SYMPOSIUM

A Lack of Crowding? Body Size Does Not Decrease with Density for Two Behavior-Manipulating Parasites

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From the symposium "Parasitic Manipulation of Host Phenotype, or How to Make a Zombie" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2014 at Austin, Texas.

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Synopsis For trophically transmitted parasites that manipulate the phenotype of their hosts, whether the parasites do or do not experience resource competition depends on such factors as the size of the parasites relative to their hosts, the intensity of infection, the extent to which parasites share the cost of defending against the host's immune system or manipulating their host, and the extent to which parasites share transmission goals. Despite theoretical expectations for situations in which either no, or positive, or negative density-dependence should be observed, most studies document only negative density-dependence for trophically transmitted parasites. However, this trend may be an artifact of most studies having focused on systems in which parasites are large relative to their hosts. Yet, systems are common where parasites are small relative to their hosts, and these trophically transmitted parasites may be less likely to experience resource limitation. We looked for signs of density-dependence in *Euhaplorchis californiensis* (EUHA) and *Renicola buchanani* (RENB), two manipulative trematode parasites infecting wild-caught California killifish (*Fundulus parvipinnis*). These parasites are small relative to killifish (suggesting resources are not limiting), and are associated with changes in killifish behavior that are dependent on parasite-intensity and that increase predation rates by the parasites' shared final host (indicating the possibility for cost sharing). We did not observe negative density-dependence in either species, indicating that resources are not limiting. In fact, observed patterns indicate possible mild positive density-dependence for EUHA. Although experimental confirmation is required, our findings suggest that some behavior-manipulating parasites suffer no reduction in size, and may even benefit when "crowded" by conspecifics.

Introduction

Many types of parasites experience competition for limiting resources (Read 1951; Holmes 1961; Dobson 1985; Poulin 1996, 2001; Bush and Malenke 2008; Mideo 2009), and these competitive interactions can have important implications for parasite fitness and regulation of their population sizes (e.g., Dobson 1985; Brown et al. 2003; Fredensborg and Poulin 2005; Heins and Baker 2011). However, resources should not be limiting for parasites that occur at low intensities (i.e., few parasites residing within a host individual), grow little, and/or are small relative to their host or small relative to the site they use within the host (e.g., Shostak et al. 2008; Benesh 2011). Further, there are situations in which parasites may benefit from being surrounded by others, and cooperation or facilitation among parasites could feasibly lead to positive density-dependence (Poulin 1994; Brown 1999; Kuris 2003; Vickery and Poulin 2010).

One group of parasites that often may not experience resource limitation and may benefit from the presence of conspecifics are the trophically transmitted parasites. These are parasites that transmit to their next host, a predator, when that predator preys upon the parasites' host (Holmes and Bethel 1972; Lafferty 1992, 1999). Trophically transmitted parasites frequently increase the probability of predation on their host by modifying the host's behavior (reviewed by Moore [2002] and Hughes et al. [2012]). Behavioral modification may be costly for parasites. If parasites can share these costs, sharing theoretically may lead to positive effects of increased parasite density (Poulin 1994; Brown 1999). Further, given their shared interests concerning transmission, we may even see selection for facilitation or cooperation among trophically transmitted parasites (Poulin 1994; Brown 1999; Kuris 2003). Table 1 depicts when negative, zero, and positive density-dependence should occur, given different combinations of resource limitation and cost sharing among parasites.

Despite some predictions for a lack of competition, examinations of trophically transmitted parasites typically document negative density-dependence, usually reflected by reduced body size (e.g., Brown et al. 2003; Fredensborg and Poulin 2005; Keeney et al. 2009; Dianne et al. 2012). However, in most cases, this can be explained by the parasites living under conditions in which resources should be limiting (i.e., parasites occur at high intensities, grow substantially, or are large relative to their host). For example, negative density-dependence occurs for nematode larvae in copepods (Benesh 2011), *Schistocephalus solidus* tapeworm

Table 1 A simple matrix depicting different combinations of resource limitation and cost sharing among parasites that can give rise to different forms of density-dependence

		"Cost" sharing	
		N	Y
Resource	N	0	0,+
limitation	Y	−	−, 0, +

Notes: Cost sharing ("Y" for yes; "N" for no) assumes that: (1) something incurs costs (energy diverted from growth), (2) the amount expended is plastic, so that (3) *per capita* costs decrease with increasing density. The cells indicate extremes of whether resource limitation or cost sharing occurs, and are best understood as bracketing the range of possibilities. For example, under no limitation of resources, we expect no dependency (0) to positive (+) dependence on density, depending on the benefits of cost sharing. The symbols indicate the general direction of the responses, not the specific shapes (e.g., linear or saturating, or even a hump-shaped response for the lower right quadrant).

plerocercoids in fish (Heins et al. 2002; Heins and Baker 2011), and acanthocephalans in isopods (Benesh and Valtonen 2007). Similarly, the documentation of negative density-dependence among small trematode metacercariae usually involves very small hosts (e.g., amphipods) (Brown et al. 2003; Fredensborg and Poulin 2005; Keeney et al. 2009). An exception to this is the apparent crowding effect documented by Fredensborg and Poulin (2005) for metacercariae in a crab. As this appears to be the only examination of competition for small, trophically transmitted parasites in relatively large hosts, data from additional systems are required to adequately determine whether trophically transmitted parasites typically experience density-dependence.

We studied the relationship between density and size in two trematode species, *Euhaplorchis californiensis* (EUHA, Heterophyidae) and *Renicola buchanani* (RENB, Renicolidae), two trophically transmitted parasites that are small relative to their intermediate host, the California killifish (*Fundulus parvipinnis*). One EUHA metacercaria is approximately 1 millionth the size of an average killifish host (calculations from mean body sizes reported in Hechinger et al. 2011), and RENB is only slightly larger. The final hosts for EUHA and RENB parasites are predatory birds (Martin 1950, 1971). Their first and second intermediate hosts are California horn snails (*Cerithidea californica*) and California killifish, respectively (Martin 1950, 1971). RENB also infects other fish species as second intermediate hosts, for example, longjaw mudsuckers (*Gillichthys mirabilis*) (Martin 1971). EUHA and RENB reach 100% prevalence in populations of adult California killifish (Lafferty and Morris 1996; Shaw et al. 2010). EUHA infects the brain surface of killifish and often reaches intensities in the thousands (Martin 1950; Shaw et al. 2010), whereas RENB infects the killifish's liver, and often reaches intensities in the hundreds (Martin 1971; Lafferty and Morris 1996). Trematodes infecting second intermediate hosts are known as metacercariae, and density-dependence has consequences for the parasite's fitness because metacercarial size is correlated with adult size and egg production in trematodes (e.g., Brown et al. 2003; Fredensborg and Poulin 2005). Metacercarial size can therefore serve as a measure of the fitness implications of limited resources or trade-offs associated with the cost of manipulation of the host or of defending against the immune system.

Concerning host resources, the limited area of the host brain surface bounded by a skull for EUHA, and, to a lesser extent, the limited host liver volume for RENB, means that available space

eventually could be limiting. But, given the relatively small sizes of the parasites and the confined portion of the host they use, nutritional resources seem less likely to be ultimate limiting factors. Further, parasites might experience positive density-dependence resulting from sharing costs of evading the immune system or of manipulating the host. EUHA appears to be an active manipulator as it is associated with changes in killifish's neurotransmission (Shaw et al. 2009; Shaw and Øverli 2012), and the combined intensity of both parasites positively associates with increased conspicuous behaviors of killifish and increased predation rates by birds (Lafferty and Morris 1996). To test these hypotheses, we asked whether density of the parasite affects the body size of EUHA or RENB metacercariae infecting wild-caught California killifish. Although experimental confirmation is necessary, our results indicate zero, or perhaps positive, density-dependence. These findings clarify that it remains an open question as to whether trophically transmitted parasites typically experience negative density-dependence under natural conditions.

Materials and methods

California killifish were collected from Carpinteria Salt Marsh Reserve (34°24'00"N, 119°31'30"W) in Santa Barbara County, CA, USA, using a two-pole seine in early May 2011. Killifish were transported to the University of California Santa Barbara, where they were held in two tanks in groups of 20–25 individuals per 38 L tank. All tanks had flow-through, filtered seawater, which maintained temperatures between 16°C and 20°C from May to July. South-facing windows provided a natural light:dark cycle. Fish were fed daily on a diet of fish flakes, frozen brine shrimp, bloodworms, and mysid shrimp.

Although we do not have specific data for EUHA or RENB, like many trematode metacercariae, EUHA and RENB appear to rapidly reach asymptotic size. The cognate species, *Euhaplorchis* sp. A, reaches final sizes in 10–14 days (L. L. McNeff 1978, unpublished MS thesis, University of Alabama). EUHA becomes competent to infect its definitive host after 14 days (Martin 1950), but observations in the laboratory suggest that the parasites' growth may continue for an additional 2–4 weeks (K. L. Weinersmith, personal observation). We maintained infected killifish for 2 months in the laboratory to permit parasites to reach maximum size. The duration of growth for RENB is likely shorter than that characterizing EUHA, given that it grows less in the fish (indicated by the smaller difference in size between cercariae,

the parasite stage that is infective to killifish, and developed metacercariae). EUHA and RENB cercariae are rare in the water column at Carpinteria Salt Marsh Reserve from December to late May (Fingerut et al. 2003), and so most parasites from killifish collected in early May likely were acquired prior to December and, consequently, probably at least 7 months old. Hence, the metacercariae in our fish were at least 2 months old and probably more than 9 months, meaning that they had reached their final sizes (this is further supported by the within-host comparison of metacercarial size described below).

Fish were euthanized with an overdose of MS-222 (250 mg/L), weighed, and sexed. The liver and brain were removed under a dissecting microscope and placed in filtered seawater (33 ppt).

Liver tissue was placed in a small quantity of seawater, and gently compressed between two glass plates. The number of RENB infecting the liver was counted, and then the top plate removed. The liver was pulled apart with teasing needles, and 10 metacercariae, or as many metacercariae as could be found in 30 min, were haphazardly removed for measurement of volume (see below).

The brain was gently removed from the brain case, and the brainstem was separated from the rest of the brain. During experimental infections, EUHA initially accumulates on the brainstem (Shaw et al. 2009), indicating that metacercariae on the brainstem are the earliest colonizers in natural infections. The brainstem is small relative to the rest of the brain, and the space available to metacercariae likely is rapidly saturated. Metacercariae that arrived earlier are both less likely to have experienced resource limitation, and are likely to be older, and so comparisons between EUHA metacercariae on the brainstem and those on the rest of the brain should allow us to explore the influence of resource limitation and slow, consistent increase in metacercarial volume. We measured 10 randomly chosen EUHA metacercariae from the brainstem and another 10 randomly picked from the rest of the brain from each fish to examine whether metacercariae that might have arrived earlier achieved a larger size than did metacercariae found elsewhere on the brain. The brain and brainstem were then compressed between glass plates, and the number of EUHA metacercariae was counted. The brain case was examined under a dissecting microscope to count the metacercariae that had separated from the meninges during removal of the brain.

Metacercariae for which measurements of volume were made were first placed on slides in a drop of seawater. Preliminary trials using a range of salinities
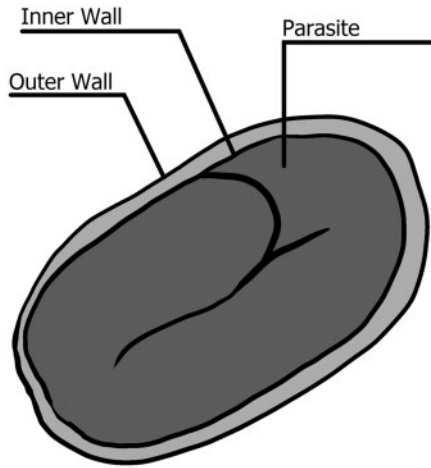
Fig. 1 Illustration of a trematode metacercaria.

(5–8 ppt) indicated that metacercariae in seawater maintained a constant size over a 2-h period, whereas lower salinities resulted in metacercariae changing size. Therefore, we used seawater to permit standardized measurements. To prevent coverslip pressure, a thin layer of vacuum grease was applied to all edges of a coverslip before placement. The metacercariae are encysted within a secreted wall (Fig. 1). For EUHA and RENB, we calculated the volume of the entire metacercaria (outer volume), and the volume of the space inside the wall of the metacercaria (inner volume). The volume inside the wall of the cyst roughly approximates the volume of the parasite's body, whereas the outer volume reflects the entire space taken up by the parasite in the host. All measurements were made on a compound microscope under $400\times$ magnification. EUHA's volume was calculated using the equation for the volume of a prolate spheroid $(V = 4/3(\pi)(r_1)(r_2^2))$, whereas the equation for the volume of an oblate spheroid $(V = 4/3(\pi)(r_1^2)(r_2))$ was used to calculate RENB volume, where r_1 is the radius of the length and r_2 is the radius of the width.

Statistical analysis

We found that the inner and outer metacercarial volumes were highly correlated $(R > 0.9)$ in the metacercariae of both trematode species. As inner volume is more likely to be closely related to the parasite's fitness, we focused our analyses on this variable.

Paired t-tests comparing the within-fish average metacercarial volume of EUHA on the brainstem, versus that from other parts of the brain, revealed no difference in volume $(t = 9847, df = 18, P = 0.34)$. The average brainstem volume was 0.00077 mm^3 (± 0.00001 SEM), and the average volume of

metacercariae on other regions of the brain was 0.00079 mm^3 (± 0.00001 SEM). This lack of difference suggests that metacercariae in our samples had reached a final size, making it possible to test our predictions.

Using the lme4 package (Bates et al. 2014) in R 3.0.2 (R Development Core Team 2013), we fit a set of linear mixed models to estimate the expected inner volume of a metacercaria. An individual fish-level random intercept accounted for non-independence of trematodes recovered from within a single host. We fit eight models for each species of parasite, using different combinations of fish's sex and metacercarial density (total number of parasites per gram of fish mass) as "fixed effect" predictors. We used parasite density rather than parasite intensity because fish body size varied and density should therefore better represent the resources available to the parasites. However, using intensity yielded qualitatively similar results (results not presented), given our relatively restricted host size range. For density calculations, we used host body mass rather than organ mass because body mass was readily and precisely attainable and it correlates with organ mass (Calder 1996). Body mass has the further advantage in that it may be better than specific organ mass at representing the ultimate pool of resources (e.g., amino acids and carbohydrates available in blood) available to parasites living in any specific host tissues. We fit all these models by maximum likelihood and evaluated their performance using corrected values of Akaike information criterion (AICc) (Burnham and Anderson 2002). Model-averaged confidence intervals were calculated using the sample.qa.posterior function in the rethinking package (McElreath 2014).

Results

We collected data on RENB and EUHA metacercarial volume (Table 2) from 19 fish, and obtained parasite

Table 2 Average metacercarial volume ($\pm$SEM) for EUHA and RENB

Parasite	N	Inner volume (mm^3)
EUHA		
Stem	172	0.00077 ± 0.00001
Other	175	0.00079 ± 0.00001
Total	347	0.00078 ± 0.00001
RENB		
	149	0.00275 ± 0.00003

Notes: Data for EUHA collected on the brainstem ("Stem"), the rest of the brain ("Other"), and all metacercariae combined ("Total") are presented.

Table 3 Mean host mass (±SEM) and mean (±SEM) intensity of EUHA and RENB infection (i.e., the mean number of each parasite found in hosts)

Host's sex	N	Host mass (g)	RENB intensity (#)	EUHA intensity (#)
Female	12	3.06 ± 0.27 (1.58–5.13)	68.25 ± 24.11 (3–260)	2434.83 ± 224.01 (1089–3770)
Male	7	2.40 ± 0.28 (1.68–3.70)	56.71 ± 18.85 (3–136)	2107.14 ± 218.13 (1025–2615)
Total	19	2.82 ± 0.21	64.00 ± 16.43	2314.1 ± 163.09

Note: Ranges in parentheses.

Table 4 Top-ranked models that are the best predictors of EUHA or RENB volume

Parameter	df	ΔAICc	Weight
EUHA volume			
=EUHA density + (1|Fish number)	4	0.0	0.42
=Sex + EUHA density + (1|Fish number)	5	1.6	0.19
=EUHA density + RENB density + (1|Fish number)	5	2.0	0.16
=1 + (1|Fish number)	3	3.3	0.08
RENB volume			
=1 + (1|Fish number)	3	0.0	0.23
=RENB density + (1|Fish number)	4	0.5	0.18
=Sex + (1|Fish number)	4	0.5	0.18

Notes: The top-three ranked models are presented; the null model (1 + (1\|Fish number)) is included if it did not rank in the top three.

intensities similar to those previously observed in this system (Table 3) (Lafferty and Morris 1996; Shaw et al. 2010).

Based on AICc, three of the four best-performing models included an effect of EUHA density on EUHA volume (Table 4 and Supplementary Table S1), and this effect was slight, but reliably positive (Table 5 and Fig. 2). According to the best-performing model, which had more than two times the weight of the second-best model, an increase of 100 parasites/g fish corresponds to an increase in expected volume of $6 \times 10^{-6} \pm 2 \times 10^{-6} \, \mathrm{mm}^3$ (mean ± SEM). Although this change in volume is relatively small compared with the within-fish variation, it does correspond to a 10% increase in inner volume across the range of observed parasite densities. The other models that included an effect of EUHA density produced similar estimates. Some of the top-ranked models also contained terms for RENB density or hosts' sex, but their confidence intervals spanned zero (Table 5) and we thus cannot confidently determine what influence (if any) they have on metacercarial volume.

We did not find any clear relationships with RENB volume (Table 4), as no effects were

consistently observed among the top models and the highest-performing model was the null.

Discussion

Our observational data indicate that EUHA and RENB do not display negative density-dependence in high-density infections ranging from 299 to 1571 individuals/g for EUHA and from 2 to 102 individuals/g for RENB. On the contrary, individual size increased with density for EUHA and appeared independent of density for RENB (Fig. 2). Although it is common to observe smaller parasites at higher intensities of infection for trophically transmitted parasites in relatively small hosts (Benesh 2011), limitation of resources may be less severe, or absent, in relatively large hosts (e.g., Michaud et al. 2006; Keeney et al. 2009), as is the case for EUHA and RENB infecting California killifish. Killifish's body condition does not decline over a broad range of EUHA densities of infection in wild-caught, naturally infected fish (Shaw and Øverli 2012). Those fish were collected from the same population as the fish in this study and were likely infected by RENB as well (although density of RENB was not quantified). The observation that killifish's body condition does not decline at high EUHA densities suggests that these parasites are not approaching the point at which parasites consume resources at a greater rate than the rate at which the host can replenish them. Hence, their small sizes, lack of obvious, substantial energy drain on hosts, and a lack of negative density-dependence on body size all combine to indicate that EUHA and RENB may be free of resource-limitations at natural densities in their second intermediate host (killifish).

The positive density-dependence observed for EUHA metacercarial volume is suggestive of cost sharing. A game theoretic model by Vickery and Poulin (2010) indicates that behavior-manipulating parasites may benefit by investing energy in growth instead of manipulation when in the presence of conspecifics. If an individual's reduction in manipulation is less than the combined increase in manipulation arising from new parasites, then the host's

Table 5 Beta coefficients and 95% confidence intervals (CIs) for the top three models and the null model estimating the volume of EUHA metacercariae

Parameter	Estimate (2.5%, 97.5%)		Estimate
=EUHA density + (1|Fish number)			
Intercept	7.2×10^{-4} (6.8×10^{-4}, 7.7×10^{-4})	(1|Fish number)	2.5×10^{-5}
EUHA density	6.0×10^{-8} (1.3×10^{-8}, 1.1×10^{-7})	Residual	1.3×10^{-4}
=Sex + EUHA density + (1|Fish number)			
Intercept	7.3×10^{-4} (6.8×10^{-4}, 7.7×10^{-4})	(1|Fish number)	2.5×10^{-5}
EUHA density	6.2×10^{-8} (1.4×10^{-8}, 1.1×10^{-7})	Residual	1.3×10^{-4}
Male	-1.3×10^{-5} (-4.9×10^{-5}, 2.3×10^{-5})		
=EUHA density + RENB density + (1|Fish number)			
Intercept	7.2×10^{-4} (6.7×10^{-4}, 7.7×10^{-4})	(1|Fish number)	2.5×10^{-5}
EUHA density	6.1×10^{-8} (1.3×10^{-8}, 1.1×10^{-7})	Residual	1.3×10^{-4}
RENB density	10.0×10^{-8} ($^{-}5.9 \times 10^{-7}$, 7.9×10^{-7})		
=1 + (1|Fish number)			
Intercept	7.8×10^{-4} (7.6×10^{-4}, 8.0×10^{-4})	(1|Fish number)	3.4×10^{-5}
		Residual	1.3×10^{-4}
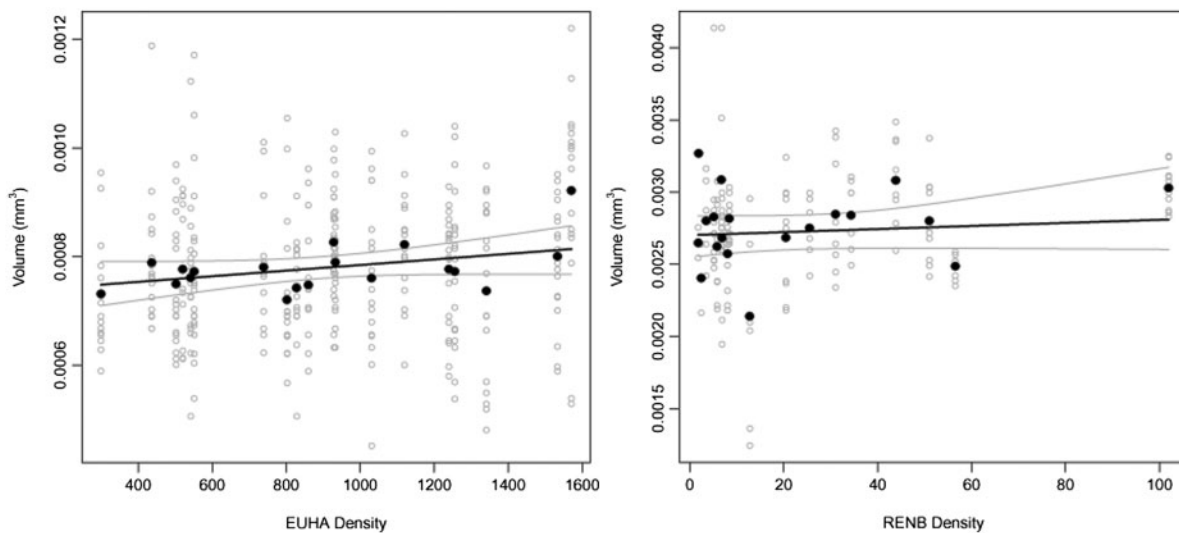
Fig. 2 Model-averaged predictions for the relationship between the parasite's density and the metacercarial inner volume. The open circles represent raw data and the dark circles indicate model-averaged predictions. The solid black line is the best-fit line, and the gray lines are 95% confidence intervals. For models that depend on sex, the best-fit line and confidence interval are for parasites in female fish. For models that depend on heterospecific density, the best-fit line and confidence interval are for fish that harbor an average density of heterospecifics.

manipulated phenotype should become more exaggerated and parasites should experience an increased probability of transmission (Brown 1999). EUHA may fit these predictions, as the volume of EUHA increases slightly with increasing density of EUHA (shown here), and a greater intensity of infection is associated with an increase in California killifish's manipulated phenotype (i.e., an increase in conspicuous behaviors) (Lafferty and Morris 1996),

suggesting that the costs of manipulation are shared among metacercariae. It is important to note that we should only observe cost sharing of manipulation if EUHA continues to grow after it has reached the point where it is competent to infect predatory birds (i.e., when it has reached patency), as predation prior to that point would result in the death of the parasite. EUHA can infect predatory birds 2 weeks post-infection of killifish

(Martin 1950), and appears to continue to grow for an additional 2–4 weeks (K. L. Weinersmith, personal observation); thus, this condition may be met in this host–parasite system.

Cost sharing can also occur either if the host mounts a maximum immune response that is "diluted" by the presence of many conspecific parasites (and thus each individual can invest less in self-defense), or if parasites actively produce compounds that suppress the immune response. Helminth parasites are known to secrete immunosuppressive compounds (Maizels et al. 2004), and Cornet (2011) observed that hosts with two parasite individuals had less immune activity than did hosts with a single parasite. EUHA metacercariae secrete fibroblast growth factors *in vitro*, and these secretions aid in the aggregation of EUHA (J. La Clair and K. D. Lafferty, unpublished data). Perhaps aggregating EUHA are able to each invest less in immunosuppression or behavioral manipulation at high densities, and are able to achieve a greater volume (thus greater fitness). Although we do not expect to observe a sharing of the costs of manipulation until parasites reach patency, cost sharing of immune system defense would immediately benefit a newly arriving parasite. Parasites may combat the immune system as they mature but they likely do not invest in manipulation yet (as they likely do not invest in manipulation before they are infectious to the next host), and so cost sharing of immune defense may be more pervasive than cost sharing of manipulation. Hence, this system should be useful for further work exploring the possibility of cost sharing among behavior-manipulating parasites.

An alternative, but unlikely, explanation for the observed increase in volume of EUHA with increased density is that the parasites continue growing with age and that those in high-density infections are older on average. This explanation is unlikely for three reasons. First, these types of encysting metacercariae experience asymptotic growth and we maintained fish in the laboratory for 2 months to ensure that even recent infections could reach maximum size. Second, metacercariae should tend to be older in larger (and probably older) fish; however, the fish's standard length was not associated with the density of parasites for RENB ($R^2 < 0.01$, $P = 0.91$, df = 17) and length was negatively associated with density for EUHA ($R^2 = 0.47$, $P = 0.001$, df = 17), indicating that there was no confound of older metacercariae in longer fish driving the observed relationships of metacercarial size with density. Third, metacercariae on the brainstem (which should be older—see the "Materials and methods" section)

were not larger than metacercariae from other areas of the brain. In fact, the raw data suggest that parasites on the brainstem may be slightly smaller than those on the rest of the brain (Table 2), further discounting the notion that continued growth of metacercaria confounded our results.

An alternative explanation, that we cannot discount, for the positive association between density and body size for EUHA is that some hosts may be particularly compatible with EUHA infection. For instance, such hosts may be relatively highly susceptible to infection (driving higher densities) and conducive for higher growth rates of the parasite. However, there is not a strong positive relationship between the average volume of EUHA and RENB metacercaria ($R^2 < 0.01$, $P = 0.91$, df = 17), which suggests that some hosts are not generally more compatible for trematodes than are others. Controlled infections would be necessary to confirm whether or not certain individual fish are specifically compatible for infection by EUHA. We know of no examples of positive density-dependence in trophically transmitted parasites, and so observing slight positive density-dependence in EUHA is noteworthy even if compatibility of the host is the mechanism underlying this relationship. If compatibility is the driving mechanism, then further work can examine why compatibility is important in this host–parasite system while it does not drive such false density-dependence in other hosts studied.

Competition can be an important factor limiting population size (Dobson 1985). Our results suggest that EUHA and RENB metacercariae are not limited by resources, or that cost sharing ameliorates the impact of limited resources. Because we did not observe negative density-dependence for either parasite, it is likely that competitive dynamics in the second intermediate host do not limit overall size of population of either EUHA or RENB. In fact, more parasites are associated with an increased probability of transmission in this system (Lafferty and Morris 1996), suggesting that conspecifics may also provide a benefit in terms of more rapid transmission. This frequently is not the case in other host–parasite systems in which parasites manipulate the hosts' phenotype. For example, metacercariae of *Microphallus papillorobustus*, infecting the gammarid *Gammarus insensibilis*, require only one parasite for complete expression of the host's manipulated phenotype (Thomas et al. 1997), and larger infrapopulations experience reduced size and reduced fecundity (Brown et al. 2003). Additionally, Dianne et al. (2012) found that the acanthocephalan, *Pomphorhynchus laevis*, does not induce stronger phenotypic

manipulation in the presence of more than one parasite (but see Franceschi et al. 2008), and average size of the parasites decreases with increasing intensity. Even if costs are shared in these systems, resource limitation results in a net reduction in fitness arising from the presence of conspecifics. Our results pertain to a different, and understudied, situation in which parasites are small, do not appear to saturate the available habitat, and share transmission interests. Although experimental confirmation is required, our findings suggest that, when resources are not limiting and there is a positive relationship between infrapopulation size and the expression of the manipulated host's phenotype, trophically transmitted parasites may actually benefit from being ''crowded'' by conspecifics.

Supplementary data

Supplementary Data available at *ICB* online.

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