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Host snail species exhibit differential *Angiostrongylus cantonensis* prevalence and infection intensity across an environmental gradient

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ABSTRACT

Diverse snail species serve as intermediate hosts of the parasitic nematode *Angiostrongylus cantonensis*, the etiological agent of human neuroangiostrongyliasis. However, levels of *A. cantonensis* infection prevalence and intensity vary dramatically among these host species. Factors contributing to this variation are largely unknown. Environmental factors, such as precipitation and temperature, have been correlated with overall *A. cantonensis* infection levels in a locale, but the influence of environment on infection in individual snail species has not been addressed. We identified levels of *A. cantonensis* prevalence and intensity in 16 species of snails collected from 29 sites along an environmental gradient on the island of Oahu, Hawaii. The relationship between infection levels of individual species and their environment was evaluated using AIC model selection of Generalized Linear Mixed Models incorporating precipitation, temperature, and vegetation cover at each collection site. Our results indicate that different mechanisms drive parasite prevalence and intensity in the intermediate hosts. Overall, snails from rainy, cool, green sites had higher infection levels than snails from dry, hot sites with less green vegetation. Intensity increased at the same rate along the environmental gradient in all species, though at different levels, while the relation between prevalence and environmental variables depended on species. These results have implications for zoonotic transmission, as human infection is a function of infection in the intermediate hosts, ingestion of which is the main pathway of transmission. The probability of human infection is greater in locations with higher rainfall, lower temperature and more vegetation cover because of higher infection prevalence in the gastropod hosts, but this depends on the host species. Moreover, severity of neuroangiostrongyliasis symptoms is likely to be greater in locations with higher rainfall, lower temperature, and more vegetation because of the higher numbers of infectious larvae (infection intensity) in all infected snail species. This study highlights the variation of infection prevalence and intensity in individual gastropod species, the individualistic nature of interactions between host species and their environment, and the implications for human neuroangiostrongyliasis in different environments.

1. Introduction

Zoonoses account for ~60% of human infectious diseases and 75% of newly discovered or emerging infectious diseases (Taylor *et al.*, 2001; Jones *et al.*, 2008). Zoonotic pathogens are transmitted to humans from wildlife and domestic animals (the hosts) when favorable behavioral, ecological, and genetic conditions occur. Therefore, detailed knowledge of the transmission ecology of the etiological agents of zoonotic diseases is key to ameliorating their impact on human health.

Environmental factors such as rainfall and temperature have direct and indirect effects on enzootic cycles (Gubler *et al.*, 2001; Paull and Johnson, 2014), thereby altering the potential for zoonotic transmission (Estrada-Peña *et al.*, 2014). Indeed, some well-known diseases, such as malaria (Kelly-Hope *et al.*, 2009; Chowdhury *et al.*, 2018) and schistosomiasis (Xue *et al.*, 2011), exhibit clear patterns related to environmental factors, suggesting that transmission and the frequency of zoonoses can be predicted to some degree by variation in these factors. Similarly, the distribution of *Angiostrongylus cantonensis* (Chen, 1935), a parasitic nematode with a complex life-cycle (Fig. 1) involving snails as intermediate hosts and rats as definitive hosts (Alicata and Jindrak, 1970; Bhaibulaya, 1975), is constrained by patterns of environmental variables incompatible with its physiological limits, with rainfall and temperature

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being key factors associated with prevalence and transmission of the disease (Lv *et al.*, 2006; Epelboin *et al.*, 2016; Kim *et al.*, 2019).

Angiostrongylus cantonensis is the causative agent of neuro-angiostongyliasis and responsible for the majority of human cases of eosinophilic meningitis; symptoms vary greatly but coma and death may occur in extreme cases (Murphy and Johnson, 2013). The main pathway of human infection is ingestion (accidental or intentional) of raw or under-cooked host snails containing infectious larvae (Cowie, 2013). Thought to have originated in Southeast Asia, *A. cantonensis* is now widespread throughout many tropical/subtropical regions of the world (Cowie, 2019), and is expanding into more temperate regions such as the southeastern United States (Stockdale Walden *et al.*, 2017), western Europe (Paredes-Esquivel *et al.*, 2019), and eastern Australia (Barratt *et al.*, 2016).

Angiostrongylus cantonensis is a generalist parasite that can infect intermediate host species across the gastropod phylogeny, although prevalence (proportion of individuals in a population infected with a given pathogen) and infection intensity (mean number of larvae per unit of tissue) vary greatly among gastropod species (Kim *et al.*, 2014). Determinants of infection prevalence in intermediate host species are poorly understood, although within species host size (a proxy for age) has a significant effect, and food and habitat preferences and other behavioral differences among species have been suggested as contributing factors (Medeiros *et al.*, 2020). Variation in *A. cantonensis* prevalence in snail populations directly affects the probability of zoonotic transmission, and the level of infection intensity in an ingested snail is probably related to the severity of human illness (Murphy and Johnson, 2013; Ansdell *et al.*, 2020).

Understanding the dynamics among snails, the environment, and *A. cantonensis* is pertinent to predicting the parasite's likely range and reducing human health risk in regions where *A. cantonensis* is present. While the influence of environmental factors has been assessed in relation to the distribution of *A. cantonensis* across rainfall and temperature gradients, and the geographical range has been predicted in a number of regions of the world (e.g. Lv *et al.*, 2011; Kim *et al.*, 2019), the impact of these factors at the level of the individual intermediate host species within a community has not been considered. We addressed this gap in knowledge by comparing the prevalence and intensity of *A. cantonensis* infection in individual species within gastropod communities located along a dramatic environmental gradient on the island of Oahu, Hawaii. Specifically, we asked 1) whether *A. cantonensis* infection intensity and prevalence were strongly correlated with environmental factors; 2) whether *A. cantonensis* infection intensity and prevalence varied in parallel among all host species along the environmental gradient; and 3) whether interactions between species and environmental variables best predict *A. cantonensis* prevalence and/or intensity in snails. To our knowledge, this is the first time interactions between intermediate hosts of *A. cantonensis* and the environment have been addressed at a species level among multi-species communities.

2. Materials and methods

2.1. Location

The great range of climates on the island of Oahu, Hawaii (Giambelluca *et al.*, 2014), provides an opportunity to investigate patterns across dramatic yet accessible environmental gradients over short distances, thereby reducing confounding factors that may be introduced in studies of gradients over necessarily much larger distances elsewhere. The climatic variation is due in part to trade winds that blow across the northern Pacific Ocean from northeast to southwest. On reaching Oahu, the air is forced upward over the eastern mountain range and cools rapidly, condensing into clouds and releasing rain over the eastern (windward) side of the island, leaving dry air to pass over the western (leeward) side of the island (Fig. 2a).

Specimens were collected from 29 sites on 39 sampling occasions on Oahu between April and November 2018. The 29 collection sites span the island and encompass an environmental gradient from the typically rainy, cool and lush windward coast to the dry and hot leeward coast, and are located in 14 distinct watersheds (Fig. 2b). Site land use varied, but was mostly restricted to residential, recreational, and agricultural land and agricultural/wildland interfaces. We agreed with landowners not to disclose the location of the study sites (many of them farms) because of the sensitive nature of rat lungworm disease, which is potentially food-borne, among local communities. Instead, we indicate the watershed (Table 1) and number of sites within each watershed (Fig. 2b), which maintains confidentiality while providing information about the broad environmental context of our study.

2.2. Gastropod collection and preparation

At each site, two to six people searched for gastropods by looking

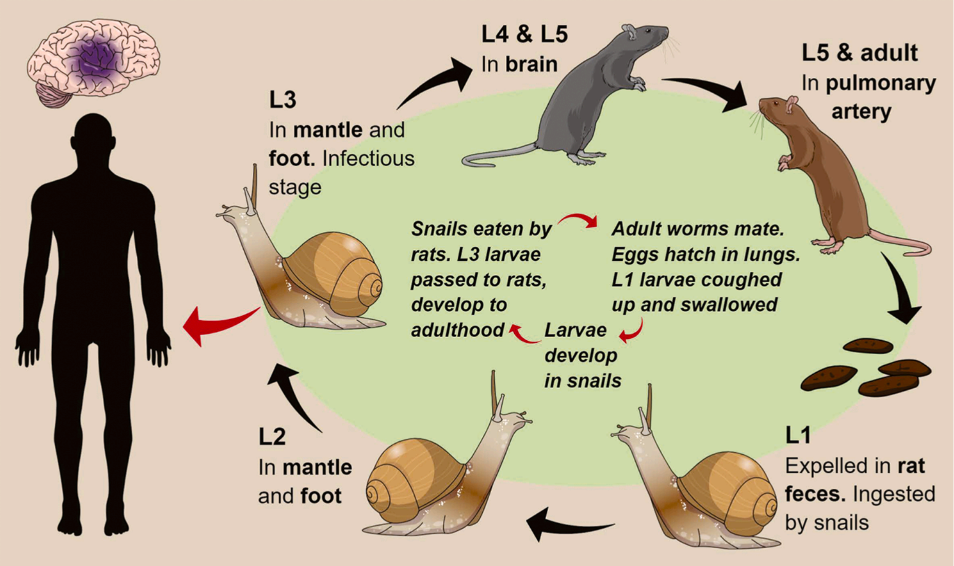

Fig. 1. The life cycle of *Angiostrongylus cantonensis* and the mode of human infection. L1 to L4 signify the first to fourth larval stages and L5 signifies the fifth (subadult) stage.

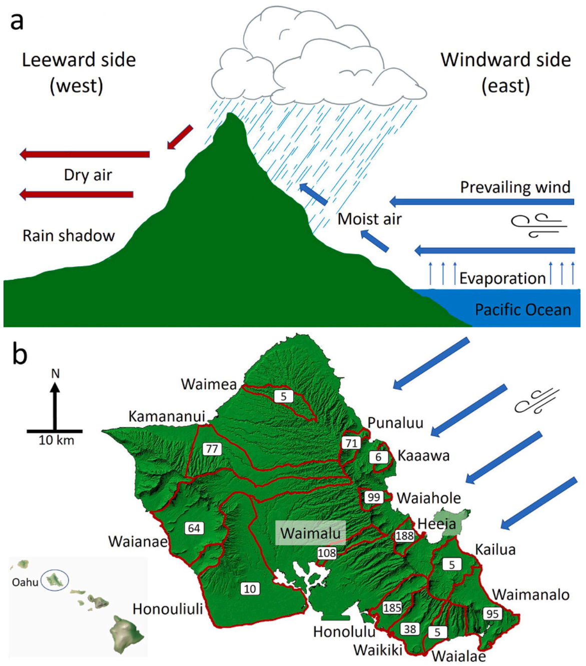

Fig. 2. Rainfall patterns and site locations on Oahu, Hawaii. a) Schematic showing the effects of island topography and north-east trade winds on rainfall. b) Topographic map of Oahu (inset: main Hawaiian Islands) depicting topography and the direction of north-east trade winds, with number of snails collected from each of the 14 watersheds where collection sites were located.

under rocks, leaf litter, and man-made objects, and inspecting tree trunks, vegetation, and on, around and under infrastructure. Eleven of the sites were sampled extensively; at these all species found were collected, up to 24 individuals per species, per site, for site totals of 59–108 specimens (Table 1, Supplementary Table 1). Collection at the remaining sites focused largely on a single species, *Lissachatina fulica*, with a goal of at least six individuals collected per site.

All specimens collected were taken to the laboratory alive for same-day processing, including all steps up to and including adding the tissue sample to the buffer solution (see below), which halted DNA degradation. Specimens were first identified to species and euthanized, and shells (if present, i.e. not in slugs) were removed following the *niku-nuki* method (Fukuda *et al.*, 2008). For specimens that weighed ≤ 1.0 g, the whole body was digested for DNA extraction. 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. Tissue was removed from the front, middle and tail end of the foot, as well as from the mantle and mantle skirt, to mitigate over/under estimation of nematode density (infection intensity) due to uneven larval distribution throughout the body (Jarvi *et al.*, 2012). All forceps and surgical scissors were autoclaved prior to handling tissue and flame sterilized between uses. Tissues from a random subset of gastropods from each site were initially viewed under a microscope for confirmation of identification of *A. cantonensis* larvae.

2.3. Molecular analysis - DNA extraction and qPCR amplification

Snail tissue was homogenized in 24 ml of Longmire's lysis buffer (0.1 M Tris, 0.1 M EDTA, 0.01 M NaCl, 0.5 % sodium dodecyl sulfate) per 1 g of minced gastropod tissue, combined with six to eight ceramic beads and disrupted with a bead beater for 3 min. Proteinase K (150 µg) was aliquoted into each sample prior to incubation at 56°C for 1–48 h, vortexing at 1 h intervals until the solution was homogeneous. Once all tissue was degraded and a homogenous solution obtained, 200 µl aliquots, one per sample, were transferred to sterile 1.7 ml microcentrifuge tubes. DNA extractions were then performed with Qiagen DNeasy Blood and Tissue Spin Column Kits following the manufacturer's standard protocol. DNA was quantified with a NanoDrop 2000 spectrophotometer. Using qPCR, the internal transcribed spacer 1 (ITS1) sequence of *A. cantonensis* rDNA was amplified using a species-specific primer set (Qvarnstrom *et al.*, 2010) in an amplification mix of 10 µl total volume: 5.0 µl TaqMan Fast Advanced Master Mix (2X), 3.5 µl nuclease-free water, 0.5 µl Taqman assay mix (20X; ACANITS1, Life Technologies assay ID No. AI39RIC), and 1.0 µl of template DNA. The qPCR cycling structure was 2 min at 50°C, 20 s at 95°C, and 40 cycles of 3 s at 95°C and 30 s at 60°C, followed by 2 min at 56°C. All qPCR samples were run with positive and negative controls. Additionally, identities of the larvae were confirmed by sequencing (Sanger) tissue from a small subset of randomly sampled gastropods from among our collections and comparing the results to the GenBank database.

Table 1
Watershed, time of year and number of snails collected from each site.

Site	Watershed	Month	Number of snails
1	Honouliuli	April	5
2	Waimea	April	5
3	Waimanalo	May	95
4	Waikiki	May	7
5	Waikiki	May	2
5	Waikiki	July	2
6	Kailua	May	1
6	Kailua	May	1
6	Kailua	October	3
7	Waimalu	May	108
8	Honolulu	May	1
9	Honouliuli	May	1
9	Honouliuli	May	1
10	Waiahole	May	99
11	Honolulu	May	1
12	Waikiki	May	3
13	Waialae	May	5
14	Waikiki	May	7
15	Waianae	June	64
16	Waikiki	June	9
17	Heeia	June	5
18	Honolulu	June	5
18	Honolulu	June	8
19	Heeia	June	7
19	Heeia	June	102
20	Waikiki	June	6
20	Waikiki	July	2
21	Honouliuli	June	3
22	Kaaawa	June	3
22	Kaaawa	August	3
23	Heeia	June	7
24	Kamananui	July	77
25	Heeia	July	2
25	Heeia	November	65
26	Honolulu	August	78
26	Honolulu	September	28
27	Honolulu	October	59
28	Honolulu	October	5
29	Punalulu	October	71

2.4. Determining prevalence and infection intensity

Infection prevalence is based on a binary variable, the presence/absence of *A. cantonensis* DNA in individual hosts. Samples with exponential amplification curves crossing a threshold of 0.2 fluorescent units were interpreted as positive for the presence of *A. cantonensis*. Samples with curves that did not cross the threshold were interpreted as negative.

Infection intensity was determined by amplification threshold (Ct) values of the qPCR. Since the ratio of gastropod tissue mass to lysis buffer was standardized during the digestion step, we interpreted Ct values as a correlate of the density of worms in gastropod tissue (infection intensity). This approach is supported by a general lack of correlation between the Ct value and the mass of the tissue sample digested across samples (Pearson's $r = 0.002$, $P = 0.98$), 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.

2.5. *Environmental variable data acquisition*

Three environmental variables were selected *a priori* as predictor variables based on working knowledge of gastropod and rat ecology: air temperature, rainfall, and vegetation cover (determined by a Landsat Surface Reflectance-derived Enhanced Vegetation Index (EVI), which quantifies vegetation greenness by calculating the amount of near infrared and red light reflected back into the atmosphere). Values of EVI range from -1 to 1 (more positive numbers represent greener areas). Data for each variable were extracted from a repository of environmental data (Giambelluca *et al.*, 2014). The repository contains data for each 234 × 250 m spatial unit of the main Hawaiian Islands. Raster files containing values for each environmental variable from every spatial unit were downloaded from the repository, then uploaded to ArcGIS Pro (version 2.5.0) to extract the monthly averages for each variable at each collection site on each collection occasion.

2.6. *Statistical analysis and model validation*

All statistical analyses were conducted in R (R Development Core Team, 2020). We examined the relationships of two dependent variables, 1) *A. cantonensis* prevalence, and 2) *A. cantonensis* infection intensity, to the set of environmental variables, and to host species, while controlling for time (month of collection) and location (watershed) (Table 1). To reduce the dimensionality of the environmental data, the variables were run through a principle component analysis using the function 'princomp' in the statistics package R (R Development Core Team, 2020). The resulting component scores for each site were compared to ground truthing of the site itself, using a combination of in person and Google Earth observations. The principle component explaining the greatest proportion of the variance (PC1) served as an independent variable in lieu of raw environmental variable data in downstream analyses.

We employed a model selection approach [Akaike Information Criterion, corrected for small sample size; AICc (Akaike, 1973; Burnham and Anderson, 2002)] and fit the data to two candidate sets of mixed models, one for each dependent variable (prevalence, intensity), using the glmmTMB package (Brooks *et al.*, 2017).

Prior work indicated snail species as an *a priori* covariate of *A. cantonensis* prevalence and intensity (Medeiros *et al.*, 2020). Therefore, species was included as a factor in all models. Also, snail size is an important predictor of *A. cantonensis* prevalence (but not intensity), and the relationship of size and *A. cantonensis* prevalence varies among species (Medeiros *et al.*, 2020). Therefore, a size*species interaction was included as a factor in all models in which prevalence was the independent variable (except the null model) but was left out of infection intensity models.

The weight of individual snails differed by orders of magnitude among species. Therefore, to allow for meaningful comparisons among species, we scaled weight (mass in grams) by calculating standard scores (z-scores) as the difference between the individual's weight and the mean weight of the species, divided by the standard deviation of weight for that species (Medeiros *et al.*, 2020). These z-scores thus represent the signed number of standard deviations that an individual mass value is from the mean value for that species in our data set and is hereafter referred to as zmass.

To avoid model convergence issues, we only included data from the snail species that were collected at five or more sites (the random grouping variable with unique environmental values) and at least four individuals of the species tested positive for *A. cantonensis*. Six species (*Lissachatina fulica, Euglandina rosea* [in fact a species complex; Meyer *et al.*, 2017], *Laevicaulis alte, Parmarion martensi, Paropeas achatinaceum* and *Veronicella cubensis*) met the criteria and were included in the models.

For analyses in which infection prevalence was the dependent variable, we assessed the fit of our data (AICc) to a set of four generalized linear mixed models with a binomial distribution (see Results section). Models included PC1 and species, a PC1*species interaction, species only, and a y-intercept model.

For analyses in which infection intensity (Ct values) was the dependent variable, data of infected individuals were fit to four linear mixed models (see Results section). The predictors mirrored those of the prevalence models, with the exception of zmass.

Watershed and month of collection were included as random factors in all models to account for time of year and location of collection. Models were ranked according to appropriateness of data fit, a $\Delta\text{AICc} > 2$ signifying a reduction in model fit (Burnham and Anderson, 2002), and evaluated to determine the influence of host species and the relative importance of PC1 and a PC1*species interaction.

3. Results

3.1. *Gastropods collected*

All specimens collected belonged to non-native species, as almost all native Hawaiian species have been extirpated from all but the higher elevation parts of the Hawaiian Islands (Meyer and Cowie, 2010). Overall, 185 of 956 individuals and 12 of the 16 species tested positive for *A. cantonensis* DNA (Table 2).

3.2. *PCA component environments*

Principle component 1 (PC1) explained ~60% of the variance and is driven by strong positive loadings for rainfall and vegetation cover (EVI), and a less strong negative loading for temperature (Table 3). Thus, a highly positive PC1 score represents rainy, cooler, heavily vegetated sites, and a highly negative PC1 score represents dry, hotter sites with little vegetation. This relationship between rainfall, temperature and vegetation cover reflects clearly the windward/leeward dynamics of the island (Fig. 2).

3.3. *Infection prevalence models*

The model including a PC1*species interaction best fit the data (Model 1; Table 4), indicating that the relationship between

Table 2

Numbers of each species screened and infected, and the number of sites at which each species was found, ordered by % infected. Species included in model analyses are asterisked.

Species	Number screened	Number (%) infected	Number of sites
*Parmarion martensi**	58	37 (63.8)	5
*Euglandina rosea**	59	23 (39.0)	12
*Laevicaulis alte**	86	33 (38.4)	13
*Lissachatina fulica**	244	69 (28.3)	35
Tauphaedusa tau	6	1 (16.7)	1
Gonaxis kibweziensis	32	5 (15.6)	4
Pallifera sp.	17	1 (5.9)	6
Ovachlamys fulgens	20	1 (5.0)	4
Liardetia doliolum	45	2 (4.4)	4
*Paropeas achatinaceum**	120	5 (4.2)	10
*Veronicella cubensis**	142	4 (2.8)	12
Subulina octona	60	1 (1.7)	6
Allopeas clavulinum	9	0 (0)	4
Bradybaena similaris	34	0 (0)	3
Meghimatium bilineatum	19	0 (0)	3
Opeas hannense	5	0 (0)	1

Table 3
PCA summary and loadings

Importance of components	Comp. 1	Comp. 2	Comp. 3
Standard deviation	1.321	0.891	0.619
Proportion of Variance	0.597	0.272	0.131
Cumulative Proportion	0.597	0.869	1.000
Loadings			
Vegetation (EVI)	0.612	0.443	0.655
Air temperature	-0.450	0.876	-0.173
Rainfall	0.651	0.189	-0.736

environment and *A. cantonensis* prevalence varies dramatically among host species, evident in the coefficients of Model 1 (Supplementary Table 2) and the heterogeneous slopes in Fig. 3. Despite the overall trend of increasing prevalence with higher PC1 scores, model predictions show three species, *P. martensi, P. achatinaceum*, and *V. cubensis*, exhibit only a minimal increase in prevalence in relation to higher precipitation and vegetation cover, and lower temperature, while prevalence in *E. rosea* and *L. fulica* increases considerably, with that of *L. alte* being intermediate (Fig. 3). This effect is strongest in *E. rosea*, a predator of other snails, with infection prevalence rising from zero to 0.85 along PC1 (-2.0 to 2.8).

3.4. *Infection intensity models*

The model including PC1 and species, but no interaction, best fit the data (Model 2.ct; Table 4), indicating that rainfall, temperature, and vegetation cover are important predictors of infection intensity, and that this relationship is not dependent on species. The lack of a PC1*species interaction in the best fit model suggests that while species have different intensity levels, as demonstrated by the coefficients of Model 2.ct (Supplementary Table 3), their infection intensities increase (Ct values decrease) at approximately the same rate in response to PC1, that is to higher rainfall levels and vegetation cover, and lower temperatures (Fig. 4).

4. Discussion

Our study shows that both *A. cantonensis* prevalence and infection intensity in the snail host vary greatly among species, and are determined by local environmental conditions. These results suggest that the risk factor of human *A. cantonensis* infection shifts in accordance with the environment and is dependent on the snail species.

A few species have garnered particular attention as important vectors of neuroangiostrongyliasis in various parts of the world, for example *L. fulica* in Brazil (Thiengo *et al.*, 2007), China (Song *et al.*, 2016) and American Samoa (Kliks *et al.*, 1982), species of Ampullariidae in China (Lv *et al.*, 2011; Song *et al.*, 2016) and Thailand (Eamsobhana *et al.*, 2010), and *P. martensi* in Hawaii (Hollingsworth *et al.*, 2007). While these species do indeed seem important vectors in these various regions, many other gastropod species can act as hosts (Kim *et al.*, 2014). Our study, which included both *L. fulica* and *P. martensi*, confirmed that a diversity of species act as hosts of *A. cantonensis* to greater or lesser degrees, but further demonstrated that the importance of individual species as hosts depends on their surrounding environment.

Table 4
Model criteria and Akaike scores for two candidate GLMM model sets, each including one of two independent variables: (*Angiostrongylus cantonensis* prevalence and infection intensity). Best fit models indicated in bold. All models also include two random effects: location of collection site (watershed) and time of collection (month).

Prevalence models (y = *Angiostrongylus cantonensis* presence +/-)				
Rank	Fixed effects	AICc	Δ AICc	weight
Model 1	y ~ species*zmass + Comp.1*species	544.0	0.0	0.860
Model 2	y ~ species*zmass + Comp.1	547.5	3.6	0.140
Model 3	y ~ species*zmass	559.5	15.6	<0.001
Model 4	y ~ 1	705.7	161.7	<0.001

Intensity Models [y = log(Ct)]				
Rank	Fixed effects	AICc	Δ AICc	weight
Model 2.ct	y ~ Comp.1 + species	-206.7	0.0	0.888
Model 3.ct	y ~ species	-202.3	4.4	0.098
Model 1.ct	y ~ Comp.1*species	-198.4	8.3	0.014
Model 4.ct	y ~ 1	-179.5	27.2	<0.001

The risk potential for human transmission of *A. cantonensis* from the various snail species can be evaluated in part based on their infection prevalence and intensity because 1) higher infection prevalence is reflected in a higher chance of human exposure to the infectious third-stage larvae (L3) in the snail, and 2) the severity of the disease is thought to be influenced by the number of L3 ingested (Murphy and Johnson, 2013; Ansdell *et al.*, 2020).

Prevalence and intensity were both positively correlated with PC1 (Figs. 3, 4), driven largely by monthly average rainfall levels (11.6–285 mm) and vegetation cover (0.136–0.618 EVI values), and less so by temperature (22.1 – 25.2°C). Wallace and Rosen (1969) noted an overall similar correlation of both prevalence and infection intensity with rainfall on Oahu, and Kim *et al.* (2019), based on climatic modeling in Hawaii, predicted greater suitability of habitat for *A. cantonensis* in areas of higher rainfall. Epelboin *et al.* (2016) found that numbers of cases of human neuroangiostrongyliasis on the island of Mayotte in the Indian Ocean were spatially correlated with areas of higher rainfall and temporally correlated with the rainy season. Taken together, these studies (and others) support the notion that infection prevalence and intensity, correlated positively with rainfall, contribute to human transmission rates.

But what drives these environmental patterns of prevalence and intensity? One possibility could be related to snail behavior. Gastropods are susceptible to desiccation because of their highly permeable integument and therefore greatly impacted by ambient humidity, which is strongly correlated with precipitation patterns and temperature. Thus, increased rainfall is correlated with reduced desiccation risk and is well known also to be correlated with increased movement and activity in many gastropod species (White, 1959; Nagabhushanam and Kulkarni, 1971; Bailey, 1975; Albuquerque *et al.*, 2008). Higher levels of activity increase a snail's chance of encountering rat feces, which may contain *A. cantonensis* larvae. This would potentially lead to increased infection prevalence and intensity in the snail hosts, and thus an increased chance of transmission and increased severity of the disease in humans. Increased gastropod activity also provides more opportunity for human-gastropod interactions, thereby also increasing the chance of transmission.

While both gastropod behavior conducive to the acquisition of first-stage larvae (L1, see Fig. 1) and human-gastropod interactions increase overall with rainfall, so may the availability of L1. These larvae can survive for an extended time outside their natural host environment when submerged in water and at no risk of desiccation (Richards and Merritt, 1967; Campbell and Little, 1988; Howe *et al.*, 2019). The only period in the *A. cantonensis* life-cycle during which the nematode is not within a host is during its transition from definitive to intermediate host (rat to snail), as L1 in rat feces. During this period, the L1 are at risk of desiccation. However, this risk is reduced in areas with higher rainfall and cooler temperatures, allowing longer survival in rat feces and therefore a higher abundance of infected feces in the environment and a higher concentration of viable L1 in those feces. This would again lead to higher infection prevalence and intensity in the snail communities, further increasing the likelihood of human infection as well as the severity of disease.

We found that prevalence of *A. cantonensis* in snails increased with higher PC1 values, but the specific trends in prevalence varied

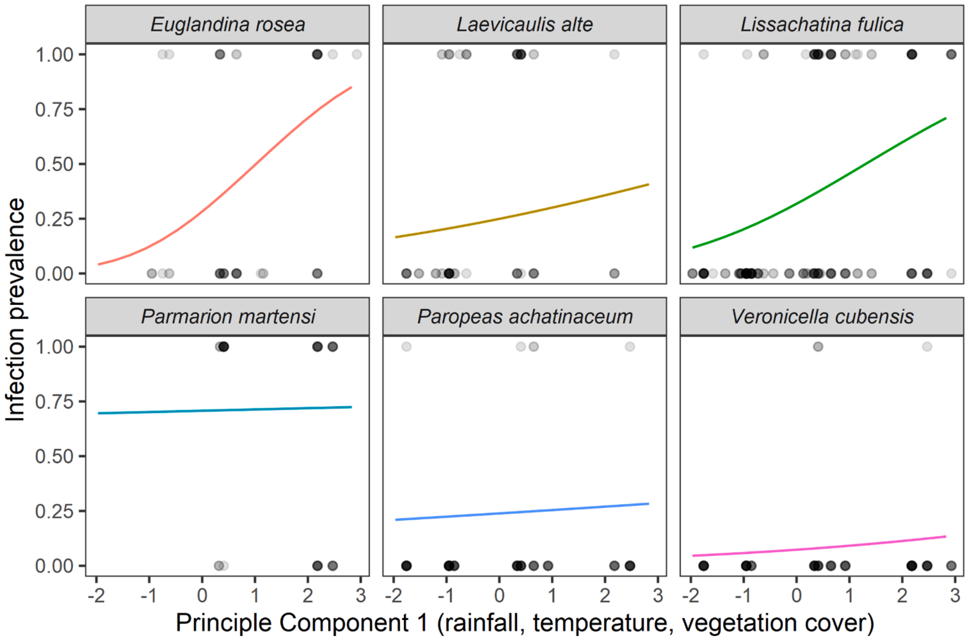

Fig. 3. Relationship of *A. cantonensis* prevalence to PC1 scores for the six gastropod species analyzed. The lines are the predicted relationships derived from the best fit model (Model 1). The dots represent individual snails collected at sites along the PC1 gradient; some dots overlapped (>1 individual at a site) and dot translucency corresponds to the number of snails collected at a site (i.e. at a particular PC1 score). Prevalence varies among gastropod species and along the PC1 gradient, driven largely by rainfall and vegetation levels, which correspond to windward and leeward sides of Oahu. Although prevalence is positively correlated with increasing rainfall and vegetation cover and decreasing temperature (positive PC1 values), the level of response is dependent on snail species.

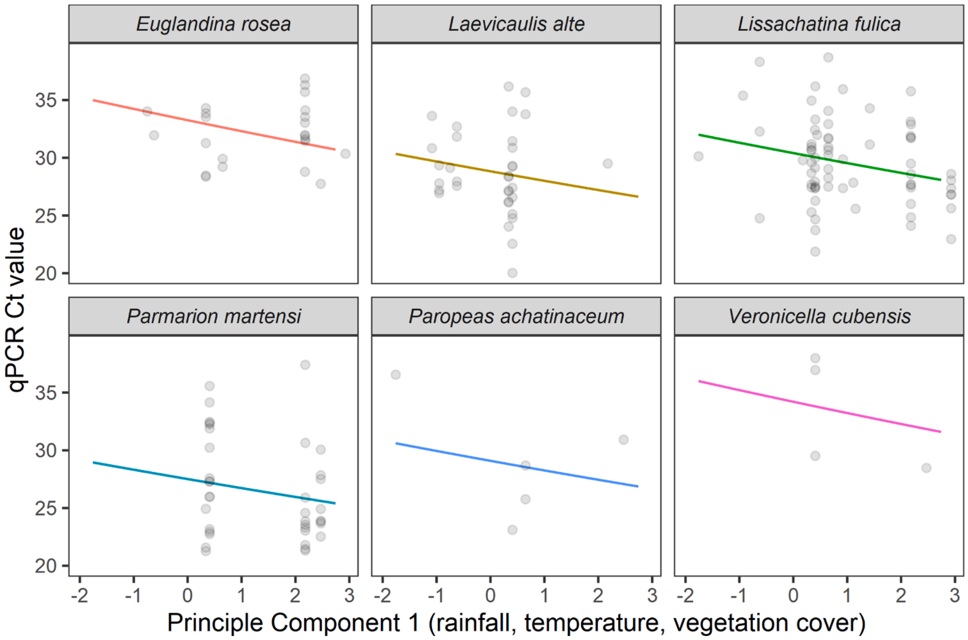

Fig. 4. Relationship of intensity of *A. cantonensis* infection to PC1 scores for the six gastropod species analyzed. The lines are the predicted relationships derived from the best fit model (Model 2.ct). The dots represent individual snails collected at sites along the PC1 gradient; some dots overlapped (>1 individual at a site) and dot translucency corresponds to the number of snails collected at a site (i.e. at a particular PC1 score). Intensity of *A. cantonensis* infection is positively correlated with rainfall and vegetation cover and decreasing temperature (positive PC1 values) and increases at the same rate in all snail species. Lower Ct values reflect higher infection intensity.

dramatically among snail species across the gradient (Fig. 3). The differences in prevalence among snail communities, the species composition of which may differ among areas differing in rainfall levels, temperature, and vegetation cover, are likely to be a result of each species' individual ecology. For example, the relative infection prevalence in *L. alte*, generally a species of drier habitats, *L. fulica*, a species with an apparently broader habitat range in Hawaii (Cowie, pers. obs.), and *E. rosea*, a predator of other snails, changed across the PC1 gradient. In drier, hotter, less vegetated areas (low PC1 values), *L. alte* exhibited a higher infection prevalence compared to *L. fulica* and *E. rosea* (Fig. 3). With increased PC values, infection prevalence of *L. alte* increased steadily until this species dropped out of the sampled communities, which may have been too wet/rainy for it. *Lissachatina fulica* infection prevalence also increased over the PC1 gradient, albeit with a steeper slope, and eventually surpassed the prevalence in *L. alte*. The greatest increase occurred in *E. rosea*, possibly resulting from the ingestion of other, possibly infected, snails. This highlights that the relative importance of snail species as significant sources of *A. cantonensis* transmission is not static across the PC1 gradient.

In contrast to the varied trends in increasing infection prevalence among species along the PC1 gradient, infection intensity increased (Ct value decreased) at roughly the same rate among species along the PC1 gradient, albeit at different levels (Fig. 4). This lack of a PC1*species interaction regarding infection intensity hints at the mechanism of larval acquisition by the snails. If increased intensity were a product of increased fecal consumption, prevalence would also increase among snail species at the same rate, which was not the case. Alternatively, if the dietary habits (amount of feces ingested) of a snail species remained the same across the environmental gradient, but the number of viable *A. cantonensis* larvae in rat feces increased, infection intensity would increase at the same rate in all species, as seen in Fig. 4. Therefore, the factor driving the increase in infection intensity across the PC1 gradient may be the number of viable larvae available, which is possibly correlated with rainfall, as discussed above.

Infection intensity, however, although increasing along the environmental gradient at the same rate across all species, differed in level among species. These differences may also be related to the snail hosts' ecology, in particular their feeding and microhabitat preferences. Different snail species feed on a wide diversity of food types (Speiser, 2001); some may readily consume rat feces, others may not eat them at all. Snail species also differ widely in their preferred microhabitats (Kemencei *et al.*, 2014); their frequency of encounter with rat feces may therefore also differ widely. Both factors could explain the overall differences in intensity among species. Furthermore, food sources may vary across the gradient, and the propensity to consume rat feces may be commensurate with the lack of availability of preferred food sources. Again, consider the comparison between *L. alte* and *L. fulica*. If *L. alte* has a greater preference for rat feces than does *L. fulica*, it may actively search for them and ingest not only more but also fresher feces than *L. fulica*. Fresh feces are more likely to contain viable, non-desiccated L1 then older feces, thereby increasing the potential for *L. alte* rather than *L. fulica* to become heavily infected in drier, hotter environments. However, in wetter, cooler, environments, *L. fulica* consumption of rat feces results in a higher chance of infection because of the extended longevity of the L1 larvae in the feces. Also, the higher activity level of *L. alte* compared to *L. fulica* in a dry and hot environment would lead to a higher frequency of encounters with fresh rat feces and therefore a higher susceptibility to *A. cantonensis* infection in such environments. This hypothetical example emphasizes the need for more information on snail host feeding and habitat preferences.

This study showed that local environment and species are both important predictors of infection prevalence and intensity in intermediate snail hosts of *A. cantonensis*, and the interactions of these factors influence prevalence. We conclude that the importance of snails in the zoonotic transmission of neuroangiostrongyliasis varies among species and is heavily influenced by the environment, specifically rainfall and vegetation cover, and to a lesser extent, temperature. This information is relevant to understanding the dynamics of human transmission of *A. cantonensis*, and as *A. cantonensis* spreads into new areas as the changing climate alters habitat availability for its intermediate hosts, this knowledge may enhance understanding of the zoonotic risk presented by various host species and facilitate targeted preventative action.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author statement

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Supplementary materials

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