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Original Contribution

Species Identity and Size are Associated with Rat Lungworm Infection in Gastropods

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Abstract: *Angiostrongylus cantonensis*, the rat lungworm, is an emerging zoonotic pathogen that cycles between definitive rat and intermediate gastropod hosts. Zoonotic infection occurs when humans intentionally or accidentally consume infectious larvae in a gastropod host, and may manifest as neuroangiostrongyliasis, characterized by eosinophilic meningitis, severe neurological impairment, and even death. Thus, the risk of *A. cantonensis* zoonoses may be related to the distribution of *A. cantonensis* larvae across gastropod hosts. We screened 16 gastropod species from 14 communities on the island of Oʻahu, Hawaiʻi, USA, to characterize the distribution of *A. cantonensis* among species and across host size. Prevalence (proportion of the population infected) and infection intensity (density of worms in host tissue) varied among gastropod species. Prevalence also varied with gastropod host size, but this relationship differed among host species. Most host species showed a positive increase in the probability of infection with host size, suggesting that within species relatively larger hosts had higher prevalence. The density of worms in an infected snail was unrelated to host size. These results suggest that variation in *A. cantonensis* infection is associated with demographic structure and composition of gastropod communities, which could underlie heterogeneity in the risk of human angiostrongyliasis across landscapes.

Keywords: *Angiostrongylus cantonensis*, rat lungworm, Angiostrongyliasis, host size, zoonosis, snails

INTRODUCTION

Zoonotic diseases may account for 75% of emerging infectious disease worldwide, a considerable proportion of

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the public health burden (Taylor et al. 2001). The multi-host life cycles and diverse animal sources of many zoonotic pathogens complicate transmission dynamics and undermine our ability to resolve the transmission pathways that lead to human infections. However, understanding these drivers is paramount as they inform public health strategies and management aimed at suppressing zoonoses.

Angiostrongylus cantonensis, the rat lungworm, is a zoonotic pathogen that circulates between gastropods and rats, respectively, the intermediate and definitive hosts. The

life cycle (Alicata and Jindrak 1970; Cowie 2013a; Barratt et al. 2016) begins when first-stage larvae (L1) in rat feces are consumed by a snail or slug (hereafter "gastropods"). The L1 larvae molt twice in the gastropod to become third-stage larvae (L3). The life cycle continues when infectious gastropods containing L3 larvae are consumed by rats, penetrate the epithelium of the rat gastrointestinal tract, and migrate to the brain via the bloodstream. In the rat brain, L3 larvae molt twice to become L5 subadults, which migrate to the pulmonary artery, where they mature, mate, and produce eggs, which are carried in the bloodstream to the lungs. Eggs hatch as L1 larvae, which are carried to the pharynx in respiratory secretions, swallowed and passed by the rat host in its feces.

Like the rat host, humans acquire *A. cantonensis* infections by ingesting L3 larvae, largely through intentional or accidental consumption of infective gastropod tissue. However, the infection is abortive in human hosts. L3 larvae reach the human brain via the same route as in rats and there molt to the L5 subadult stage (e.g. Alicata and Jindrak 1970; Cowie 2013a; Barratt et al. 2016). However, these subadults generally do not escape the human brain and fail to mature into reproductive adults. Their presence and ultimate death in the brain are associated with neurological damage and inflammation that can lead to the disease known as angiostrongyliasis (Graeff-Teixeira et al. 2009), or more specifically neuroangiostrongyliasis (Murphy and Johnson 2013; Prociv and Turner 2018) to distinguish it from the gastrointestinal syndrome caused by *Angiostrongylus costaricensis* (Graeff-Teixeira et al. 1993, 2005). Symptoms of infection in humans range from indiscernible to debilitating. Mild cases generally involve headache and fever. More serious cases manifest as eosinophilic meningitis, with severe neuro-impairment, coma, and even death (Murphy and Johnson 2013). The severity of the symptoms is presumed to be associated with the number of larvae initially ingested that ultimately reach the brain (Tsai et al. 2001; Murphy and Johnson 2013). This infective dose is the product of the amount of gastropod tissue consumed and the density of L3 larvae in the consumed tissue.

The distribution of *A. cantonensis* infection across gastropods of different sizes has direct implications for the prevalence of neuroangiostrongyliasis in human populations. A few studies have addressed the possibility that *A. cantonensis* infection in the gastropod hosts increases with host size, possibly related to accumulation of the parasite over the host's life, but with mixed results (Wallace and Rosen 1969; Yousif and Lämmler 1975; Ibrahim 2007; Tesana et al. 2009; Chen et al. 2011; Oliveira et al. 2015). High infection prevalence (proportion of the population infected) in larger snails has implications for the potential of zoonotic infection. In areas where gastropods are intentionally consumed (Lv et al. 2009; Eamsobhana et al. 2010), human infection occurs when infectious tissue is consumed raw or undercooked. The volume of larger gastropods directly increases the potential for ingestion of undercooked tissue. Alternatively, in areas where human infections primarily occur through accidental consumption of tissue associated with the presence of gastropods on produce (Alicata 1964; Slom et al. 2002; Tsai et al. 2004; Cowie 2013b), smaller gastropods may be more difficult to detect and remove, especially on leafy greens (Yeung et al. 2013). In this case, a positive relationship between host size and *A. cantonensis* infection would decrease the opportunity for zoonotic infection.

Previous studies have demonstrated both interspecific and intraspecific variation in infection prevalence and load (number of parasites in an individual) of larval *A. cantonensis* in infected gastropods (e.g. Wallace and Rosen 1969; Hollingsworth et al. 2007; Chen et al. 2011; Kim et al. 2014; Oliveira et al. 2015; Stockdale-Walden et al. 2015, 2017; Waugh et al. 2016). Mechanisms causing this heterogeneity are unknown but might involve variation in the competence or permissiveness of certain host species for L1 *A. cantonensis* infection, or behavioral differences that increase host exposure to the parasite (e.g. coprophagy). Kim et al. (2014) demonstrated that *A. cantonensis* infection occurs widely across numerous distantly related gastropod lineages, but prevalence was higher in ground dwelling species that presumably have more opportunity to interact with infectious rat feces. The same study reported interspecific variation in the density of worms across infected gastropod host species, although this was not approached statistically to control for confounding effects (e.g. site and time of sampling). Given the likely relationship between infective dose and the severity of human angiostrongyliasis (Tsai et al. 2001; Murphy and Johnson 2013), factors that drive variation in the number of worms in infectious gastropod tissue will greatly influence *A. cantonensis* zoonotic infection rates.

Here, we report a survey of infection levels in intermediate gastropod hosts on Oʻahu, Hawaiʻi, an area with endemic rat lungworm transmission and angiostrongyliasis that has been emerging since around 1960 (Horio and Alicata 1961; Cowie 2017). We interrogate this dataset to

assess relationships among intermediate host size, *A. cantonensis* prevalence, and larval worm load across 16 gastropod host species to better understand and mitigate risks of zoonotic *A. cantonensis* infection.

METHODS

Sample Collection and Processing

Terrestrial gastropods were collected during 39 sampling events in 14 replicate communities across Oʻahu from April through November 2018 by searching microhabitats such as leaf litter, under natural and man-made objects and materials, leaf axils of large plants, and low vegetation. All were non-native species, as native species have been almost entirely extirpated from the lowland areas of the study. Most are widely distributed in Hawaiʻi and readily identified by experienced malacological researchers (e.g. Cowie 1998; Cowie et al. 2008, 2019). Field-collected specimens were brought to the laboratory and killed using the *nikunuki* method (Fukuda et al. 2008). All but some *Lissachatina fulica* specimens were weighed; shell length was measured for all specimens except slugs (for more detail, see Statistical Analyses, below). For specimens that weighed approximately ≤ 1.0 g, the whole gastropod body was digested in Longmire's lysis buffer (0.1 M Tris, 0.1 M EDTA, 0.01 M NaCl, 0.5% sodium dodecyl sulfate). For specimens that weighed > 1.0 g, a 0.5–1.0 g sample comprising approximately a 1:1 ratio of mantle and foot tissue was removed and digested. All tissue digestion was done at a volume of 24 µl of buffer per mg of tissue and 150 µg of proteinase K. DNA was extracted from 200 µl of digested lysate with Qiagen Blood and Tissue Spin Column Kits following the manufacturer's standard protocol.

Gastropod DNA samples were screened for *A. cantonensis* with a Taqman qPCR assay (ACANITS1, Life Technologies assay ID #AI39RIC) with oligonucleotides specific for the parasite's internal transcribed spacer 1 (ITS1) gene (Qvarnstrom et al. 2010). Each 10 µl qPCR reaction consisted of 5 µl of Taqman Fast Advanced master mix (2X), 3.5 µl of nuclease-free water, 0.5 µl of the Taqman assay mix (20X), and 1.0 µl of template DNA. The qPCR run conditions consisted of a 2-min incubation at 50°C, a 20-s denaturation period at 95°C, and 40 cycles of 95°C for 1 s and 60°C for 20 s. Samples with exponential amplification curves crossing a threshold of 0.2 florescent units were interpreted as positive for the presence of *A. cantonensis*. Since the ratio of gastropod tissue mass to lysis buffer was standardized during the digestion step, we interpret amplification threshold (Ct) values as a correlate of the density of worms in gastropod tissue across hosts. Our assumption is supported by a general lack of correlation between the Ct value and the mass of the tissue sample digested (Pearson's $r = 0.002$, $P = 0.98$) across samples, even after controlling for host species (F-test with Kenward–Roger approximation, $P = 0.57$). After an initial screen, positive samples were run twice more with the same protocol to permit calculation of an average Ct value.

Statistical Analyses

Our principle statistical objective was to characterize the relationships of the probability of infection and of infection intensity with gastropod species and size. Wet mass was determined for 872 of the 955 gastropods collected, but only shell length, not mass, was measured for 83 of the 244 *Lissachatina fulica* specimens. To integrate these *L. fulica* samples into the dataset, we used a model to predict mass based on shell length. The model was trained with the 161 *L. fulica* individuals in which both shell length and mass were estimated. To conform to a normal distribution, wet mass was cube root transformed. To predict mass, we selected among five models (linear shell length, quadratic polynomial shell length, cubic polynomial shell length, cubic shell length, and a y-intercept model) and used the best-fit model (quadratic polynomial shell length model, AIC weight = 0.999, $R^2 = 0.99$).

The mass of the gastropods differed by orders of magnitude among species. To generate a mass metric that differentiated individuals within a species based on mass but that was also comparable among species, we scaled mass by calculating standard scores as the difference between the individual's mass and the mean mass of the species divided by the standard deviation of mass for that species. Thus, these standard scores (z-scores) represent the signed number of standard deviations that an individual mass value is from the mean value for that species in our sample.

To estimate the influence of gastropod species, mass (z-score), and the species × mass interaction on the probability of *A. cantonensis* infection and Ct values, we used mixed logistic regression models and linear mixed models, respectively. For an overall analysis of prevalence across species, we used all species for which we had a total sample of at least five individuals. For analyses in which Ct

values were the dependent variable or host mass was one of the independent candidate variables, we used species that had at least four infected individuals. Site and month of collection were added as random effects in all mixed models or covariates in fixed effect models. We selected among full models and all combinations of nested models, including a y-intercept model, using AIC corrected for small sample size (AICc) for both dependent variables. We assume that models with a ΔAICc of greater than 2 signify a reduction in model fit. While all-subset model selection has been criticized (Anderson and Burnham 2002; Fieberg and Johnson 2015), single-model approaches (not presented) yielded very similar results in our study.

RESULTS

Of the 955 gastropods from 16 species, 182 were infected with *A. cantonensis*, an overall prevalence of 0.19. A logistic regression model that included month, site, and species (AICc weight = 1.0) fitted the infection data better than a nested null model that only included the covariates of month and site (ΔAICc = 154.4, AICc weight = 0), indicating that the probability of infection varied across species (Fig. 1). Of the 16 species, 12 (*L. fulica*, *Euglandina rosea* [in fact a species complex; Meyer et al. 2017], *Gonaxis kibweziensis*, *Laevicaulis alte*, *Liardetia doliolum*, *Ovachlamys fulgens*, *Pallifera* sp., *Parmarion martensi*, *Paropoeas achatinaceum*, *Subulina octona*, *Tauphaedusa tau*, and *Veronicella cubensis*) tested positive for *A. cantonensis*. Infection was not detected in four species (*Allopeas clavulinum*, *Bradybaena similaris*, *Meghimatium bilineatum*, and *Opeas hannense*). After generalizing across the covariates of site and season, the best-fit logistic regression model estimated that the probability of infection was greatest in *P. martensi* (0.33) and *L. alte* (0.33), followed by *L. fulica* (0.25), *E. rosea* (0.23), *G. kibweziensis* (0.16), *P. achatinaceum* (0.06), *O. fulgens* (0.05), *Pallifera* sp. (0.05) and *L. doliolum* (0.05), *T. tau* (0.04), *V. cubensis* (0.03), and *S. octona* (0.01).

The probability of infection with *A. cantonensis* in the seven host species with at least five individuals sampled was related to gastropod size but the nature of the relationship varied among species. Among the set of candidate logistic mixed regression models (Supplementary Table 1), the full model fitted the data best (AICc weight = 0.87, Table 1). This interaction model was differentiated from the nested models that included the main effects of (1) species and host mass (ΔAICc = 3.7, AICc weight = 0.13), (2) species only (ΔAICc = 34.7, AICc weight = 0), and (3) host mass

only (ΔAICc = 117.5, AICc weight = 0). The intercept-only model was a comparably poor fit to the data (ΔAICc = 143.8, AICc weight = 0). *Parmarion martensi*, *Lissachatina fulica*, *Paropeas acatinaceum*, *Veronicella cubensis*, and *Euglandina rosea* generally exhibited a positive relationship between infection probability and host mass (Fig. 2). In contrast, the probability of infection in *Laevicaulis alte* was unrelated to host size, and the relationship was weakly negative in *Gonaxis kibweziensis* (Fig. 2).

We performed an ad hoc analysis of *A. cantonensis* prevalence among individual gastropods with absolute mass below 200 mg. This dataset comprised (1) individuals of small species, i.e. species that reach only a small size (< 200 mg) as adults (*A. clavulinum*, *L. doliolum*, *O. hannense*, *O. fulgens*, *Pallifera* sp., *P. achatinaceum*, *S. octona*, and *T. tau*) and (2) small (< 200 mg and presumably young) individuals of large species, i.e. species that reach a larger size as adults (*B. similaris*, *L. fulica*, *E. rosea*, *G. kibweziensis*, *L. alte*, *M. bilineatum*, *P. martensi*, and *V. cubensis*). Within this reduced dataset, only individuals of small species were infected with *A. cantonensis* (Table 2). A binomial regression (qAICc weight = 0.94) that modeled prevalence in gastropod species as a function of species size (small or large) in this reduced dataset better fitted the data than a null, y-intercept-only model (ΔqAICc = 5.4, qAICc weight = 0.06), and the coefficient of this best-fit model suggested that for snails of a specific (i.e. smaller) size the probability of infection was higher in small species. The same analysis with the whole dataset showed that a binomial regression (qAICc weight = 0.94) modeling gastropod species prevalence as a function of species size fitted the data better than the y-intercept model (ΔqAICc = 5.5, qAICc weight = 0.06). However, in contrast to the analysis of the reduced dataset, this model estimated that the overall probability of infection was 8.4 times greater for large species than for small species.

Average Ct values varied among the seven species that had more than four infections (Fig. 3, Supplementary Table 2). A linear mixed model that only included a fixed effect of species fitted the data best (AICc weight = 0.73). After generalizing across the random effects of site and season, this species-only model estimated that Ct values were lowest for *P. martensi* (26.7), similar among *L. fulica* (29.6), *L. alte* (28.7), and *P. achatinaceum* (28.7), and highest for *G. kibweziensis* (33.0), *E. rosea* (32.0), and *V. cubensis* (33.1). Unlike prevalence, average Ct values were unrelated to host mass. The species-only model outperformed a model that included both the main effects of
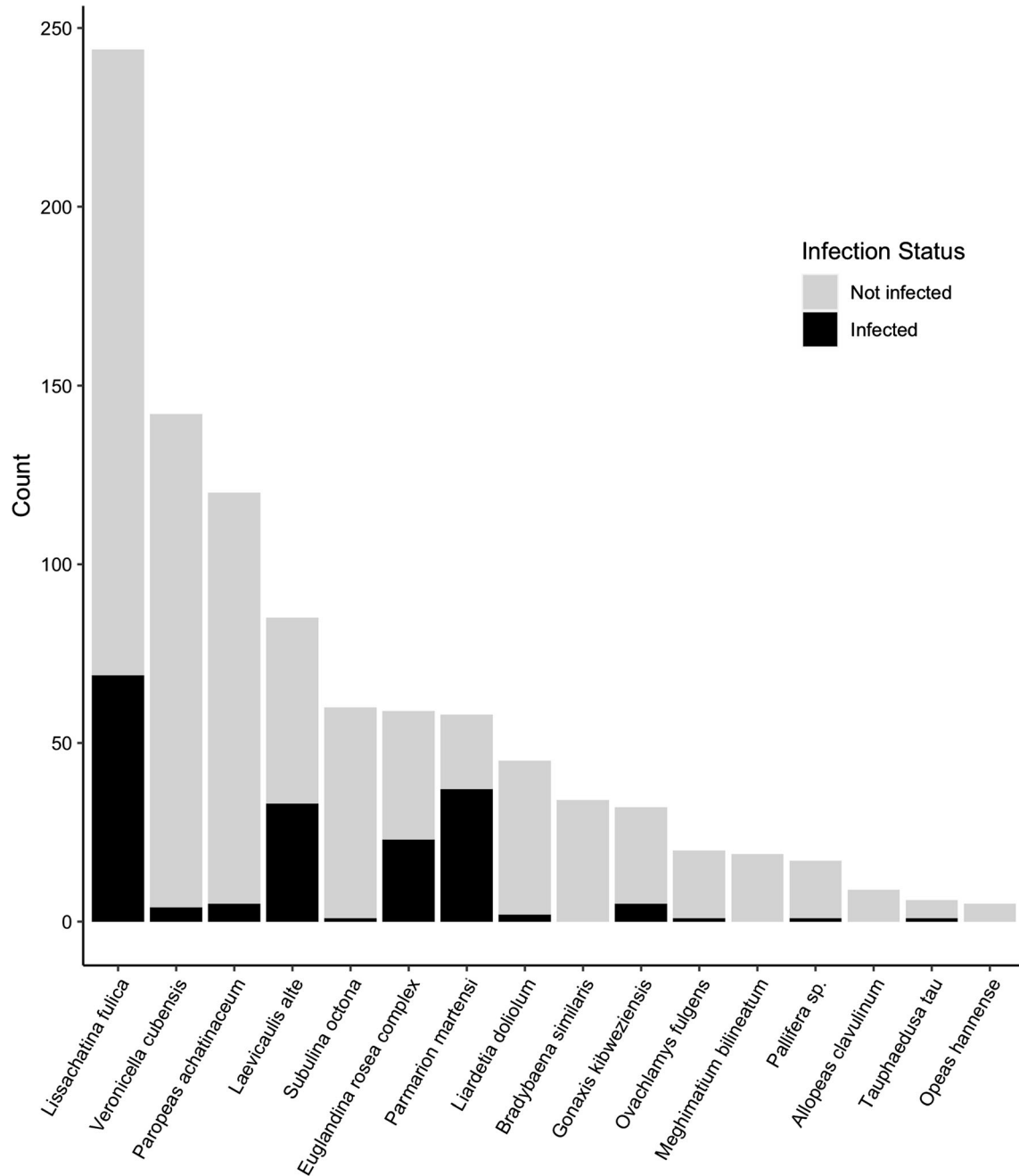
Figure 1. The number of gastropod hosts infected with *A. cantonensis* and uninfected across all gastropod species included in the dataset.

species and host mass (ΔAICc = 2.1, AICc weight = 0.26). Models that included a host mass × species interaction (ΔAICc = 8.0, AICc weight = 0.01) or host mass only (ΔAICc = 25.6, AICc weight = 0) fitted the data poorly, the latter performing worse than the intercept-only model (ΔAICc = 23.7, AICc weight = 0). Low sample sizes of infected individuals in several small species (*L. doliolum*, *O. fulgens*, *Pallifera* sp., *S. octona*, and *T. tau*) precluded a species level statistical comparison of Ct values with larger species. However, average Ct values associated with these

five species were generally similar to or lower than those of several larger species, with the exception of *P. martensi* (Table 2).

DISCUSSION

We screened 16 gastropod species for *Angiostrongylus cantonensis*, the causative agent of neuroangiostrongyliasis, and showed that *A. cantonensis* prevalence varied across

Table 1. Coefficients of the Main Effects of the Best-Fit Model Explaining the Dependent Variable, i.e. *A. cantonensis* Prevalence.

Main effects	Estimate	2.5% confidence limit	97.5% confidence limit
Intercept	− 1.70	− 3.26	− 0.61
Gonaxis kibweziensis	− 1.32	− 10.59	0.32
Laevicaulis alte	0.69	− 0.31	1.82
Lissachatina fulica	− 0.12	− 1.01	1.01
Parmarion martensi	1.21	− 0.14	2.71
Paropeas achatinaceum	− 2.75	− 10.92	− 1.24
Veronicella cubensis	− 3.17	− 12.42	− 1.55
Mass (*z*-score)	0.76	− 0.01	2.36
Gonaxis kibweziensis * mass	− 1.41	− 8.98	0.47
Laevicaulis alte * mass	− 0.82	− 2.55	0.24
Lissachatina fulica * mass	− 0.03	− 1.50	0.95
Parmarion martensi * mass	0.87	− 0.83	2.57
Paropeas achatinaceum * mass	0.54	− 1.51	2.70
Veronicella cubensis * mass	0.01	− 2.18	3.61

Random effects	Variance		
Community	1.82	0.55	2.16
Month	0.03	0.00	0.84

Confidence limits (CL) are estimated with parametric bootstrap implemented in the program R package lme4 (Bates et al. 2015).
For the fixed effect of species, *Euglandina rosea* is the reference value.

species. The probability of infection also varied with gastropod host mass, but this relationship was species dependent. Amplification threshold (Ct) values also varied among infected gastropod host species, suggesting that the average density of worms in infected hosts differs among host taxa. However, the relative density of *A. cantonensis* larvae across infected individuals did not vary with host size. These data have important implications for *Angiostrongylus* transmission and the risk of zoonotic infections that result in neuroangiostrongyliasis.

Host species effects greatly influenced the distribution of *A. cantonensis* larvae across hosts, being consistently important across analyses. The distribution of any parasite across a community of hosts is a function of host compatibility and the parasite–host encounter rate. Several studies (see the introduction) have demonstrated variability in the prevalence of *A. cantonensis* among host species. Kim et al. (2014) extended these analyses with a comprehensive synthesis of recorded *A. cantonensis* intermediate hosts and a phylogenetically explicit analysis of host status. They demonstrated that a phylogenetically diverse assemblage of gastropods can serve as intermediate hosts for *A.*

cantonensis, suggesting that host breadth is not limited by evolutionarily conserved traits that limit more specialized parasites. While the causes of the variation in intermediate host compatibility in this system remain almost unknown, currently available data (Kim et al. 2014, 2019; this study) imply that variation in *A. cantonensis* prevalence and the density of worms per infection across sympatric intermediate host species result from a heterogeneous encounter rate between host species and parasite.

In the *A. cantonensis* transmission cycle, the encounter rate between parasite and intermediate host is probably mediated by the host's dietary preferences (especially the propensity to consume rat feces) and/or microhabitat choices that overlap those of competent definitive hosts. While our analyses do not explicitly test a link between microhabitat, diet, and indices of *A. cantonensis* infection, two species of predatory snails (*E. rosea* and *G. kibweziensis*) that consume snails had relatively high prevalence, but a low density of worms per infection. These predatory snails probably acquire larval worms from infected prey. The low density of worms per infected host might result from elevated larval worm mortality as they escape the
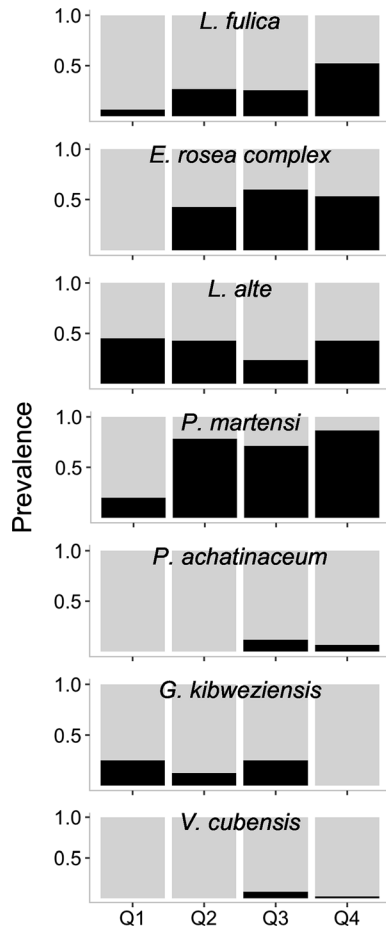
Figure 2. The relationship between size class and prevalence of infection with *A. cantonensis* across seven intermediate host species (*L. fulica*, *E. rosea*, *L. alte*, *P. martensi*, *P. achactinaceum*, *G. kibweziensis*, *V. cubensis*). Black bars represent the proportion of infected individuals in each size (mass) class. Size classes (Q1, Q2, Q3, and Q4) correspond to less than the 25th‰, between the 25th‰ and the median, between the median and the 75th‰, and greater than the 75th‰, respectively.

digestive tract and colonize the predator's muscle tissues and/or the preferential consumption of small prey items with low prevalence or fewer larvae per host. Campbell and Little (1988) demonstrated experimentally that most larvae were able to colonize *E. rosea* following the consumption of an infectious prey individual, suggesting that *E. rosea* can be an efficient host with minimal larval mortality, although the relative contributions of L1, L2, and L3 stage larvae in this study were unclear. Future work must focus on determining the proximate environmental or physiological factors and the ultimate evolutionary forces that influence the distribution of *A. cantonensis* across host species. Analyses of dietary preference and controlled infection experiments in gastropods that vary in infection prevalence

and parasite load may resolve the relative strength of encounter and host compatibility filters (Combes 1991) in modulating the distribution of parasites across hosts in this system. Such information would augment the capacity to predict the host competence of novel or invasive gastropod species, especially as *A. cantonensis* spreads to new regions in response to climate change (Lv et al. 2011; York et al. 2015; Kim et al. 2019).

Angiostrongylus cantonensis prevalence varied according to host size, although the slope of this relationship differed among host species. Most species exhibited an increasing probability of infection with size. This pattern might result from a scenario in which ingestion of L1 larvae is uncommon, exposure probability accumulates slowly with age as the host grows, and the probability of mortality as a result of infection is low, eventually resulting in a correlation between infection and body size. This hypothesis is supported by the observation that the only infected individuals among those hosts weighing < 200 mg were of small species. In this reduced dataset, the small individuals of large species would be very young individuals that had been exposed to the parasite for a relatively short time. In contrast, individuals of small species in this reduced dataset would have included a mixture of young and old individuals.

Some species did not exhibit a positive relationship between infection probability and mass. For instance, *L. alte* showed similar rates of infection across size classes. This could imply that such host taxa might have different rates of (1) encounter with the parasites over their life history, (2) infection-induced mortality, or (3) immune-mediated clearance of the infection. In contrast to prevalence, the density of *A. cantonensis* worms in infected tissue was not related to age (size). This might suggest that worms are acquired by gastropod hosts at a rate that is roughly proportional to growth.

Our results have several implications that illuminate factors associated with the zoonotic risk of neuroangiostrongyliasis in humans. The capacity for any organism to vector a zoonotic disease is, in part, a function of the frequency of contact between the vector and humans. Gastropods vector *A. cantonensis* to vertebrates primarily through the vertebrate's consumption of the gastropod. In areas with endemic *A. cantonensis* infection in humans where snails are not a common food item (e.g. Hawai'i, other Pacific islands, and the subtropical and tropical Americas), the majority of these events may be accidental. Smaller snails present an inherently greater risk of acci-

Table 2. The Numbers of Infected and Uninfected Individuals in Gastropod Species Reaching a Maximum Size of Less Than and More Than 200 mg in Our Study, in Two Datasets: Individuals with an Absolute Mass < 200 mg and the Whole Dataset of 955 Individuals.

Species	Individuals < 200 mg in mass			All individuals		
	Infected	Uninfected	Prevalence	Infected	Uninfected	Prevalence
Maximum size < 200 mg						
Allopeas clavulinum	0	9	0.00	0	9	0.00
Liardetia doliolum	2	43	0.04	2	43	0.04
Opeas hannense	0	5	0.00	0	5	0.00
Ovachlamys fulgens	1	19	0.05	1	19	0.05
Pallifera sp.	1	11	0.08	1	16	0.06
Paropeas achatinaceum	5	115	0.04	5	115	0.04
Subulina octona	1	59	0.02	1	59	0.02
Tauphaedusa tau	1	5	0.17	1	5	0.17
Total	11	266	0.04	11	271	0.04
Maximum size > 200 mg						
Bradybaena similaris	0	2	0.00	0	34	0.00
Euglandina rosea	0	7	0.00	23	36	0.39
Gonaxis kibweziensis	0	1	0.00	5	27	0.16
Laevicaulis alte	0	1	0.00	33	52	0.39
Lissachatina fulica	0	2	0.00	69	175	0.28
Meghimatium bilineatum	0	4	0.00	0	19	0.00
Parmarion martensi	0	8	0.00	37	21	0.64
Veronicella cubensis	0	12	0.00	4	138	0.03
Total	0	37	0.00	171	502	0.25

dental consumption as they are less easily detected on food items such as leafy greens. We have shown that among small individual gastropod hosts (small species and small individuals of large species), small species had a higher probability of infection with *A. cantonensis* than did large species. This trend was reversed in the whole dataset, in which several large species had dramatically higher prevalence than that in small ones. This result is important as it suggests that although larger species have much higher overall prevalence, smaller species may be disproportionately more likely to transmit neuroangiostrongyliasis to humans because of their size when they are infectious. This implies that small gastropod host species may be an important source of human infection, even though their *A. cantonensis* prevalence is relatively low.

Host species effects impact zoonotic disease risk across several important emerging infectious disease systems. For instance, changes in host species community composition are associated with the spatiotemporal risks of zoonotic infection with West Nile virus (Allan et al. 2009; Hamer et al. 2011; Kilpatrick et al. 2006a, b) and with *Borrelia burgdorferi*, the causative agent of Lyme disease (LoGiudice et al. 2003). In this system, we demonstrate that intermediate hosts differ fundamentally in their capacity to vector *A. cantonensis* zoonoses. These analyses highlight the importance of local gastropod community structure in modulating the risk of neuroangiostrongyliasis and suggest that the structure of local gastropod communities may account for heterogeneity in the relative risk of neuroangiostrongyliasis across the landscape of human populations.
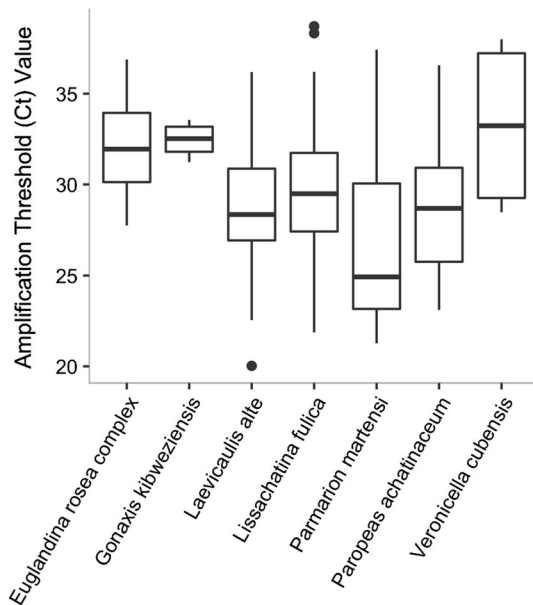
Figure 3. Boxplot showing the distribution of amplification threshold values across the seven intermediate host species with the greatest number of infections. Lower and upper limits of the boxes denote the first and third quartiles of the Ct value distribution for each species. Upper and lower whiskers extend to the largest and smallest values within 1.5 times the distance between the first and third quartiles. Dots beyond the whiskers represent outlying or extreme data points for each distribution. The line within the box represents the median of each distribution.

ACKNOWLEDGEMENTS

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