final mass respectively at higher densities, but actually increased final mass of both size classes at lower densities relative to containers without parasites. The analysis of survival showed that LG survival was lowest at the highest density, while the effects of predators, parasites and all interactions were not significant, although a marginally non-significant density x parasite interaction occurred. In the analysis of LG final developmental stage, a significant predator x density interaction occurred, because LG developed more rapidly in the presence of predators at the lowest density (Figure 2a, Table 1). For SG, parasite presence had a positive effect, although a marginally non-significant interactive effect of predators occurred which counteracted the parasite effect (Figure 2b, Table 1). SG survival did not depend on density, parasite presence or predator presence, and no interactions were significant, while LG survival was negatively affected by increased density, but no other effects were significant (Figure 3, Table 2). In tanks exposed to parasites, individual infection intensities of LG (mean ± SE = 175.6 ± 14.3 metacercariae) were much higher than SG (29.3 ± 2.6 metacercariae) (paired t-test, $t = 7.94$, $df = 19$, $p < 0.001$; Figure 4a & b). LG and SG infection did not depend on density, predator presence, or the density x predator interaction (Table 3).

Experiment 2 – Effects across a broader density gradient

Despite being covered with shade cloth, nine mesocosms in two blocks were colonized by predaceous libellulid dragonfly larvae (*Leucorrhinia intacta*). The presence of *L. intacta* strongly reduced survival of SG (quasi-binomial GLM, $p < 0.001$), so those nine containers were

excluded from further analyses. One additional tank in the "uninfected snail" treatment (100 SG density) was excluded from analysis because LG in that tank were infected with low numbers of metacercariae. Three or four remaining replicates of each treatment combination were thus included in analyses. In addition, at the 25 SG density, the smallest one or two of the five LG were indistinguishable from the largest SG in some containers at the end of the experiment. The tank median rather than mean mass for both LG and SG in all tanks was therefore used in analyses, and tadpoles were selected for dissection and staging to avoid potential biases due to misclassifying SG and LG individuals (i.e. the largest three LG were selected from each container and the largest few SG individuals from all containers were not selected).

Median final mass of LG decreased with greater density, and there was a negative effect of parasites, but the density x parasite interaction was not significant (Table 2). Median final mass of SG decreased at higher densities but did not depend on the presence of parasites, and the density x parasite interaction was not significant (Table 2). In the analysis of LG and SG survival and developmental stage, the effects of density, parasites, and the parasite x density interaction were not significant (Tables 1 & 2, Figure 3). Infection intensity was again higher in LG (28.3 ± 5.1 metacercariae) than SG (mean ± SE = 8.6 ± 0.9 metacercariae; $t = 4.11$, $df = 10$, $p = 0.002$; Figure 4c & d). Infection intensity did not depend on density for either size class (Table 3).

Comparison with previous experiments

Despite similar experimental designs, our results suggest that parasitism and host density interacted to affect growth in Experiment 1 but not in Experiment 2. Furthermore, we did not observe a negative effect of parasitism on survival that we had previously observed (Marino & Werner 2013). We hypothesized that differential growth conditions and the range of densities used may offer an explanation. To test this hypothesis, we combined results from two previous experiments with the results from our two new experiments in a meta-analytical framework (see Appendix S1). This allowed us to test explicitly how parasite effects changed across experimental contexts. Across experiments, the effects of parasites on SG growth became more positive with higher absolute growth rates (Figure 5a, slope = 0.05, QM = 5.08, df = 1, p = 0.024) but did not depend on initial density (QM = 0.018, df = 1, p = 0.89). The effects of parasites on SG survival became more negative as initial densities increased (Figure 5b, QM = 5.37, df = 1, p = 0.021) but did not depend on absolute growth rates (QM = 0.35, df = 1, p =

0.55). These results are consistent with the hypothesis that differences in growth conditions and densities used contributed to different parasite effects observed in these experiments.

Discussion

Our results show that consideration of the context of individual host-parasite interactions is important when evaluating parasite effects. Conditions for growth (reflected in the overall growth rate) and host density, which can depend on or also determine the strength of competition, influenced the fitness effects of parasites. Furthermore, parasite transmission and effects of parasites on host fitness components depended on individual size. Such changes in parasite transmission and the fitness consequences of infection as a result of density-dependent processes and host variation are likely to mediate the dynamic effects of parasitism on host populations (Dwyer, Elkinton & Buonaccorsi 1997; Begon 2008).

Our results are consistent with an interactive effect of competition and parasitism on host fitness. Competitive stress can reduce host condition (e.g. due to elevated corticosterone stress hormone levels; Glennemeier & Denver 2002), which may impair host defenses against pathogens (Apanius 1998; Belden & Kiesecker 2005; Echaubard *et al.* 2012). Such an effect may explain why parasite presence and high host density jointly reduced LG growth in Experiment 1 and SG survival at higher densities in the cross-experiment comparison. The former result is in line with a marginally non-significant interactive effect ($p = 0.056$) of echinostome infection and competition on northern leopard frog (*Rana pipiens*) growth (Koprivnikar, Forbes & Baker 2008), which suggests that an interactive effect may occur broadly across host taxa. Our results thus emphasize the importance of considering the influence of density in disease models not only with respect to parasite transmission but also competition, which is seldom considered.

An intriguing result was that parasites positively affected host growth under low densities in Experiment 1. Thinning (i.e. a parasite-induced reduction in host density) is unlikely to be responsible here, as parasites did not affect survival in Experiment 1. Edema could also have influenced final mass but was not apparent in animals and would be unlikely to explain the observed interactions. Instead, a possible explanation is that hosts adaptively respond to the presence of parasites by increasing growth rates through elevated foraging rates or altered metabolism, when environmental conditions allow. Increased growth rates could be adaptive, because tolerance of parasitism increases with size (Schotthoefer *et al.* 2003; Holland *et al.*

2007). In the absence of parasites, intrinsic or extrinsic costs associated with accelerated growth rates (e.g. a growth-mortality tradeoff, Schiesari, Peacor & Werner 2006) may restrict growth. However, in the presence of parasites, growth costs may be outweighed by the risks and costs associated with parasitism. An interactive effect of parasitism and competition may result because an adaptive growth response is only possible when resource levels are sufficient to counteract the costs of infection.

For a growth response to be adaptive by itself, the fitness benefits of increased tolerance would need to outweigh the costs of greater infection associated with larger size. Alternatively, a growth response may be part of an adaptive response to allow tadpoles to reach metamorphosis more quickly and thus escape the threat of parasitism, although we only observed a positive effect of parasites on final Gosner stage in SG in Experiment 1. Another alternative is that parasite exposure or infection may influence behaviour (e.g. boldness, foraging) that affects growth (Kortet, Hedrick and Vainikka 2010; Barber & Dingemanse 2010). Although per capita infection did not significantly differ across densities, the total number of cercariae removed from the water column was greater at higher densities. Perceived risk from parasites may thus have been greater at lower densities, which may have influenced foraging or other behaviours. A final possibility is that post-infection parasite-induced trait changes benefit the parasite, if behaviours or larger size increase the likelihood of successful transmission to the definitive host. Positive effects of parasites on growth have been reported previously. For example, infection with the trematode, *Ribeiroia ondatrae*, increases size at metamorphosis of the Oregon spotted frog, *Rana pretiosa* (Johnson *et al.* 2012), and positive effects of parasites on growth have been documented in other systems (Phares 1996; Arnott, Barber & Huntingford 2000).

Despite evidence from the laboratory that parasites have strong negative effects on small green frog tadpole growth at comparable infection intensities, parasites did not substantially decrease SG growth in any of the four mesocosm experiments compared. Instead, effects of parasites on SG were near to neutral or positive. The difference between studies probably relates to dynamical changes in and feedbacks between resource levels, infection rates and densities that were not present in studies at smaller scales. Furthermore, in contrast to SG, a negative effect of parasites on LG occurred under some circumstances (i.e. at the 100 SG density in Experiment 1 and across densities in Experiment 2). LG thus experienced detectable negative effects of parasites under conditions where SG did not, despite evidence that larger tadpoles experience

fewer effects of infection under individual exposures in the laboratory (Schotthoefer *et al.* 2003; Holland *et al.* 2007). The much higher infection intensities in LG likely provide an explanation, as effects of echinostomes on growth are intensity-dependent (Marino *et al.* 2014).

With respect to our first hypothesis that increased density reduces infection, we found no evidence for a negative effect of density on infection of small tadpoles, despite examining a broad gradient of densities. Our sample sizes for dissection were limited and necessarily did not include animals that died during the experiments, as dead tadpoles are typically not visible in the large mesocosms and rapidly degrade. Nevertheless, other recent studies have similarly reported no effect (Raffel *et al.* 2010; Marino & Werner 2013) or even a positive effect (Johnson *et al.* 2013; Wojdak *et al.* 2014) of density on larval amphibian trematode infection at the mesocosm scale. The lack of a negative effect of density on infection is surprising given that the opposite effect has been observed in aquaria (Johnson *et al.* 2013) and because simple arithmetic dictates that the ratio of parasites to hosts decreases with the addition of more hosts. Furthermore, increased host densities can reduce host size through competition, and larger tadpoles experience higher infection rates (Holland *et al.* 2007), which would also be expected to lead to negative density-infection relationship. However, two other mechanisms may work to counteract the aforementioned effects and result in a neutral or positive density-infection relationship. First, increased host densities can reduce host condition due to elevated stress hormone levels (Glennemeier & Denver 2002), which may impair parasite resistance (i.e. ability to reduce parasite burden; Belden & Kiesecker 2005, Raberg *et al.* 2009). Second, increased host densities may increase the likelihood of contact between parasites and hosts. Such a spatial effect may arise because, at higher densities, competitive interactions may constrain some hosts to areas where cercariae are more abundant. Our results thus suggest a potential balance between negative and positive effects of density on infection. These mechanisms are likely factors across a broad range of ecological systems, yet most studies fail to address the interplay between them. Importantly, the upshot at a population scale would be that increased density increases the total number of parasites that successfully transmit to a new host even if infection at the individual host level is unchanged.

Despite evidence that size structure influenced host-parasite interactions, no support was found for our second hypothesis that predicted density- or trait-mediated indirect effects of parasitism. Direct effects of parasites on LG apparently outweighed any indirect benefit

mediated through effects on SG, likely due to the unexpectedly high infection intensities in LG. Several factors may contribute to differences among size classes in infection intensity, including better detection of larger hosts by cercariae, less intraspecific competition among parasites due to more kidney tissue available in larger hosts, size-dependent differences in host behaviour, and host choice by parasites (Wojdak *et al.* 2013). From the parasite perspective, transmission to definitive hosts may be more likely for metacercariae in larger tadpoles, because larger tadpoles are more tolerant of infection than smaller tadpoles (Holland *et al.* 2007). Larger tadpoles also likely experience lower background mortality (Werner 1986) and may be preferred prey by mammal and avian definitive hosts due to greater visibility and nutritional content. However, the fitness advantages of infecting a larger host are not necessarily greater, as larger tadpoles are also more efficient at eliminating cysts (Holland 2009).

With respect to our final hypothesis that predator presence influences relative competitive ability and effects of parasites for different size classes, the results of Experiment 1 were generally consistent with a trait-mediated indirect effect of predators on LG growth, mediated through effects on SG (Peacor and Werner 2000). However, we found no evidence for an effect of predator presence on infection or a consistent interactive effect with parasites on fitness. Variation in the way tadpoles assess relative risk from parasites and predators at different spatial scales is a possible explanation for why our experimental results do not support our hypothesis, which was based in large part on evidence from the laboratory. Although predator cue effects on transmission have been shown at a small scale (e.g. Thiemann and Wassersug 2000), our results align with other studies that have failed to show an effect of predator cue on echinostome transmission at the mesocosm scale (Raffel *et al.* 2010; Marino & Werner 2013).

Context (i.e. density and growth conditions) and trait (i.e. size) dependence pose challenges to incorporating parasites into population and community models. Nevertheless, such factors are crucial and merit additional research, as our results suggest that the magnitude and even direction of parasite effects can change, and such interactions are likely to be common. Many animals tolerate low resource levels in the absence of disease, but the combined effects of competition and parasitism can act synergistically to reduce host fitness (Bedhomme *et al.* 2004; Sadd 2011). In future it will be useful to identify whether consistent trade-offs (e.g. resource allocation to parasite defenses vs. other fitness components) exist and what traits (e.g., growth

rates) are involved, in order to incorporate competition into a broad theory of host-parasite interactions.

Finally, the observed context dependence of parasite effects may have important consequences for how host-parasite interactions play out in nature. First, parasite effects on growth and survival may mediate apparent competition and keystone effects (Hudson, Dobson & Newborn 1998; Hatcher, Dick & Dunn 2006), comparable to effects of predators (Paine 1966; Werner & Peacor 2003). Second, a positive effect of competition on infection rates mediated through physiology or space may counteract potential encounter-dilution effects, because reduced contact rates caused by higher host densities may be offset by impaired resistance to infection due to competitive stress or spatial effects. Finally, effects of competition and size structure on parasite transmission and persistence (e.g. due to host death) may also influence transmission to definitive hosts, with potential downstream consequences. Interactions between competitive and host-parasite interactions may thus have important implications for the relationships between host density, size structure and disease.

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558 **Supporting Information**
559 Additional Supporting Information may be found in the online version of this article:
560 **Appendix S1.** Supplementary methods and results for cross-experiment comparison.
561

Table 1: Results of analyses of log-transformed final mass and Gosner (1960) stage of large (LG) and small (SG) green frog tadpoles using general linear models.

		LG Final Mass			SG Final Mass			LG Gosner			SG Gosner		
		F	***df***	***p***	***F***	***df***	***p***	***F***	***df***	***p***	***F***	***df***	***p***
Experiment 1	Parasite	0.011	1, 43	0.92	0.59	1, 27	0.45	0.65	1, 43	0.46	5.27	1, 27	**0.030**
	Density	9.87	2, 43	**<0.001**	2.59	1, 27	0.12	6.85	2, 43	**0.0026**	2.01	1, 27	0.17
	Predator	3.60	1, 43	0.065	0.44	1, 27	0.51	2.03	1, 43	0.16	1.88	1, 27	0.18
	Para x Dens	4.11	2, 43	**0.023**	5.31	1, 27	**0.029**	0.48	2, 43	0.62	0.42	1, 27	0.29
	Para x Pred	0.84	1, 43	0.36	2.20	1, 27	0.15	0.53	1, 43	0.47	4.05	1, 27	0.520
	Pred x Dens	2.81	2, 43	0.071	0.01	1, 27	0.92	4.43	2, 43	**0.018**	0.27	1, 27	0.054
	Para x Pred x Dens	1.39	1, 43	0.26	0.043	1, 27	0.84	2.74	1, 43	0.076	0.27	1, 27	0.61
	Block	3.46	4, 43	**0.016**	5.31	4, 27	0.093	0.98	4, 43	0.43	1.31	4, 27	0.61
Experiment 2	Parasite	5.97	1, 10	**0.035**	0.65	1, 10	0.44	1.75	1, 10	0.21	0.049	1, 10	0.83
	Density	4.35	1, 10	**0.044**	7.95	1, 10	**0.0086**	6.01	1, 10	**0.017**	2.28	1, 10	0.15
	Para x Dens	0.20	1, 10	0.82	0.36	1, 10	0.71	0.13	1, 10	0.88	1.11	1, 10	0.36
	Block	5.96	4, 10	0.010	1.03	4, 10	0.44	2.19	4, 10	0.14	0.42	4, 10	0.79

Table 2: Results of analysis of proportion survival of large (LG) and small (SG) green frog tadpoles using a quasi-binomial generalized linear model.

		LG Survival			SG Survival		
		Deviance	***df***	***p***	***Deviance***	***df***	***p***
Experiment 1	Parasite	1.41	1	0.27	3.31	1	0.34
	Density	14.81	2	**0.0015**	0.29	1	0.78

	Predator	1.37	1	0.27	1.31	1	0.55
	Para x Dens	4.44	2	0.14	0.22	1	0.81
	Para x Pred	1.01	1	0.35	0.17	1	0.83
	Pred x Dens	4.53	2	0.14	0.51	1	0.71
	Para x Pred x Dens	<0.001	2	~1.00	0.16	1	0.83
	Block	12.54	4	**0.027**	14.58	4	0.40
Experiment 2	Parasite	2.22	1	0.19	11.04	1	0.58
	Density	2.36	2	0.41	13.85	2	0.24
	Para x Dens	1.07	2	0.43	18.97	2	0.39
	Block	5.02	4	0.67	89.92	4	0.07

Table 3: Results of analysis of infection (number of metacercariae) of large (LG) and small (SG) tadpoles using a negative binomial generalized linear mixed effects model.

		LG Infection			SG Infection		
		Deviance	***df***	***p***	***Deviance***	***df***	***p***
Experiment 1	Predator	1.41	1	0.24	0.26	1	0.61
	Density	0.86	2	0.65	0.05	1	0.82
	Pred x Dens	0.052	2	0.97	0.47	1	0.49
	Block	6.01	4	0.20	4.01	4	0.40
Experiment 2	Density	4.24	2	0.15	1.01	2	0.60
	Block	13.59	4	**<0.001**	13.73	4	**0.0080**

Table 4: Summary of four mesocosm experiments that were compared to examine the dependence of parasite effects on growth and survival on density and growth rates.

Experiment	Referred to as:	SG Density	LG Density	Duration	Replicates	Infection (mean ± SE metacercariae)
A	Experiment 1 in Marino and Werner 2013	200	0	26 d	5	19.4 ± 1.7
B	Experiment 3 in Marino and Werner 2013	250	0	14 d	8	41 ± 9.4
C	Experiment 1 here	0, 50, 100	5	28 d	5	30.15 ± 3.5
D	Experiment 2 here	25, 100, 200	5	28 d	3-4	8.6 ± 0.9

Figure Legends

Figure 1: Results from Experiment 1; points show means ± s.e.m, averaged across other treatments. a) The effects of parasites on large green frog tadpole (LG) growth depended on density (parasite x density interaction: $p = 0.023$). b) The effects of parasites on small green frog tadpole growth also depended on density (parasite x density interaction: $p = 0.029$). c) Predators

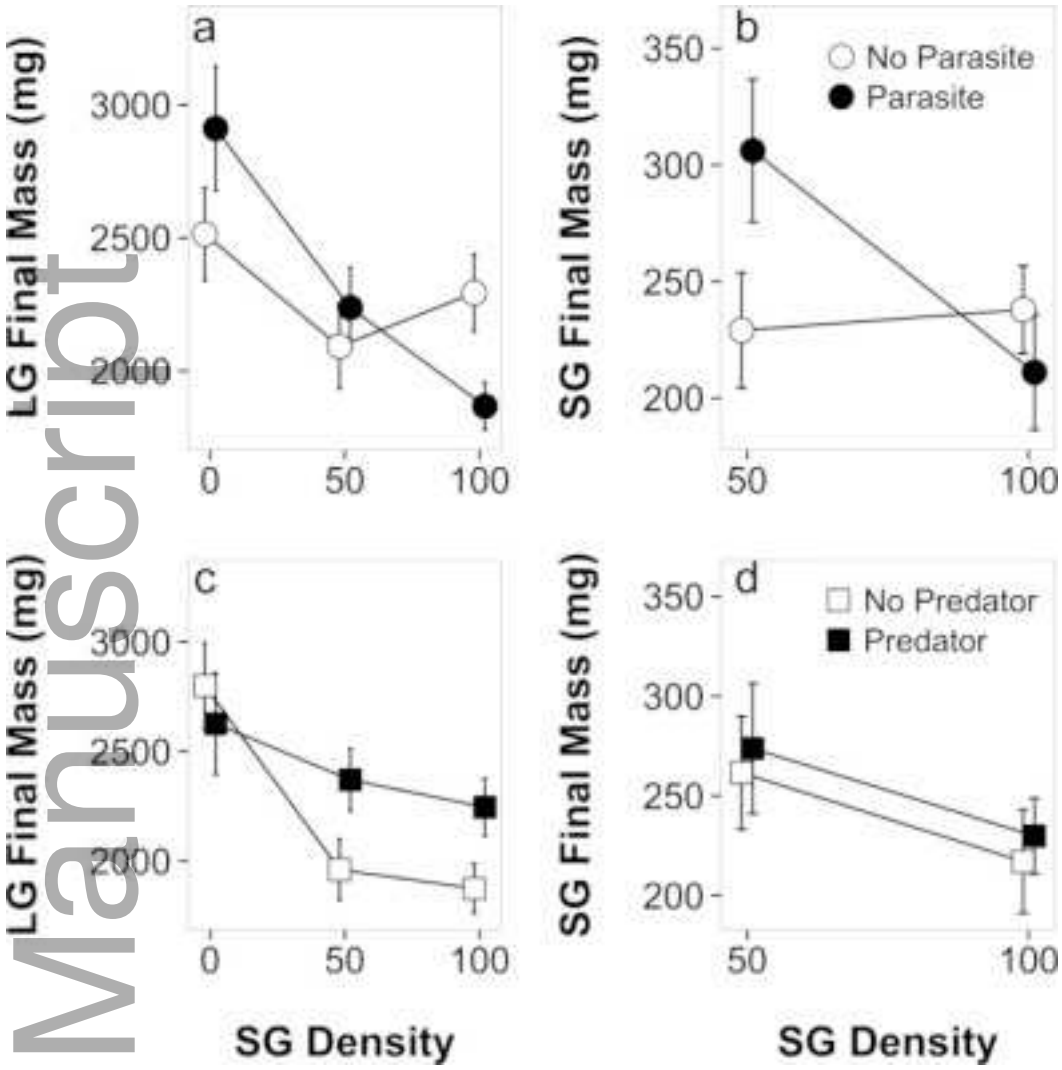
tended to have a positive indirect effect on LG growth at higher densities (predator x density interaction: *p = 0.071*), but d) SG growth did not change due to predator presence.

Figure 2: a) Final Gosner (1960) stage of large green frog tadpoles (LG) across density treatments (predator x density interaction: p = 0.018) and b) final Gosner stage of small green frog tadpoles (SG) across parasite treatments (parasite x predator interaction: p = 0.054) in Experiment 1. Points show means ± s.e.m, averaged across other treatments.

Figure 3: Final survival (proportion) of large (a, c) and small (b, d) green frog tadpoles in Experiments 1 (a, b) and 2 (c, d) across densities in the presence or absence of parasites. For Experiment 1, points show means ± s.e.m, averaged across predator treatments.

Figure 4: Boxplots of tank median infection (number of metacercariae per tadpole) in large (a, c) and small (b, d) green frog tadpoles in Experiments 1 (a, b) and 2 (c, d).

Figure 5: a) Across four mesocosm experiments, effects of parasites on small green frog (SG) growth were more positive at higher absolute growth rates ($p = 0.024$). b) Parasites also reduced SG survival more at higher densities ($p = 0.021$). Letters indicate experiment (summarized in Table 4), and numbers in (a) indicate density. Effect sizes are the log response ratio (parasites/control) for growth rates and survival, calculated for each density within each experiment. Bars show ± s.e.m.



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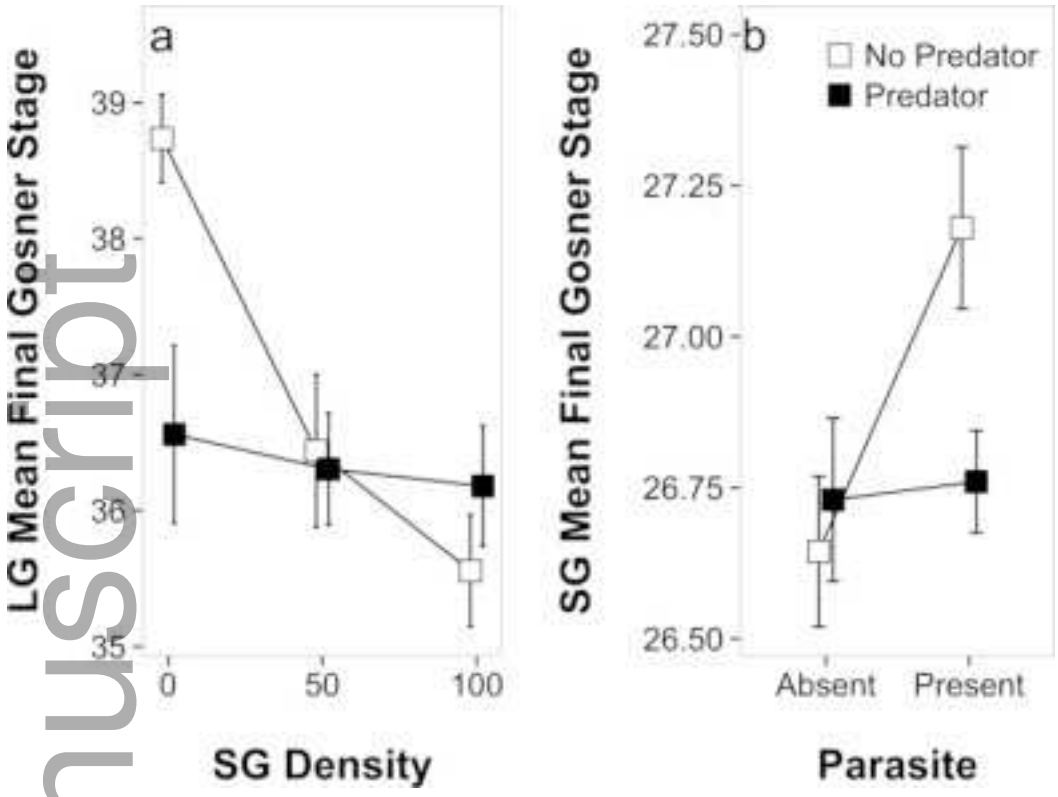

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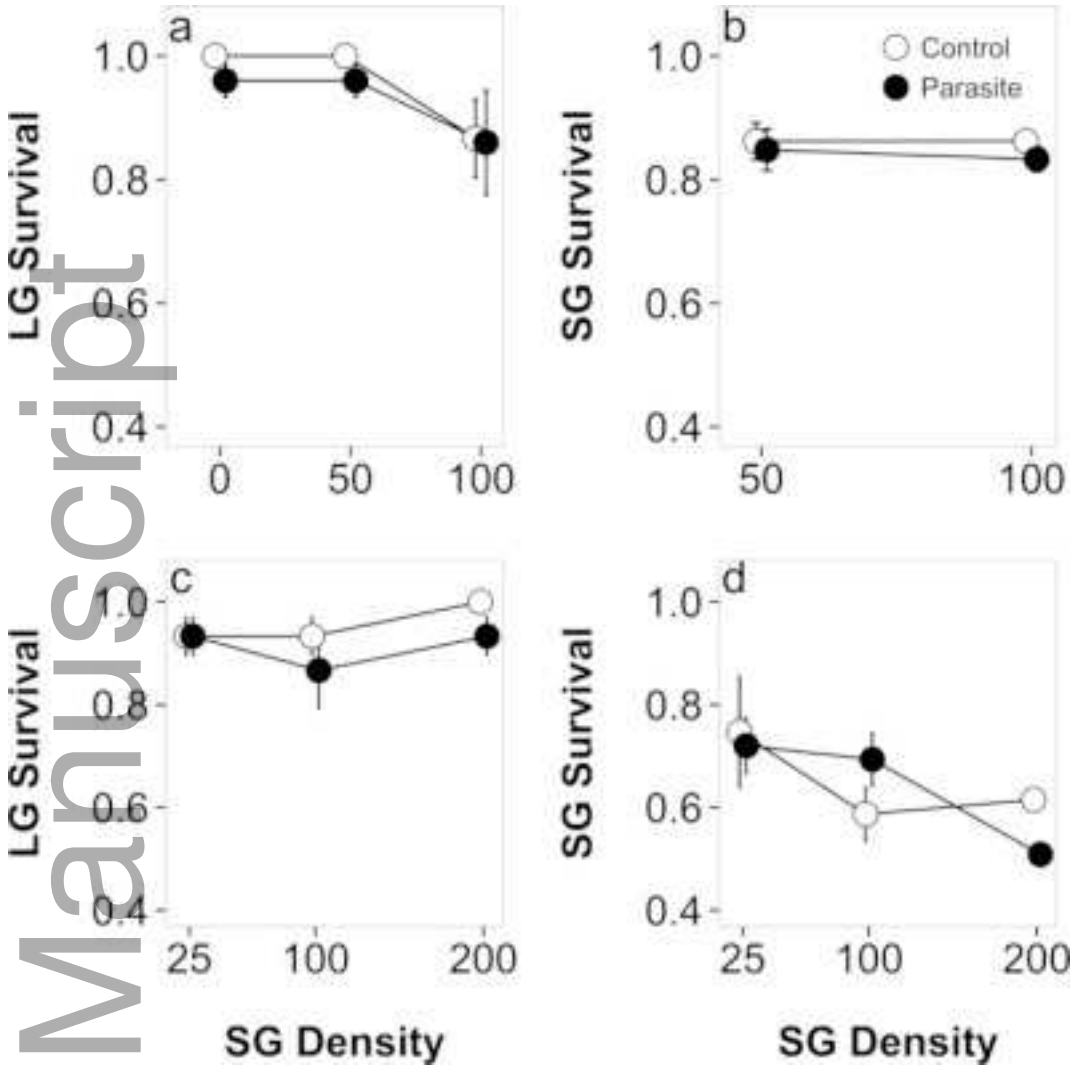

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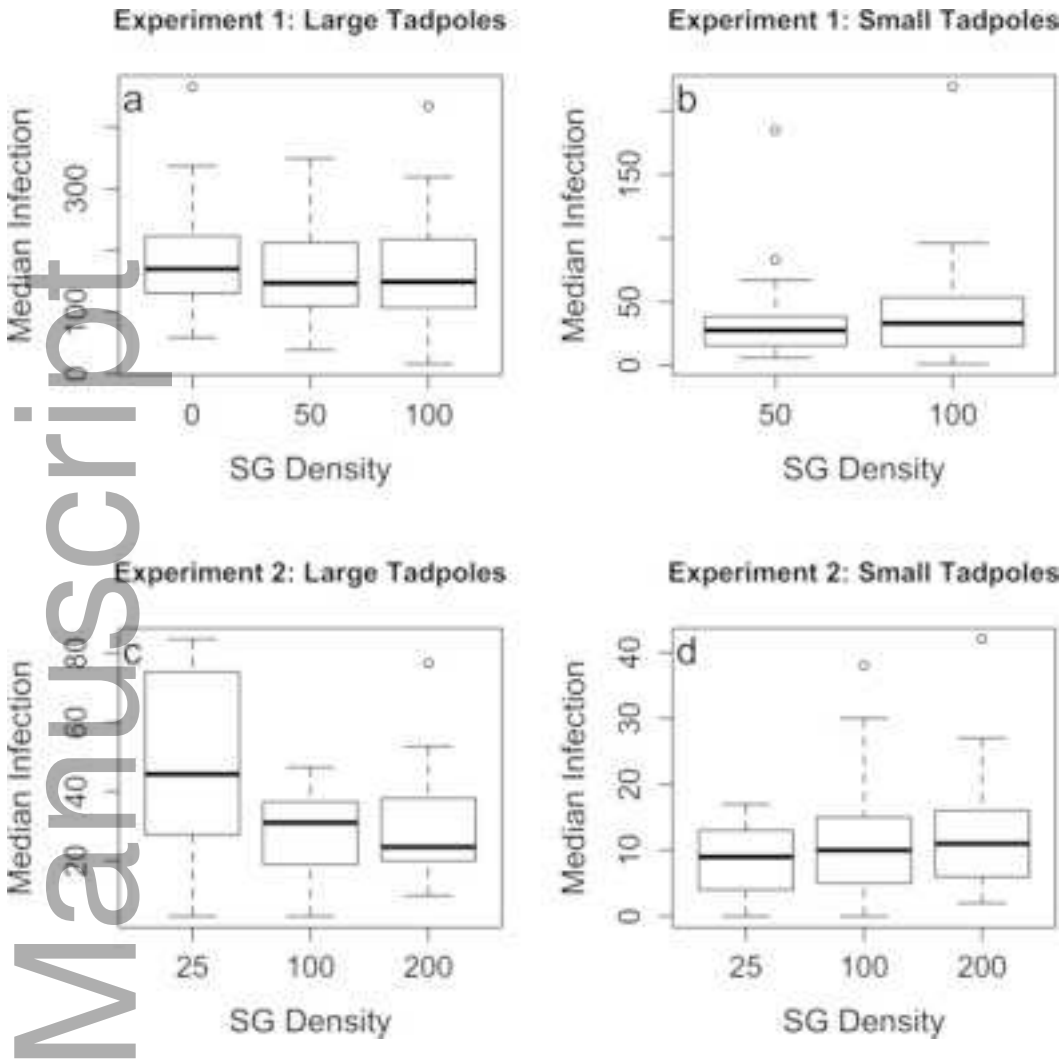

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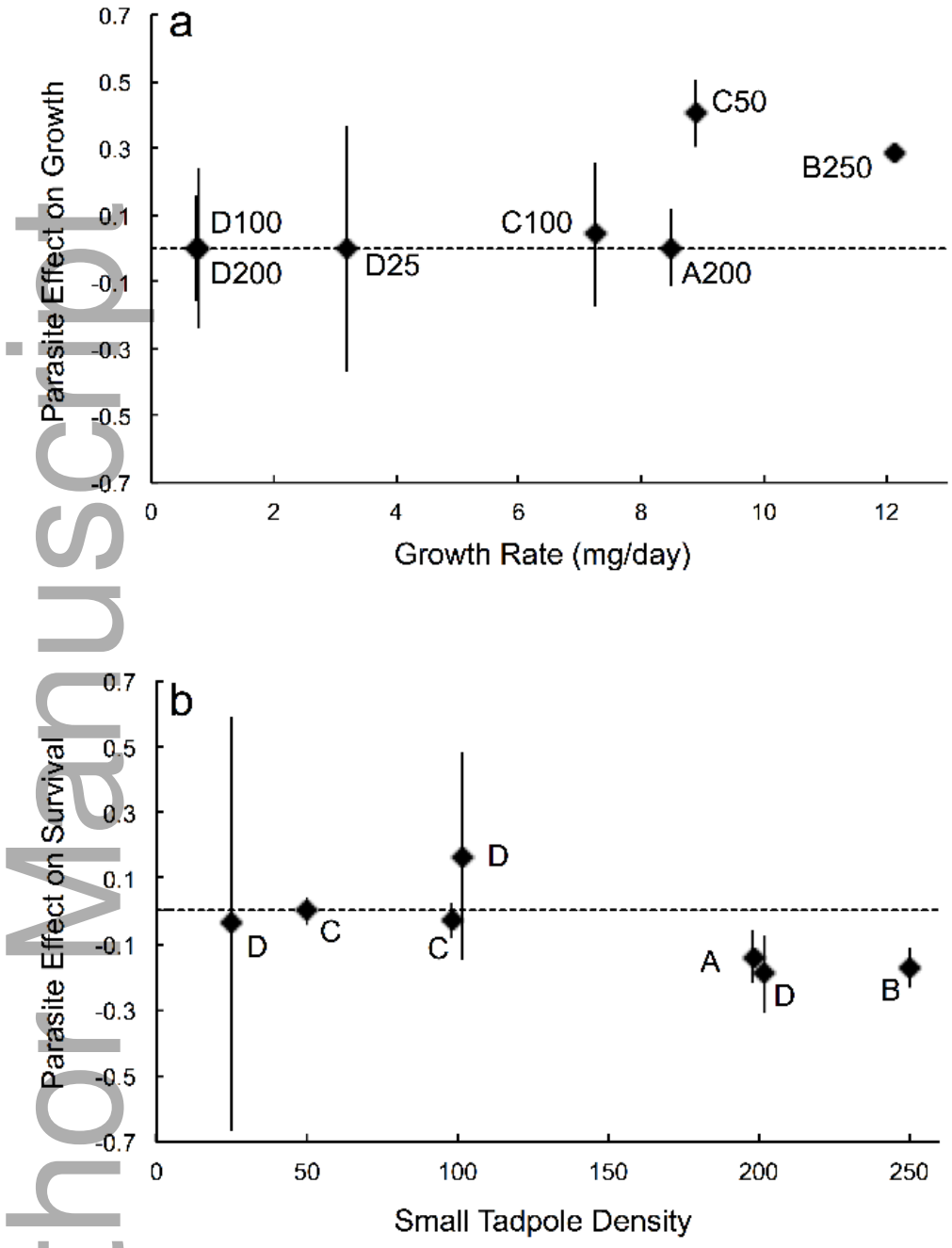

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