One Health 17 (2023) 100658

Contents lists available at ScienceDirect

One Health

journal homepage: www.elsevier.com/locate/onehlt

Stressed snails release *Angiostrongylus cantonensis* (rat lungworm) larvae in their slime

Randi L. Rollins [a,b,*], Matthew C.I. Medeiros [c], Robert H. Cowie [a]

[a] Pacific Biosciences Research Center, University of Hawai'i at Mānoa, 3050 Maile Way, Gilmore 408, Honolulu, HI 96822, USA
[b] School of Life Sciences, University of Hawai'i at Mānoa, 3190 Maile Way, St. John 101, Honolulu, HI 96822, USA
[c] Pacific Biosciences Research Center, University of Hawai'i at Mānoa, 1800 East West Road, LSB 316, Honolulu, HI 96822, USA

ARTICLE INFO

Keywords:
Parasite release
Zoonotic disease
Snail borne disease
Host stress
Transmission pathways

ABSTRACT

This study investigated the influence of stress on release of *Angiostrongylus cantonensis* larvae from a snail host, *Parmarion martensi*. We subjected 140 infected, wild-caught *P. martensi* to three stress-inducing treatments (heat, molluscicide, physical disturbance) and an unstressed control treatment for 24 h, after which larval presence and abundance in the slime were quantified by qPCR targeting the ITS1 region of the parasite's DNA, and compared among treatments. The significance of stress and host infection load on larval release was determined by generalized linear mixed models and permutation tests. The results indicated that stress significantly increased the probability of larval presence in slime and the number of larvae released, and highly infected snails were also more likely to release larvae. Among stressed snails, 13.3% released larvae into slime, the number of larvae present in the slime ranging from 45.5 to 4216. Unstressed controls released no larvae. This study offers a partial explanation for conflicting results from prior studies regarding *A. cantonensis* presence in snail slime and sheds light on the broader One Health implications. Stress-induced larval release highlights the potential role of slime as a medium for pathogen transmission to accidental, paratenic, definitive and other intermediate hosts. These findings emphasize the importance of considering stress-mediated interactions in host-parasite systems and their implications for zoonotic disease emergence. As stressors continue to escalate because of anthropogenic activities and climate change, understanding the role of stress in pathogen shedding and transmission becomes increasingly important for safeguarding human and wildlife health within the One Health framework.

1. Introduction

Host-parasite relationships are dynamic and modulated by their surrounding environments [1]. Shifts in these relationships can be consequential for the life-cycle of the parasite and its host. In zoonotic parasite-host systems, these shifts also have implications for the health and well-being of wildlife, domestic animals, and humans [2]. The host environment is crucial in determining a parasite's success, as it directly impacts parasite survival and reproduction. For instance, favorable conditions for the host may mitigate the negative impacts of parasitism [3]. Alternatively, stressors applied to the host can amplify the negative consequences of parasitism [4,5], altering the interplay between host and parasite and changing the dynamics of parasite transmission.

Environmental stressors such as temperature changes, pollutants, or physical disturbance can impact the host, the parasite, or both, in diverse ways [6–8]. Stress can alter host behavior, which may influence the likelihood of encountering (and subsequent infection with) a parasite [9]. When encounters occur, stress-induced immune suppression of the host can result in higher host parasite abundance and increased parasite egg release [10,11], which can increase intensity of infection in subsequent hosts and infection prevalence at the population level, and can also increase host morbidity and mortality [12]. Fecundity, of both the host and the parasite, can be influenced by, for example, drought-induced stress [13,14]; parasite development can be stunted by nutritional and crowding stress [15]; and both host and parasite growth may be impacted by temperature stress [16]. Among zoonotic parasites, increased pathogen prevalence and intensity of infection in hosts, which can be attributed to these stress mediated functions, are often correlated with an increase in human infection [17] and the possibility of spillover events [18].

Specifically, regarding snail-borne parasitic diseases (SBPD) host stress caused for instance by high temperatures and drought can increase

* Corresponding author at: Pacific Biosciences Research Center, University of Hawai'i at Mānoa, 3050 Maile Way, Gilmore 408, Honolulu, HI 96822, USA.
E-mail address: rrollins@hawaii.edu (R.L. Rollins).

https://doi.org/10.1016/j.onehlt.2023.100658
Received 3 October 2023; Accepted 25 November 2023
Available online 27 November 2023

release of infectious stage parasites from the snails [13,19]. Transmission, in particular of nematodes that cause SBPD, often involves ingestion of an infected snail [20]. In these systems, shedding of infectious parasite larvae into the environment, for instance in snail slime trails or into water resources, may serve as additional human transmission routes. Therefore, understanding if and how many larvae are shed from infected snails, and under what conditions, including stress, is an important component of preventing human illnesses caused by these snail-borne parasites.

We address this issue of stress-mediated pathogen shedding and its potential as an alternative transmission pathway in the context of an emerging human health issue caused by a parasitic nematode, *Angiostrongylus cantonensis* (the rat lungworm), and one of its snail host species, *Parmarion martensi*. *Angiostrongylus cantonensis* is the causative agent of neuroangiostrongyliasis and arguably the leading cause of eosinophilic meningitis globally [21]. Symptoms of infection in humans are diverse and occasionally fatal [22]. *Angiostrongylus cantonensis* completes its life cycle in snails (intermediate hosts) and rats (definitive hosts) (Fig. 1). Ingesting an infected intermediate snail host, intentionally or accidentally, is a primary pathway of transmission to humans [23] and other accidental hosts, including domestic dogs [24]. However, presence of infectious *A. cantonensis* larvae in snail slime has been proposed as another potential route of infection. Several papers have addressed this issue, but the combined results are largely inconclusive and snail slime has been considered not to be a major transmission pathway and therefore not a major human health concern [25,26]. However, large numbers of *A. cantonensis* larvae have occasionally been observed in the slime of wild captured *Parmarion martensi* (Rollins, unpublished), suggesting that this may not always be the case, and that certain factors/circumstances may induce larval release, but have not been investigated.

We hypothesized that host stress influences the release of infectious *A. cantonensis* larvae from snails. Specifically, we predicted that 1) *A. cantonensis* larvae are present in the slime of *Parmarion martensi*, and if so, 2) stressed snails release more larvae than non-stressed snails, and 3) heavily infected snails release more larvae than less heavily infected snails. We tested these predictions by subjecting wild caught, naturally infected snails to a variety of stressors (heat, molluscicide, and physical disturbance) and quantifying their larval output by qPCR, and then comparing the larval output of stressed snails and non-stressed (control) snails. In addition to answering these key questions, gaining an understanding of the effect of stress on this specific host-parasite relationship is important for public health, especially as the increasing frequency and intensity of environmental stressors are features of anthropogenic global change [27,28]. This study aligns with the holistic concept of One Health as an example of how interactions between humans, animals, and the environment can alter the risk of disease.

2. Methods

2.1. Snail collection and maintenance

Adult size (> 600 mg) *Parmarion martensi* (196 individuals) were collected on the island of Oʻahu, Hawaiʻi, and brought to the laboratory. The snails were placed in individual petri dishes (90 mm diameter) lined with damp paper towels, fed dry dog food (Wholehearted) and romaine lettuce ad libitum and kept at their optimal temperature, 21 °C [29], with a 12 h day/night light cycle for no longer than 5 days before exposure to stress treatments.

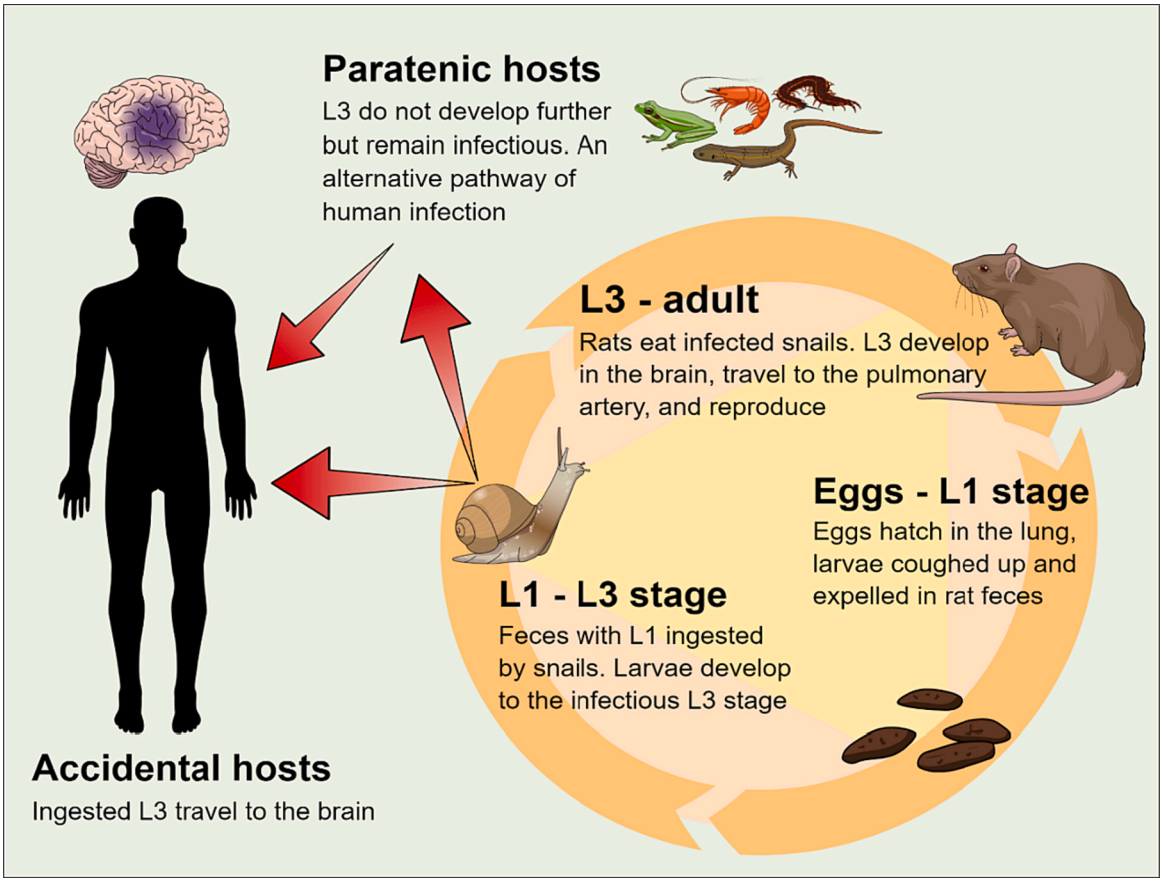

Fig. 1. *Angiostrongylus cantonensis* life cycle in intermediate snail hosts and definitive rat hosts. Humans and other mammals and birds can become accidental hosts, and a variety of other kinds of animals can serve as paratenic hosts. Graphic from Cowie et al. 2022.

2.2. Release of larvae in snail slime

To determine if stress induces release of L3 larvae into snail slime, the snails were randomly assigned to one of three stress-inducing experimental treatments (heat, molluscicide, disturbance) and a control (49 snails each). Treatment level (temperature, amount of bait, and physical disturbance) had been determined in preliminary experiments in which a treatment was applied in increasing increments until the lethal stress level was reached (see Supplemental Information), allowing all stressors to be standardized to a similar sub-lethal level.

All snails were weighed, put into sterile 50 mL tubes, which were also weighed, immediately before being placed into incubation chambers for the 24 h experimental period. All snails (including snails in the control group) were handled in a similar manner, except for their independent treatments. Snails treated with molluscicide ingested 30.0–40.0 mg of sodium ferric EDTA bait (Corry's Slug & Snail Killer) ~1 h prior to being placed into 50 mL tubes. Physically disturbed snails were subjected to constant shaking in a tube rack (Benchmark Scientific Roto-Mini Plus Tube Rotator R2020) set to 25 rpm on mixing mode 'pause' during the 24 h incubation period. Heat-treated snails were incubated at 30.5 °C. Snails in the molluscicide, physical disturbance, and control groups were incubated at 21 °C. The 24 h experimental incubation period began with 12 h day, followed by 12 h night.

After 24 h, the tubes containing the snails were removed from the incubation chambers and weighed to detect any changes in weight over the treatment period. If feces were present in the tube, they were removed with sterile forceps and weighed, so as not to include fecal weight in the weight of the slime samples. Next, snails were removed from the tubes, and the tubes were re-weighed to determine a) the weight of the snail post-treatment, and b) the amount of slime (in mg) excreted by the snail during the 24 h experimental period.

The snails were tested in six batches (listed as 'Run' in the data file included with the R code materials) to allow timely handling and processing of each sample.

2.3. Molecular detection of larvae

2.3.1. Digestion of snail tissue, slime and rat feces

To determine the infection status and infection load (number of larvae present in a snail), after the experiment each snail was euthanized, minced, and digested in 15 mL of Longmire's lysis buffer (0.1 M Tris, 0.1 M EDTA, 0.01 M NaCl, 0.5% sodium dodecyl sulfate) per gram of snail tissue, and Proteinase K (150 μg), then incubated at 56 °C overnight.

To detect presence and number of larvae in the slime, Longmire's lysis buffer and Proteinase K were added to the 50 mL tubes containing slime from the experimental period (same ratio as listed above for snail tissue) and incubated at 56 °C for 1–2 h. Fecal samples were prepared the same way as the slime samples.

2.3.2. DNA extraction and qPCR

DNA was extracted from 200 μL aliquots of each digested snail, slime and fecal sample using the Qiagen Blood & Tissue spin column kit, following the manufacturer's protocol. A Taqman qPCR assay (ACANITS1, Life Technologies assay ID #A139RIC) targeting the internal transcribed spacer 1 (ITS1) sequence of *A. cantonensis* rDNA [30] was conducted, following the protocol of Rollins et al. [31]. All qPCR samples were tested in duplicate and run with positive and negative controls. Samples with Ct amplification curves that crossed the 0.2 fluorescent unit threshold were considered positive for *A. cantonensis* infection. Data from non-infected snails were omitted.

2.4. Standard curve

A standard curve was created to convert the resulting qPCR cycle threshold (Ct) values into numbers representative of the number of *A. cantonensis* larvae present in the snails or their slime. To create the standard curve, DNA was extracted from ~12,860 *A. cantonensis* L3 larvae, freshly obtained from wild caught *Parmarion martensi* following the larval isolation method of Rollins et al. [32]. The larvae were added to 200 μL of Longmire's lysis buffer and processed using a Qiagen Blood & Tissue spin column kit following the manufacturer's protocol. Twelve dilutions of the DNA isolate (Supplemental Table 1) provided a range of larval concentrations for qPCR, performed following the same qPCR protocol used for tissue and slime, above. The resulting Ct values of each dilution were plotted against their respective number of larvae, which were natural log transformed. The best fit line served as a standard curve to convert the Ct values from snail tissue and slime samples into numbers of larvae, and to determine the total parasite load in each snail or slime sample by extrapolation to the total weight of the snail or slime. Total parasite load, rather than Ct values, was included in analyses investigating the effect of host infection intensity.

We standardized the dilution of each sample in buffer solution prior to DNA extraction at 15 mL buffer per 1 g sample, so each of the 200 μL aliquots used for DNA extraction contained 0.01333 g of sample material. Therefore, *sample weight (g) / 0.01333* = *x*. Then, *x* * exp*(number of larvae)* = *total number of larvae present in a snail or slime sample*.

2.5. Statistical analysis

All statistical analyses were conducted in Posit, formerly known as RStudio [33]. Our principle statistical objectives were to estimate the effects of host stress and host infection (total parasite load) on both presence and number of *A. cantonensis* larvae in host snail slime. The following variables were measured and included in the analyses: larval presence in snail slime (binary variable), number of larvae in snail slime (positive whole-number count variable), total snail larval load (count variable), treatment group (categorical variable with 4 levels), and weight of slime produced (continuous variable). Preliminary analysis found that snail size and the weight of slime produced were collinear, so snail size was not included in the analyses and any potentially confounding effects of snail size had been controlled during the DNA extraction protocol.

2.5.1. Generalized linear mixed models

In addition to their respective response and predictor variables, all models included slime weight as a covariate, and experimental batch (i.e., block) as a random effect. The slime weight variable was square-root transformed, and both snail larval load and slime weight variables were rescaled by z-score transformation (subtract the mean and divide by the standard deviation) using the R function 'scale' to improve model fit and convergence.

To test the effects of the various stress treatments (including the control) and snail larval load, we ran four model comparisons (marginal likelihood ratio tests) using the R function 'anova'. Here, we compared a model including the variable of interest to a model without that variable (the two models considered a 'model set'). If the model containing the variable of interest was a significantly better fit, then that variable was considered significant. Eight generalized linear mixed models (glmms) were built using the R package glmmTMB (v1.1.7 [34]).

Model set 1: Both models assumed a binomial distribution and were fit to the data with larval presence in slime as the response variable and snail larval load as an additional predictor variable. Treatment was the variable of interest.

Model set 2: Both models assumed a zero-inflated quasi-Poisson distribution of the data ("nbinom1" implemented in the R package, glmmTMB) with the total number of larvae in the slime samples as the response variable and snail larval load as an additional predictor variable. Treatment was the variable of interest.

Model sets 3 and 4: Two additional model sets were built, each identical to the models outlined above, except snail larval load was the variable of interest. Therefore, we removed the snail larval load variable instead of the treatment variable in the comparison models.

2.5.2. Permutation tests for stress

Because of the low number of snails that released larvae in slime, the complete and quasi separation of the data, and the non-parametric distribution of the infection data among treatments, we combined snails from the three stress treatments (heat, molluscicide, disturbance) into a single group, and compared them to non-stressed snails using permutation tests. This allowed us to determine the effect of stress in general, as opposed to specific kinds of stress.

We ran two permutation tests using the R package mosaic (v1.8.3 [35]) to determine 1) whether the effect of stress on the release of larvae from infected snails was significant, and 2) whether stress induces a significantly higher proportion of the larvae within an infected snail to be released into the snail's slime. Permutation test 1 included stress (yes/no) and larval presence in slime (yes/no) as variables, with the null hypothesis: *stress does not increase the presence of larvae in slime*. Permutation test 2 included stress and the proportion of larvae within an infected snail that are released into the slime as variables, with the null hypothesis: *stress does not increase the proportion of larvae released in slime*. The proportion of larvae released into slime was calculated by dividing the total number of larvae in a slime sample by the combined total number of larvae inside that snail and larvae released by that snail: *slime larval load / (snail larval load + slime larval load)*. Each test was permuted 100,000 times.

3. Results

3.1. Angiostrongylus cantonensis infection of snails, slime, and rat feces

Of the 196 *Parmarion martensi* individuals exposed to experimental stress treatments and controls, ten died during treatment, leaving 186 that completed the experimental period (Table 1). Of these 186, 140 tested positive for *A. cantonensis* infection (75.3%). The 46 non-infected snails and their data were omitted from further consideration. *Angiostrongylus cantonensis* larvae were detected in the slime of 14 of the 105 infected, stressed snails (13.3%) and none of the 35 infected, non-stressed snails (Table 1).

The Ct values of the 140 infected snails ranged from 38.70 to 20.48. Estimated numbers of larvae within a snail, extrapolated from snail weight and snail Ct values compared to the standard curve (Supplemental Table 1, Supplemental Fig. 1), ranged from 41.5 to 2,011,820 larvae (see data file in R code materials). The Ct values of the 14 slime samples ranged from 34.22 to 25.85, and the estimated number of larvae in the slime produced during the 24 h experimental period ranged from 45.5 to 4216 per snail (Table 2).

During the experiment 78 snails produced feces. Of these 78, feces from 11 snails tested positive for *A. cantonensis* infection (14.1%). Larval release in slime was not correlated with larval release in feces, as only one of the snails that released larvae in its slime also released larvae in its feces. Both stressed and unstressed snails released larvae in their feces; one in the heat treatment, one in the molluscicide treatment, four in the disturbance treatment, and five in the control group. Further analyses were not conducted because of the low number of positive fecal samples.

Table 1
Number of snails tested, number infected, and number (percentage) of infected snails in each treatment that released larvae in their slime.

Treatment	Snails tested*	Snails infected	Snails that released larvae
Molluscicide (21 °C)	48	36	6 (16.7%)
Heat (30.5 °C)	44	35	2 (5.7%)
Disturbance (21 °C)	47	34	6 (17.7%)
Control (21 °C)	47	35	0 (0.0%)

* The number of wild-collected snails tested is the 49 snails assigned to each of the four groups minus the snails that died during the experimental period.

Table 2
The amount of slime released from each of the 14 snails that released larvae into their slime in the three stress treatments, with qPCR Ct values and numbers of larvae extrapolated from those values.

Treatment	Slime weight (mg)	Slime Ct value	Slime larval load
Disturbance	106.4	25.85	4216
Molluscicide	189.6	28.99	857
Heat	215.3	29.85	626
Disturbance	98.8	30.20	607
Molluscicide	216	30.88	573
Disturbance	388.5	31.00	556
Molluscicide	146	31.06	501
Disturbance	682.6	31.65	299
Molluscicide	710.9	31.67	213
Molluscicide	314.8	32.15	206
Molluscicide	970.1	32.41	182
Disturbance	210.6	33.71	66
Disturbance	149.9	33.78	60
Heat	240.6	34.22	45

3.2. Model results

The GLMM fits indicate the significance of treatment on presence and number of larvae in snail slime. Among treatment groups (including the control) we found significant variation in the number of larvae released into the slime (model set 2, $p = 0.008$), and marginally nonsignificant variation in the prevalence of larvae found in slime (model set 1, $p = 0.054$) (Table 3). More heavily infected snails were more likely than less heavily infected snails to have larvae present in their slime (model set 3, $p = 0.001$) and to have more larvae in their slime (model set 4, $p = 0.025$). The relationship of snail larval load and the number of larvae excreted in snail slime is shown in Fig. 2. However, model coefficients were uninterpretable because of complete and quasi separation in the data, so modelled effect sizes were not estimated.

3.3. Permutation results for stress

The permutation test results highlighted the significance of stress in both the presence and ratio of larvae released in snail slime: stressed snails were more likely to release larvae into their slime (permutation test 1, $p = 0.013$) and stressed snails had more larvae in their slime (permutation test 2, $p = 0.015$). Comparison with their null distributions indicates that if stress were unrelated to larval presence or to number of larvae in slime, then our data would only occur 1.4% of the time, for

Table 3
Models and model comparison results.

Model	Response and predictor variables	df	AIC	loglik	Pr (>Chisq)
Models testing stress treatment groups (treatment)					
1a	larvae in slime ~ snail load + slime weight + **treatment**	4	83.976	−37.988	
1b	larvae in slime ~ snail load + slime weight	7	82.331	−34.165	0.054
2a	slime load ~ snail load + slime weight + **treatment**	6	308.09	−148.05	
2b	slime load ~ snail load + slime weight	9	302.21	−142.1	0.008
Models testing snail infection load (snail load)					
3a	larvae in slime ~ treatment + slime weight + **snail load**	6	91.439	−39.719	
3b	larvae in slime ~ treatment + slime weight	7	82.331	−34.165	0.001
4a	slime load ~ treatment + slime weight + **snail load**	8	328.78	−144.62	
4b	slime load ~ treatment + slime weight	9	328.68	−142.1	0.025

The variable of interest in each model set is bolded.

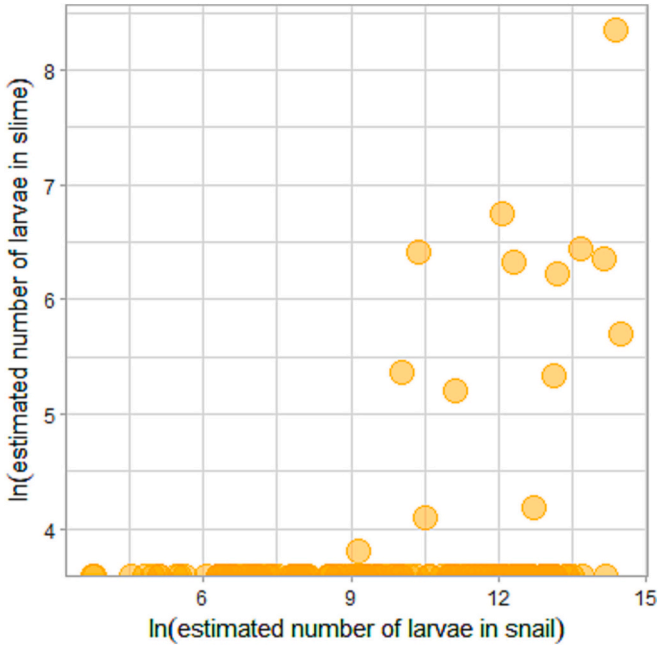

Fig. 2. Relationship of snail infection load and number of larvae released into snail slime. Each infected snail is represented by an orange circle. Snail infection load varies across the x-axis, but larvae were only released into the slime of 14 snails, all of which have a larval load greater than ln(9).

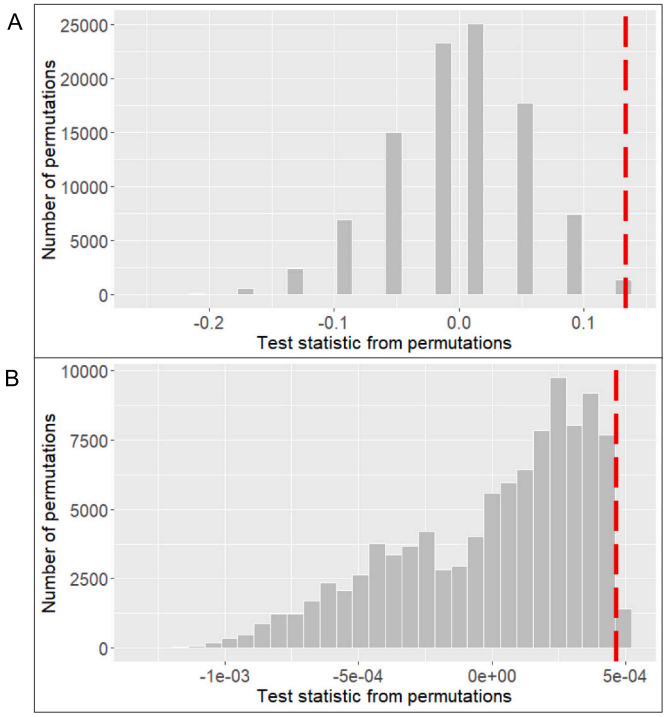

Fig. 3. Results of permutation test 1 (A) and 2 (B). The initial test statistic is overlayed (red dashed line) on the null distribution created from 100,000 iterations for each test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

both larval presence in slime and proportion of larvae released from infected snails into slime (p = ~0.01 in both cases), indicating a clear effect of stress on the release of larvae into snail slime. Therefore, we reject the null hypotheses that 1) stress does not increase the presence of larvae in slime, and 2) stress does not increase the proportion of larvae released in slime. The initial test statistics overlain on the null distributions are shown in Fig. 3.

4. Discussion

We found that *Angiostrongylus cantonensis* larvae can be excreted into the slime of snails, and stress has a distinct and significant effect on this larval release. While 13.3% of stressed snails released larvae into their slime, no larvae were detected in the slime of non-stressed snails. This finding is supported by highly significant permutation tests results and demonstrates the influence of stress on the release of parasites from their hosts, as well as the potential for increased zoonotic transmission under stressful conditions. Specifically, this study highlights the role of stress in altering the quiescent period of *A. cantonensis* L3 in intermediate hosts, leading to their release into the environment in slime (as opposed to into a new host once an infected snail is ingested) and shows that heavily infected snails tend to release more larvae than less heavily infected snails. The permutation results and statistical models both found that larval release from the host is related to stress.

The number of larvae detected in the snail slime was not trivial. We estimated between 45.5 and 4216 larvae in the slime, which was not correlated with the amount of slime (0.24 g and 0.11 g, respectively) (Table 2). However, further studies are needed to determine the risk of this slime as an alternative pathway for transmission to accidental hosts (e.g. humans, non-human primates, dogs, horses, various marsupials, bats, certain birds [e.g. [24,36–38]), paratenic hosts (frogs, lizards, flatworms etc. [39]), or definitive rat hosts [e.g. [40,41]). Although the number of larvae required to cause illness in humans is not known, experimental infection of one rhesus monkey (*Macaca mulatta*; as *M.* "*rhesus*") and 36 Taiwan monkeys (*Macaca cyclopsis*) resulted in death after ingestion of several hundred or 5000–10,000 infectious third stage *A. cantonensis* larvae, respectively [42,43]. The number of larvae we detected in the slime suggests that slime could occasionally be a viable transmission pathway and a cause of human illness but how many constitute an infectious dose in humans is difficult to determine.

This is the first study to identify the relationship between stress and *A. cantonensis* presence in snail slime. When stress is considered in the context of this host-parasite relationship, it offers a partial explanation regarding the conflicting results of previous studies. While some studies observed no larvae, other studies found larvae in the slime (Supplemental Table 2), albeit mostly in small numbers, which differs from the higher numbers of larvae found in snail slime in this study. Several other factors could also contribute to the conflicting evidence in prior studies, leading to a conclusion that slime is of little significance in transmission to humans [23,26], including the following.

Infection load of host snail: This varied greatly in one of the studies of naturally infected snails [44], and in the other cases was either not determined or not indicated, which may have contributed to the inconclusive results. As our results demonstrate, infection load of the host is an important factor in larval release.

Sample size: We found larvae in the slime of 13% of stressed snails, but none in the non-stressed snails. A small effect size such as this requires a sample size large enough to detect the effect, which was not the case in those prior studies in which sample size was indicated (Supplemental Table 2).

Period of observation, and length of time the larvae are present in the snail: Observations of slime varied greatly among studies, from three times a day for two months [45], to a single 10-min period [46]. Two studies infected snails experimentally. One of these observed snails for 2 months after L1 developed into L3 (21 days post-infection) [45]. The other did not state the length of time between infection and observation of slime [47].

Different snail species were investigated, and often only one species in a study: The differences in integument thickness among species may play a role in larval release [48]. Although our study sought to address the uncertainty of *A. cantonensis* release in slime by incorporating a large

sample size and evaluating the effects of stress, we only tested a single species, and determined that stress is important in this species. Although stress may have a similar effect on other host snail species, further studies are needed to address this.

Therefore, stress in the intermediate snail hosts may influence human *A. cantonensis* infection rates. In the *A. cantonensis* life cycle, larvae are transferred from the intermediate snail host into the definitive rat host when a rat ingests an infected snail (Fig. 1). Prior studies have shown that *A. cantonensis* L3 shed from snails were infectious to rats [45,49,50]. Infectivity of shed larvae has also been confirmed in the congeneric *A. vasorum* [51]. However, it is unclear if *A. cantonensis* detects host stress/death and leaves the host, or if the host itself expels the parasite when stressed. *Caenorhabditis elegans* can detect mechanical and temperature stress [52], but further studies are needed to determine the stress-detecting ability of *A. cantonensis*. Regardless, demonstrated infectivity of *A. cantonensis* larvae shed in snail slime or into the environment, coupled with our results showing larvae present in slime of 13% of stressed *P. martensi*, indicate the possibility of slime as a further transmission pathway (in addition to the ingestion of an infected host), and a potential medium for encountering the parasite. Of note, *A. cantonensis* larvae were also found in the feces of some of the snails, which may also serve as a viable transmission pathway.

The clear impact of stress in this study demonstrates its importance in host-parasite systems, emphasizing the broader One Health perspective and the integral connection between animal, human, and environmental health. This study underscores the role of host stress in the transmission of zoonotic disease, which is particularly pertinent given that approximately 60% of infectious diseases that emerged between 1940 and 2004 have been attributed to zoonoses [53]. Moreover, 58% of all known human pathogens are zoonotic [54], highlighting the significant overlap between animal and human health. Meanwhile, zoonoses are predicted to increase with continued exploitation of wildlife, unsustainable farming practices and land use [55], and climate change [56,57], which will reshuffle environments and alter host stressor regimes. For example, it has been suggested that COVID-19 emerged in the human population in close association with a wet market in Wuhan, China, an environment ripe for zoonotic spillover [58]. In this environment, animals are under constant stress while being kept in small cages (in which they often travel long distances), crowded and commingled in unnatural species combinations, and surrounded by a noisy human environment. Stress can increase saliva, feces and urine production, all of which can carry pathogens shed from the host and facilitate zoonotic transmission [59,60]. The dangers of such environments are compounded by the stress imposed on animals infected with zoonotic pathogens. Furthermore, in a time of rapid global change, animal hosts are increasingly subject to environmentally and anthropogenically induced stress that may surpass their tolerance thresholds [61], further increasing the risk of pathogen spillover into wildlife, domestic animals, and human communities.

Funding

This work was supported by the George F. Straub Trust of the Hawai'i Community Foundation (grant number 20CON-102164) and by a University of Hawai'i Graduate Student Organization Research Grant.

CRediT authorship contribution statement

Randi L. Rollins: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing – original draft. **Matthew C.I. Medeiros:** Conceptualization, Formal analysis, Writing – review & editing. **Robert H. Cowie:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

None.

Data availability

Data and associated R code are available as files in the Supplementary data.

Acknowledgments

We thank the Hawai'i Nature Center and the Lyon Arboretum for access to lands for collection of many *Parmarion martensi* used for this study. Daniel Rubinoff provided an environmental chamber from his lab for the duration of the study. Nicole Yoneishi processed the qPCR samples in the University of Hawai'i Microbial Genomics and Analytical Laboratory Core facility, which is supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health (grant number P20GM125508). This is publication number 11752 of the University of Hawai'i School of Ocean and Earth Science and Technology, and a publication of the Manoa Angiostrongylus Research Group.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2023.100658.

References

[1] J. Wolinska, K.C. King, Environment can alter selection in host–parasite interactions, Trends Parasitol. 25 (5) (2009) 236–244.
[2] J. Mishra, P. Mishra, N.K. Arora, Linkages between environmental issues and zoonotic diseases: with reference to COVID-19 pandemic, Environ. Sustain. 4 (3) (2021) 455–467.
[3] R.M. Overstreet, J.M. Lotz, Host–symbiont relationships: understanding the change from guest to pest, in: C.J. Hurst (Ed.), The Rasputin effect: when commensals and symbionts become parasitic. Advances in Environmental Microbiology 3, 2016, pp. 27–64.
[4] K.D. Lafferty, A.M. Kuris, How environmental stress affects the impacts of parasites, Limnol. Oceanogr. 44 (1999) 925–931.
[5] I. Barber, B.W. Berkhout, Z. Ismail, Thermal change and the dynamics of multi-host parasite life cycles in aquatic ecosystems, Integr. Comp. Biol. 56 (4) (2016) 561–572.
[6] M.B. Thomas, S. Blanford, Thermal biology in insect-parasite interactions, Trends Ecol. Evol. 18 (7) (2003) 344–350.
[7] P.M. Beldomenico, M. Begon, Stress-host-parasite interactions: a vicious triangle? Rev. FAVE – Sección Cienc Veterinarias 14 (1–2) (2016) 6–19.
[8] J. Cable, I. Barber, B. Boag, A.R. Ellison, E.R. Morgan, K. Murray, E.L. Pascoe, S. M. Sait, A.J. Wilson, M. Booth, Global change, parasite transmission and disease control: lessons from ecology, Philos. Trans. R. Soc. B 372 (2017) 20160088.
[9] D.G. Barron, S.S. Gervasi, J.N. Pruit, L.B. Martin, Behavioral competence: how host behaviors can interact to influence parasite transmission risk, Curr. Opin. Behav. Sci. 6 (2015) 35–40.
[10] L. Corlatti, S. Béthaz, A. von Hardenberg, B. Bassano, R. Palme, S. Lovari, Hormones, parasites and male mating tactics in Alpine chamois: identifying the mechanisms of life history trade-offs, Anim. Behav. 84 (4) (2012) 1061–1070.
[11] C. Romeo, L.A. Wauters, F. Santicchia, B. Dantzer, R. Palme, A. Martinoli, N. Ferrari, Complex relationships between physiological stress and endoparasite infections in natural populations, Curr. Zool. 66 (5) (2020) 449–457.
[12] Z. Luo, L.F. Liu, Y.N. Jiang, L.P. Tang, W. Li, S.H. Ouyang, L.F. Tu, Y.P. Wu, H. B. Gong, C.Y. Yan, S. Jiang, Y.H. Lu, T. Liu, Z. Jiang, H. Kurihara, Y. Yu, X.S. Yao, Y.F. Li, R.R. He, Novel insights into stress-induced susceptibility to influenza: corticosterone impacts interferon-β responses by Mfn2-mediated ubiquitin degradation of MAVS, Signal Transduct. Target. Ther. 5 (2020) 202.
[13] K. O'Dwyer, R. Poulin, Taken to the limit – is desiccation stress causing precocious encystment of trematode parasites in snails? Parasitol. Int. 64 (6) (2015) 632–637.
[14] A.M. Gleishsner, J.A. Cleveland, D.J. Minchella, One stimulus—two responses: host and parasite life-history variation in response to environmental stress, Evolution 70 (11) (2016) 2640–2646.
[15] T. Habtewold, A.A. Sharma, C.A.S. Wyer, E.K.G. Masters, N. Windbichler, G. K. Christophides, *Plasmodium* oocysts respond with dormancy to crowding and nutritional stress, Sci. Rep. 11 (2021) 3090.
[16] F. Franke, S.A.O. Armitage, M.A.M. Kutzer, J. Kurtz, J.P. Scharsack, Environmental temperature variation influences fitness trade-offs and tolerance in a fish-tapeworm association, Parasit. Vectors 10 (2017) 252.

[17] B.J. Johnson, M.V.K. Sukhdeo, Drought-induced amplification of local and regional West Nile virus infection rates in New Jersey, J. Med. Entomol. 50 (1) (2013) 195–204.

[18] R.K. Plowright, C.R. Parrish, H. McCallum, P.J. Hudson, A.I. Ko, A.L. Graham, J. O. Lloyd-Smith, Pathways to zoonotic spillover, Nat. Rev. Microbiol. 15 (2017) 502–510.

[19] S.H. Paull, T.R. Raffel, B.E. LaFonte, P.T.J. Johnson, How temperature shifts affect parasite production: testing the roles of thermal stress and acclimation, Funct. Ecol. 29 (7) (2015) 941–950.

[20] B.S. Mathison, B.S. Pritt, A systematic overview of zoonotic helminth infections in North America, Lab. Med. 49 (4) (2018) e61–e93.

[21] R.H. Cowie, V. Ansdell, C. Panosian Dunavan, R.L. Rollins, Neuroangiostrongyliasis: global spread of an emerging tropical disease, Am. J. Trop. Med. Hyg. 107 (6) (2022) 1166–1172.

[22] V. Ansdell, K.J. Kramer, J.K. McMillan, W.L. Gosnell, G.S. Murphy, B.C. Meyer, E. U. Blalock, J. Yates, L. Lteif, O.A. Smith, M. Melish, Guidelines for the diagnosis and treatment of neuroangiostrongyliasis: updated recommendations, Parasitology 148 (2) (2021) 227–233.

[23] R.H. Cowie, Pathways for transmission of angiostrongyliasis and the risk of disease associated with them, Hawai'i J. Med. Public Health 72 (6 Suppl 2) (2013) 70–74.

[24] J. Odani, E. Sox, W. Coleman, R. Jha, R. Malik, First documented cases of canine neuroangiostrongyliasis due to *Angiostrongylus cantonensis* in Hawaii, J. Am. Anim. Hosp. Assoc. 57 (1) (2021) 42–46.

[25] R.H. Cowie, Biology, systematics, life cycle, and distribution *of Angiostrongylus cantonensis*, the cause of rat lungworm disease, Hawai'i. Med. Public Health 72 (6 Suppl 2) (2013) 6–9.

[26] K.J. Kramer, J. Posner, W.L. Gosnell, Role of gastropod mucus in the transmission of *Angiostrongylus cantonensis*, a potentially serious neurological infection, ACS Chem. Neurosci. 9 (4) (2018) 629–632.

[27] N. Lacetera, Impact of climate change on animal health and welfare, Anim. Front. 9 (1) (2019) 26–31.

[28] K.L. Ebi, J. Vanos, J.W. Baldwin, J.E. Bell, D.M. Hondula, N.A. Errett, K. Hayes, C. E. Reid, S. Saha, J. Spector, P. Berry, Extreme weather and climate change: population health and health system implications, Annu. Rev. Public Health 42 (2021) 293–315.

[29] L.J. Hamilton, Y. Tagami, L. Kaluna, J. Jacob, S.I. Jarvi, P. Follett, Demographics of the semi-slug *Parmarion martensi*, an intermediate host for *Angiostrongylus cantonensis* in Hawai'i, during laboratory rearing, Parasitology 148 (2) (2021) 153–158.

[30] Y. Qvarnstrom, A.C.A. da Silva, J.L. Teem, R. Hollingsworth, H. Bishop, C. Graeff-Teixeira, A.J. da Silva, Improved molecular detection of *Angiostrongylus cantonensis* in mollusks and other environmental samples with a species-specific internal transcribed spacer 1-based TaqMan assay, Appl. Environ. Microbiol. 76 (15) (2010) 5287–5289.

[31] R.L. Rollins, R.H. Cowie, M.V. Echaluse, M.C.I. Medeiros, Host snail species exhibit differential *Angiostrongylus cantonensis* prevalence and infection intensity across an environmental gradient, Acta Trop. 216 (2021), 105824.

[32] R.L. Rollins, M. Qader, W.L. Gosnell, C. Wang, S. Cao, R.H. Cowie, A validated high-throughput method for assaying rat lungworm (*Angiostrongylus cantonensis*) motility when challenged with potentially anthelmintic natural products from Hawaiian fungi, Parasitology 149 (6) (2022) 765–773.

[33] Posit Team, RStudio: Integrated Development Environment for R, Posit Software, PBC, Boston, MA, 2022.

[34] M.E. Brooks, K. Kristensen, K.J. van Benthem, A. Magnusson, C.W. Berg, A. Nielsen, H.J. Skaug, M. Mächler, B.M. Bolker, glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling, R Journal 9 (2) (2017) 378–400.

[35] R. Pruim, D.T. Kaplan, N.J. Horton, The mosaic package: helping students to 'think with data' using R, The R Journal 9 (1) (2017) 77–102.

[36] D.M. Spratt, Species of *Angiostrongylus* (Nematoda: Metastrongyloidea) in wildlife: a review, Int. J. Parasitol. Parasites Wildl. 4 (2) (2015) 178–189.

[37] S. Gelis, D.M. Spratt, S.R. Raidal, Neuroangiostrongyliasis and other parasites in tawny frogmouths (*Podargus strigoides*) in south-eastern Queensland, Aust. Vet. J. 89 (1–2) (2011) 47–50.

[38] H.D.S. Walden, J. Slapcinsky, J. Rosenberg, J.F.X. Wellehan, *Angiostrongylus cantonensis* (rat lungworm) in Florida, USA: current status, Parasitology 148 (2) (2021) 149–152.

[39] H.C. Turck, M.T. Fox, R.H. Cowie, Paratenic hosts *of Angiostrongylus cantonensis* and their relation to human neuroangiostrongyliasis globally, One Health 15 (2022), 100426.

[40] H.S. Yong, P. Eamsobhana, Definitive rodent hosts of the rat lungworm *Angiostrongylus cantonensis*, Raffles Bull. Zool. Suppl. 29 (2013) 111–115.

[41] R.H. Cowie, R. Malik, E.R. Morgan, Comparative biology of parasitic nematodes in the genus *Angiostrongylus* and related genera, Adv. Parasitol. 121 (2023) 65–197.

[42] J.E. Alicata, G. Loison, A. Cavallo, Parasitic meningoencephalitis experimentally produced in a monkey with larvae of *Angiostrongylus cantonensis*, J. Parasitol. 49 (1963) 156–157.

[43] J.H. Cross, Experimental studies on *Angiostrongylus* species and strains in monkeys and laboratory animals, in: J.H. Cross (Ed.), Studies on Angiostrongyliasis in Eastern Asia and Australia, US Naval Medical Research Unit No. 2, Taipei, 1979, pp. 118–137.

[44] S.I. Jarvi, M.E.M. Farias, K. Howe, S. Jacquier, R. Hollingsworth, W. Pitt, Quantitative PCR estimates *Angiostrongylus cantonensis* (rat lungworm) infection levels in semi-slugs (*Parmarion martensi*), Mol. Biochem. Parasitol. 185 (2) (2012) 174–176.

[45] B.G. Campbell, M.D. Little, The finding of *Angiostrongylus cantonensis* in rats in New Orleans, Am. J. Trop. Med. Hygiene 38 (3) (1988) 568–573.

[46] C.A. Waugh, J.F. Lindo, J. Lorenzo-Morales, R.D. Robinson, An epidemiological study of *A. cantonensis* in Jamaica subsequent to an outbreak of human cases of eosinophilic meningitis in 2000, Parasitology 143 (2016) 1211–1217.

[47] D. Chan, J. Barratt, T. Roberts, R. Lee, M. Shea, D. Marriott, J. Harkness, R. Malik, M. Jones, M. Aghazadeh, J. Ellis, D. Stark, The prevalence of *Angiostrongylus cantonensis/mackerrasae* complex in molluscs from the Sydney region, PLoS One 10 (5) (2015), e0128128.

[48] L.R. Ash, Observations on the role of mollusks and planarians in the transmission of *Angiostrongylus cantonensis* infection to man in New Caledonia, Rev. Biol. Trop. 24 (1) (1976) 163–174.

[49] T.C. Cheng, J.E. Alicata, Possible role of water in the transmission of *Angiostrongylus cantonensis* (Nematoda: Metastrongylidae), J. Parasitol. 50 (3) (1964) 39–40.

[50] T.-Y. Pai, W. Meyer, F.R. Torpy, S.L. Donahoe, J. Ellis, R. Malik, R. Lee, Host transmission dynamics of first- and third-stage *Angiostrongylus cantonensis* larvae in *Bullastra lessoni*, Parasitology 149 (8) (2022) 1034–1044.

[51] W. Robbins, G. Conboy, S. Greenwood, R. Schaper, Infectivity of gastropod-shed third-stage larvae of *Angiostrongylus vasorum* and *Crenosoma vulpis* to dogs, Parasit. Vectors 14 (2021) 307.

[52] M.B. Goodman, P. Sengupta, How *Caenorhabditis elegans* senses mechanical stress, temperature, and other physical stimuli, Genetics 212 (1) (2019) 25–51.

[53] K.E. Jones, N.G. Patel, M.A. Levy, A. Storeygard, D. Balk, J.L. Gittleman, P. Daszak, Global trends in emerging infectious diseases, Nature 451 (2008) 990–993.

[54] M.E.J. Woolhouse, S. Gowtage-Sequeria, Host range and emerging and reemerging pathogens, Emerg. Infect. Dis. 11 (12) (2005) 1842–1847.

[55] L. Magouras, V.J. Brookes, F. Jori, A. Martin, D.U. Pfeiffer, S. Durr, Emerging zoonotic diseases: should we rethink the animal-human interface? Front. Vet. Sci. 7 (2020), 582743.

[56] S.J. Ryan, C.J. Carlson, B. Tesla, M.H. Bonds, C.N. Ngonghala, E.A. Mordecai, L. R. Johnson, C.C. Murdock, Warming temperatures could expose more than 1.3 billion new people to Zika virus by 2050, Glob. Chang. Biol. 27 (1) (2020) 84–93.

[57] R. Rupasinghe, B.B. Chomel, B. Martinez-Lopez, Climate change and zoonoses: a review of the current status, knowledge gaps, and future trends, Acta Trop. 226 (2022), 106225.

[58] M.M. Naguib, R. Li, J. Ling, D. Grace, H. Nguyen-Viet, J.F. Lindahl, Live and wet markets: food access versus the risk of disease emergence, Trends Microbiol. 29 (7) (2021) 573–581.

[59] A.L. Smith, J. Leung, S. Kun, R. Zhang, I. Karagiannides, S. Raz, U. Lee, V. Glovatscka, C. Pothoulakis, S. Bradesi, E.A. Mayer, L.V. Rodríguez, The effects of acute and chronic psychological stress on bladder function in a rodent model, Urology 78 (4) (2011), 967.e1–7.

[60] G. Penakalapati, J. Swarthout, M.J. Delahoy, L. McAliley, B. Wodnik, K. Levy, M. C. Freeman, Exposure to animal feces and human health: a systematic review and proposed research priorities, Environ. Sci. Technol. 51 (20) (2017) 11537–11552.

[61] K. Wells, R. Flynn, Managing host-parasite interactions in humans and wildlife in times of global change, Parasitol. Res. 121 (2022) 3063–3071.