

RESEARCH ARTICLE

Shell-shocked: parasite-induced behaviour and development in an invasive dead-end snail host

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ABSTRACT

Parasites with complex life cycles produce large numbers of free-living infectious stages to overcome the low odds of successful transmission between hosts. These stages often infect non-competent or 'dead-end' hosts, which cannot support parasite development or transmission. While typically viewed as ecological cul-de-sacs, dead-end hosts may still experience meaningful effects from parasite exposure. Here, we examined how exposure to *Paragordius varius* hairworm larvae influences behaviour and development of *Physella acuta*, an invasive freshwater snail likely functioning as a dead-end host in this system. Using a dose–response design under controlled conditions, we exposed juvenile snails to increasing larval concentrations and tracked activity over 24 h in relation to water, total home range and core activity areas. Snails were then reared to assess impacts on shell development. Infection intensity scaled with larval dose. At the highest exposure, snails showed an almost one-third reduction in home range, spent substantially more time submerged and entered water less frequently but for longer durations. These shifts indicate reduced exploratory behaviour and altered water use, potentially limiting ecological flexibility. Although shell size and shape were unaffected, shell crush resistance increased markedly with dose, suggesting altered properties tied to behavioural or physiological responses. Our results show that parasites can impose sublethal yet ecologically significant costs on dead-end hosts. This challenges traditional views of dead-end hosts as passive endpoints and highlights their potential role in shaping host–parasite dynamics. For invasive species such as *P. acuta*, even non-transmissible infections may carry fitness and ecological consequences.

KEY WORDS: Host–parasite interactions, Behavioural ecology, Non-host effects, Freshwater invertebrates, Developmental plasticity, Invasion biology, Dilution effect

INTRODUCTION

Parasite life cycles involving multiple hosts are frequently associated with high reproductive capacity in the intermediate host, the definitive host, or both (Auld and Tinsley, 2015; Benesh et al., 2021). This is often counterbalanced by very low transmission success rates in free-living immature stages. Digenean trematodes provide a classic example: under favourable conditions, infected snail intermediate hosts can shed hundreds of clonal cercariae per day. For instance,

snails infected with the human blood fluke *Schistosoma mansoni* can release over 2000 cercariae daily (Le Clec'h et al., 2019). Although field data are limited, mathematical models typically require these transmission rates to be in the low single digits to accurately replicate real-world disease dynamics (Anderson and May, 1991; Anderson et al., 2021). Predation, physical barriers and environmental pollutants all decrease transmission success of immature parasite stages (Koprivnikar et al., 2023; Sures et al., 2017; Welsh et al., 2024). Additionally, local biodiversity can dilute transmission (the dilution effect) by reducing the density of competent hosts and increasing interference from non-competent or 'dead-end' hosts (Civitello et al., 2015), which are unable to support parasite development or transmission.

Dead-end hosts theoretically eliminate parasite fitness, as the parasite either cannot develop within them or cannot be transmitted to the next host through trophic or other transmission pathways (Stewart Merrill et al., 2022; Thielges et al., 2008). For example, humans engaging in open-water activities act as dead-end hosts for avian schistosomes, where cercariae penetrate the skin and cause an inflammatory response, resulting in cercarial dermatitis or swimmer's itch (Verbrugge et al., 2004). However, this condition is mild: the parasite cannot mature in humans and does not cause further complications. Other parasites cause more serious pathological conditions in dead-end hosts, resulting in severe disease and even death (e.g. Verocai et al., 2020). To date, research on dead-end hosts has often limited their ecological role as population sinks disrupting parasite transmission in competent hosts (Civitello et al., 2015), as well as the health impacts they may experience, particularly in humans and economically or ecologically important species. Therefore, data are lacking on the ecological consequences of parasites and their sublethal effects in dead-end hosts. In the current study, we move beyond these endpoints to test how infection by a parasite that typically requires multiple hosts affects the ecology of a dead-end host.

The bladder snail *Physella* (syn. *Physa*) *acuta* (Draparnaud 1805) is an invasive species from North America now found in freshwater habitats globally (Ebbs et al., 2018; Lydeard et al., 2016). Because of its remarkable ability to rapidly adapt to novel environments and displace native snails (Dobson, 2004; Núñez, 2010), it plays a key role as an intermediate host in the spread of zoonotic diseases (Dumidae et al., 2024; Lawton et al., 2018). Impacts of parasites on *P. acuta* as an intermediate host include enhanced grazing and anti-predator behaviours and a reduction in shell strength (Bernot, 2003; Bernot and Lamberti, 2008; Gustafson and Bolek, 2016). In other systems where snails act as intermediate hosts, parasites are known to increase snail size (known as gigantism) and alter behaviours such as vertical displacement to favour transmission to the next host (Ballabeni, 1995; Miura and Chiba, 2007; Ni and Doherty, 2022). Such changes are expected in intermediate hosts, but what happens when the host disrupts rather than facilitates parasite transmission?

In North America, *P. acuta* is frequently infected with parasitic hairworms (phylum Nematomorpha), and current evidence suggests

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it may function as a low-transmission paratenic or dead-end host (Hanelt et al., 2001; Harkins et al., 2016). Paratenic hosts are animals that harbour parasites without supporting further parasite development; development must occur in obligate intermediate hosts. Hairworms do not have intermediate hosts; rather, free-living aquatic larvae can form dormant cysts in a wide variety of aquatic fauna, including aquatic insects, snails, crustaceans, fishes and tadpoles (Bolek et al., 2015). Although encystment is not strictly necessary for completion of the life cycle, some of these hosts act as paratenic hosts that facilitate transmission from water to land. For example, aquatic insect larvae such as mayflies and stoneflies can transport viable cysts onto land when they emerge as terrestrial adults, where they may be consumed by definitive arthropod hosts. In contrast, hosts that spend their lives in water, such as fish and freshwater snails, rarely encounter the terrestrial arthropods required for hairworm development, and thus can be considered ecological dead ends. Although *P. acuta* is a pulmonate snail capable of leaving the water to breathe, it spends most of its time submerged (Gulanicz and Poznańska-Kakareko, 2018), thus making routine transfer of cysts to terrestrial arthropods unlikely. Experimental studies have demonstrated that cysts within snails can be passed to other hosts under laboratory feeding conditions (de Villalobos et al., 2003; Hanelt and Janovy, 2004), and snails may be consumed by aquatic predators that themselves could serve as paratenic hosts. However, the frequency and ecological relevance of such ingestion events in natural settings remain unclear. Field surveys show high infection prevalence of hairworm cysts in *P. acuta* at 70% of sampled sites, with some snails harbouring hundreds of cysts (Hanelt et al., 2001). This suggests that *P. acuta* may act as an important population sink for hairworm larvae: a host that readily accumulates infective stages but contributes minimally to onward transmission. Larvae penetrate the digestive tract and encyst within host tissues using specialised mouthparts (Bolek et al., 2015), and heavy infections can increase mortality in aquatic hosts (de Villalobos et al., 2006; Poinar and Doelman, 1974). The repeated accumulation of cysts may thus exert substantial stress on both competent paratenic hosts and likely dead-end hosts such as *P. acuta*.

The main goal of this study was to investigate the sublethal effects of hairworm infection on a likely dead-end host, the invasive freshwater snail *P. acuta*. While dead-end hosts are traditionally viewed as transmission cul-de-sacs, they may still experience significant physiological or behavioural consequences due to parasite exposure. Given the common occurrence of hairworm cysts reported in *P. acuta* populations, and the physical disruption caused by encystment, we hypothesised that infection will alter both snail behaviour and development. Specifically, we predicted that infected snails would exhibit reduced growth patterns due to energetic costs associated with the immune response or tissue damage. Additionally, we predicted that infected individuals would show a decrease in general activity, potentially due to parasite-induced disruption of normal physiological processes. These outcomes would suggest that, even without transmission potential, parasites can exert meaningful ecological effects on dead-end host populations.

MATERIALS AND METHODS

Snail rearing

Egg masses were selected randomly from a lab population of *P. acuta* maintained since 2022 (originally sourced from The University of New Mexico, USA). Out of these, 220 eggs were placed in 2 l containers with Vancouver tap water and monitored daily for hatching. After 5 days, most of the snails had hatched, and 160 specimens were carefully extracted using fine soft-tipped forceps. The newly hatched snails were moved to 2 l rearing containers with air

stones to oxygenate the water and ground oyster shells for calcium (Baptiste et al., 2024). At this point, snails were fed frozen lettuce and Nutrafin Max (Hagen Group, Baie-d'Urfé, QC, Canada) sinking pellets *ad libitum*; water was changed weekly. This was done to ensure snails started feeding on day 0 of the experiment. All snails were kept at 27±1°C with a 12 h:12 h light:dark photoperiod. Two submersible HOBO MX2201 loggers (Onset, Bourne, MA, USA) were used throughout the experiment to record temperature hourly in separate containers mimicking snail containers. Snails were kept in these rearing containers for 7 days before experimental infections.

Experimental infections

As a result of the recording setup configuration, 104 snails were randomly allocated to six cohorts consisting of 15 individuals each, with a seventh cohort comprising 14 individuals. From days 7 to 13 inclusive, a cohort was established daily to undergo starvation and exposure to *Paragordius varius* (Leidy 1851) hairworm larvae. This design effectively staggered the cohorts by 1 day for infections, behavioural recordings and measuring developmental traits. We assumed that age differences between cohorts would have a negligible effect on snail behaviour and development in response to treatment. The following infection protocol was therefore repeated daily at approximately the same time for each cohort: Snails were individually placed into wells of a 24-well plate filled halfway with Vancouver tap water and starved for 24 h. Hairworm larvae were obtained from a laboratory stock stored in microtubes at -80°C according to Bolek et al. (2013b). The larvae used were full siblings of the F1 generation, thereby limiting genetic variability. After thawing, the larval stock was mixed thoroughly, and three 2 µl droplets per microtube were examined under a microscope at 40× magnification to count larvae. The average of these counts was used to estimate larval concentration (larvae µl⁻¹), enabling consistent dosing across treatments. We used a micropipette to deliver larval stock into wells at fixed volumes calculated from the larval concentrations to create four treatments: control (2 µl of Vancouver tap water), low (100 larvae), medium (200 larvae) and high (400 larvae). Following exposure, snails remained in the wells for an additional 24 h to ingest the larvae. Afterwards, snails were individually transferred to marked 250 ml containers filled halfway with Vancouver tap water, each supplied with the same substrate and food *ad libitum*; water was changed weekly. The lids of these containers were perforated with small holes to allow air circulation. During the experiment, snails were only removed temporarily from these containers for the 24 h behavioural recordings.

Behavioural recordings

Five days after exposure to hairworm larvae (days 14–20 inclusive), snail cohorts were placed into behavioural arenas for 24 h recordings. This timeline was based on an estimation of hairworm encystment from previous infection trials (J.-F.D., unpublished data) and from observations previously made on experimental infections in *P. acuta* (Hanelt and Janovy, 2004). One snail was randomly assigned to each arena, and treatments were randomly distributed across cohorts. The arenas measured 50×100 mm with a depth of 15 mm and were made from 3 mm thick acrylic. The back and side panels were white acrylic, while the front panel was clear acrylic; all panels were joined using structural adhesive, except for the top panel which was removable. This design restricted the visual field of each snail. For each recording, three fixed rows of five arenas were stacked vertically. The midway point was marked on the outside of each arena, and water was filled to this level to establish the waterline. Arenas were enclosed in a box lined with white plastic

sheeting to improve lighting and minimise disturbance (Fig. S1). A full-spectrum LED light, connected to a timer, simulated daylight conditions matching the photoperiod of the room. Infrared lights remained on continuously to allow night-time recordings. The infrared lights and recording setup were connected to a Raspberry Pi computer (Raspberry Pi Holdings PLC, Cambridge, UK), which housed an infrared-sensitive 8-megapixel camera (Pi Camera Module 2 NoIR). To capture all 15 arenas simultaneously, the camera was mounted on a stand approximately 50 cm from the setup. Because of the narrow depth of the arenas, blind spots were effectively eliminated. A Python script run on the Raspberry Pi captured images every 5 s and compiled them into a time-lapse video (MP4 format) at 30 frames s⁻¹ for movement tracking. Arenas were washed with mild dish soap between recordings to remove snail residue. We also used this time to change the container water of the snails that had just been recorded. This cleaning and setup process staggered each recording by approximately 30 min, thus ensuring that the room remained undisturbed during active recordings.

Counting cysts and measuring shell size and shape

After behavioural recordings, snails were returned to their respective containers for an additional 15 days of development, after which they were placed in labelled tubes and frozen at -20°C (days 30–36 inclusive) until further processing. To count hairworm cysts, snails were thawed, and tissues were carefully separated from the shell with fine-tipped forceps. Doing this after freezing allowed for a clean extraction of tissues from the shell in most snails (Bolek et al., 2015). Following previously described methods, host tissues were flattened between a microscope slide and cover glass, and cysts were counted under 100× magnification (Doherty et al., 2019). To visualise the distribution of infection intensity per treatment, we created a boxplot in R version 4.5.1 (<http://www.R-project.org/>) using the Tidyverse package *ggplot2* (Wickham et al., 2019). Dried snail shells were then photographed next to a ruler with the aperture facing up using a C-mount microscope camera. Images were uploaded into *tpsDig2* (<https://life2.bio.sunysb.edu/morph/soft-dataacq.html>), where 22 landmarks were digitised following Gustafson et al. (2014) for geometric morphometric analysis (Fig. S2). Shells that lacked clear homologous landmarks as a result of damage or snails that had died before the full development period ended were excluded from this analysis. Landmark data were imported into R using the *geomorph* package (Baken et al., 2021) and aligned via generalised Procrustes analysis (GPA) to remove non-shape variation related to size, position and orientation. A principal components analysis (PCA) was performed on the aligned Procrustes coordinates, and convex hulls were drawn around PCA scores using *ggplot2* to visualise treatment-specific variation in shell shape. Centroid size, a proxy for overall shell size calculated as the square root of the summed squared distances from each landmark to the centroid, was extracted as part of the GPA.

Shell crush resistance

After photographing the shells, we assessed crush resistance using a method adapted from Gustafson and Bolek (2016). Each shell was placed aperture-down on a flat glass beaker, and a smaller flat plastic container was positioned on top. Sand was gradually poured into the container until the shell cracked. The total mass of the container and sand at the point of shell failure was recorded as crush mass, a proxy for crush resistance. Shells that were visibly damaged or snails that had died before the full development period ended were excluded from this assay.

Movement tracking and space use analysis

To track snail movement in the time-lapse videos, we used *AnimalTA* (Chiara and Kim, 2023), which applies background subtraction to detect moving targets. Briefly, we defined each arena and generated a background image free of snails using a subsample of video frames. Using the drawing tool, we also delineated the water region and marked the top of each arena. The scale was standardised by setting the arena width to 50 mm. Tracking parameters, including threshold (brightness cutoff for detecting movement), erosion and dilation, were optimised for each video. After automatic tracking, we manually reviewed and corrected mislabelled targets or omitted frames. *AnimalTA* then produced a set of standard quantitative behavioural metrics through its analysis function (total distance travelled, average speed, meander, proportion of time moving), as well as our custom metrics of interest based on the water area and top of the arena (proportion of time in water, number of entries into water, average time per entry, average distance from top). Both standard and custom metrics were included in the statistical analyses. *AnimalTA* also exported a full set of time-stamped coordinates for each snail. To standardise space use comparisons across arenas, we centred coordinates for each snail around its mean location using the Tidyverse packages *dplyr*, *tidyr* and *purrr*. We then calculated two-dimensional kernel density estimates (KDE) using the *MASS* package (Venables and Ripley, 2002) and created smoothed density heatmaps averaged by treatment group with *ggplot2*. To define areas of space use, we computed kernel utilisation distributions (KUD) using the *adehabitatHR* package (Calenge, 2006), extracting the 50% (core activity area) and 95% (total home range) isopleths. These isopleths represent the estimated spatial regions where individuals spent 50% and 95% of their time, respectively. The resulting spatial polygons were converted to simple features (sf) objects (Pebesma, 2018) and superimposed onto the heatmaps using *ggplot2*.

Statistical analysis

All statistical analyses were performed in R version 4.5.1 (<http://www.R-project.org/>). For behavioural modelling, we focused on six response variables derived from movement tracking: proportion of time in water, number of entries into water, average time per entry, average distance from top, core activity area (50% KUD threshold) and total home range (95% KUD threshold). We also examined treatment-level responses for the four standard behavioural metrics produced by *AnimalTA* (total distance travelled, average speed, meander, proportion of time moving). Bayesian multilevel models were fitted using the *brms* package (Bürkner, 2017). Data were cleaned using *dplyr* and *tidyr*, filtering missing values. Treatment was modelled as a categorical population-level effect (Bayesian ‘fixed’ effect) and cohort was included as a group-level intercept (Bayesian ‘random’ intercept) to account for between-cohort variance. Distribution families were chosen based on the nature of each outcome: beta distribution for proportional data, negative binomial distribution for overdispersed count data and Gaussian distribution for continuous data. We also modelled shell centroid size and crush resistance in response to treatment. To evaluate whether centroid size influenced crush resistance, multiple models were compared with leave-one-out cross-validation (LOO-CV) using the *loo* package (Vehtari et al., 2017), assessing whether the inclusion of centroid size or its interaction with treatment improved model fit. These developmental traits could not be included as covariates in the behavioural models as they were measured 15 days post-recordings. All models were estimated using Hamiltonian Monte Carlo (HMC) with four chains and 4000 iterations per chain,

including a 25% warmup. Weakly informative priors were used throughout. For models with sampling issues, the number of iterations was increased to 6000 or the control parameter 'adapt_delta' was raised to 0.99 to improve convergence. Model diagnostics included the potential scale reduction factor (\hat{R}) and effective sample size (ESS) ratios to assess convergence, while model fit was evaluated using posterior predictive checks, Bayesian R^2 and LOO-CV. We also used the Pareto shape parameter k to identify potential outliers that might disproportionately influence posterior distributions. Observations with $k > 0.7$ were flagged for further inspection (Vehtari et al., 2017). Posterior means (Bayesian 'effect sizes') $\pm 95\%$ credible interval (CI) and posterior contrasts were computed along with directional probabilities, e.g. $\text{Pr}(\text{High} > \text{Control})$. We classified directional probabilities of 0.90–0.95 as moderate evidence (potentially meaningful, warranting further investigation), 0.95–0.99 as strong evidence (real biological effect) and > 0.99 as very strong evidence (high certainty) for a directional effect (Makowski et al., 2019). Directional probabilities were interpreted alongside posterior means and fitted values to draw biologically relevant conclusions. Results were visualised using ggplot2, overlaying raw data points with posterior estimates and annotated probabilities of moderate evidence or higher. To examine variation among cohorts, posterior estimates of group-level intercepts were extracted and plotted with 95% CI to assess whether any cohorts deviated meaningfully from the overall mean. Finally, to assess the effect of treatment on shell shape, Procrustes linear modelling was conducted using the geomorph package, with aligned landmark configurations as the response variable. As shell shape can change with size (allometry), centroid size was included as a covariate. The model summary was computed via permutation tests with 10,000 iterations, which were used to evaluate the contribution of each predictor to shape variation.

RESULTS

Snail survival and data exclusions

Of the initial 104 snails, 88 were retained for analysis following quality control procedures. Eleven individuals were excluded because they were inadvertently exposed to hairworm larvae in dry wells, and five because of inaccurate larval dosing. During the course of the experiment, 10 snails (11.4%) died (4 in control, 2 in medium, 4 in HIGH): 6 prior to behavioural recordings and 4 after. Two of these snails died on the final day and were therefore included in developmental analyses. During behavioural recordings, water leakage occurred in 10 arenas, and these were excluded from behavioural analyses. For developmental measurements, 22 shells were excluded from geometric morphometric analyses because of damage or a lack of clear homologous landmark features, and 28 shells were excluded from the crush resistance assay because of breakage. As a result, final sample sizes were as follows: 72 snails for behavioural recordings, 66 for landmark-based shape analysis and 60 for crush

resistance assays. While these exclusions reduced the total number of observations per analysis, they were evenly distributed across treatment groups and cohorts, and sample sizes remained within acceptable ranges for the planned Bayesian models. Posterior distributions were adequately resolved, and convergence diagnostics confirmed model stability (Table 1; Table S1). As such, these exclusions did not compromise our ability to draw inferences from the data. Data from both loggers confirmed that temperatures remained within 1°C between containers throughout the experiment.

Infection intensity by treatment

Hairworm cyst counts increased with larval dose, confirming a dose-dependent infection response. The mean number of hairworm cysts per treatment group (\pm s.e.m.) was: 8 ± 1 in the low treatment, 24 ± 2 in medium and 39 ± 3 in high. No cysts were observed in control snails, and only one individual in the low treatment was uninfected. Distribution data showed mild overlap between treatment groups (Fig. S3). Based on the initial number of larvae exposed to each group, approximately 10% of larvae on average were found to be encysted within snail tissues.

Snail space use

Across treatments, snail total home ranges (95% KUD isopleths) were approximately centred on the waterline, whereas their core activity areas (50% KUD isopleths) appeared to be mostly under the water (Fig. 1A). KDE heatmaps did not reveal any consistent areas of high activity, except for one location in the high treatment group, which was driven by a single snail that remained largely stationary throughout the recording period (Fig. 1A). While there is some uncertainty regarding whether the high treatment negatively affected core activity area, reflected by a CI that slightly overlapped zero (Table 2), there is moderate and strong evidence that core activity areas were smaller in the high group compared with the medium and control groups, respectively (Fig. 1B). There is strong support that the high treatment negatively impacted the total home range of snails (Table 2). Snails in the high group exhibited a 29.2% smaller total home range compared with those in the control group, and their total home range was also smaller relative to that of snails in the low (19.7%) and medium (21.5%) treatment groups (Fig. 1C).

Snail activity metrics

Over 80% of snails spent more than 95% of the 24 h observation period moving. Among the four core behavioural metrics computed in AnimalTA, total distance travelled and proportion of time moving showed slight negative trends with increasing treatment level. However, the posterior means indicated no clear treatment effects on these metrics, including meander (Table S2 and Figs S4–S6). For average speed, the upper bound of the CI for the high treatment group just included zero, indicating some uncertainty (Table S2). However,

Table 1. Summary of fit and convergence diagnostics for Bayesian multilevel models assessing the effects of *Paragordius varius* hairworm larvae exposure on *Physella acuta* snail behavioural and developmental traits

Model	R^2	LOO-IC	Pareto k (> 0.7)	\hat{R} (max.)	ESS (min.)	Notes
Average distance from top (cm)	0.098	223.6	0	1.000	7594	Fully converged
Average time per entry (min)	0.110	673.6	0	1.000	7516	Fully converged
Centroid size	0.332	328.3	0	1.000	4400	Fully converged
Crush mass (g)	0.515	827.8	0	1.000	13,094	High explained variance
Number of entries into water	0.128	712.7	0	1.000	6992	Fully converged
Proportion of time in water	0.234	-54.1	1 (1.4%)	1.000	3809	One influential observation (verified)
Core activity area (cm ² at 50% KUD)	0.118	217.3	0	1.000	6503	Fully converged
Total home range (cm ² at 95% KUD)	0.137	432.2	0	1.000	7240	Fully converged

LOO-IC, leave-one-out information criterion; k , Pareto shape parameter; \hat{R} , scale reduction factor; ESS, effective sample size; KUD, kernel utilisation distribution.

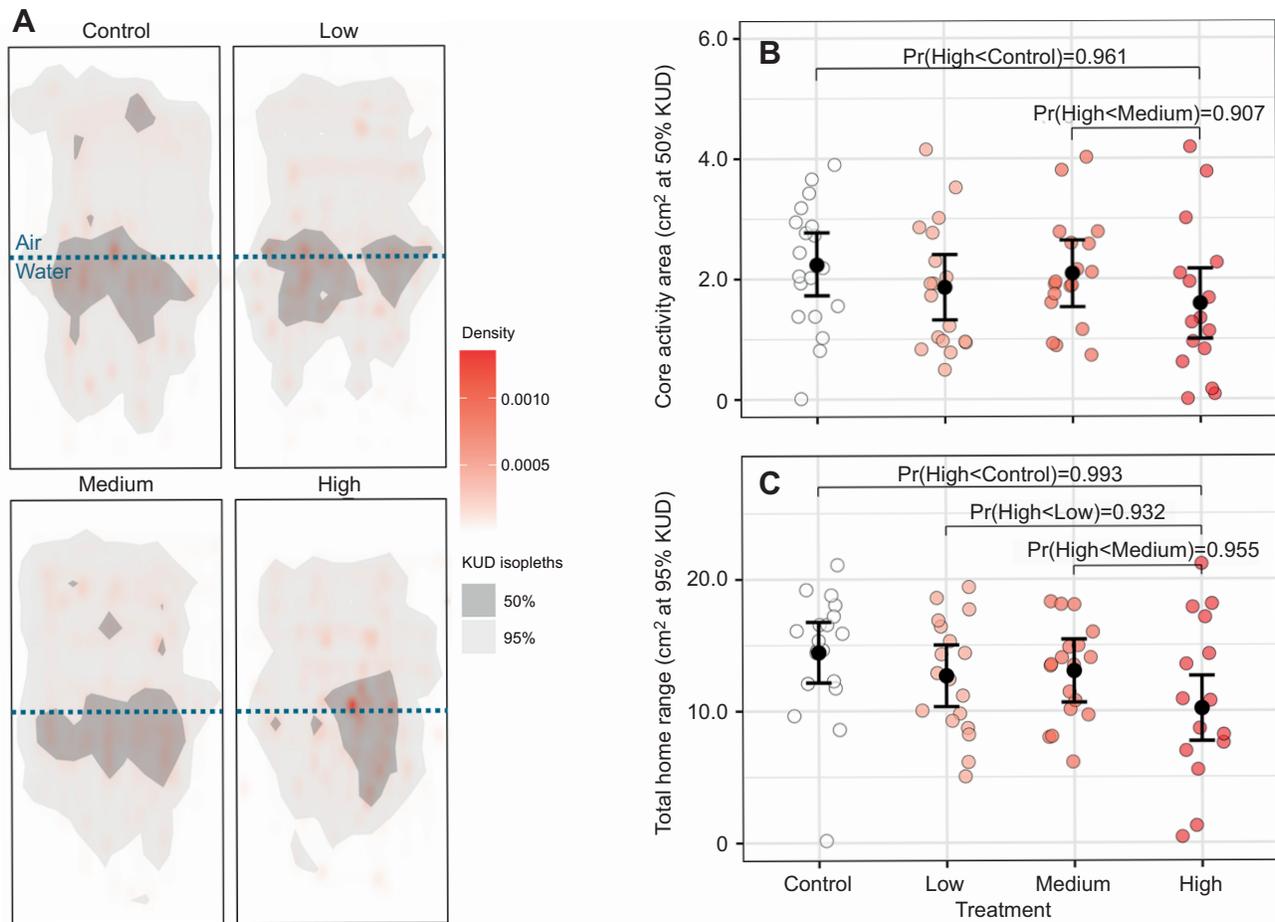


Fig. 1. Effect of *Paragordius varius* hairworm larvae exposure (treatment) on *Physella acuta* snail spatial occupancy using two-dimensional kernel density estimates (KDE). (A) Smoothed occupancy heatmaps for snails in the four treatments (control and low, medium and high larval concentration) derived from KDE, where density represents the likelihood of a snail occupying each pixel during the observation period. Superimposed shaded areas are kernel utilisation distribution (KUD) isopleths representing the estimated spatial regions where individuals spent 50% (core activity area) and 95% (total home range) of their time. Blue dotted lines indicate the waterline. (B) Area occupied by each snail at the 50% KUD threshold. (C) Area occupied at the 95% KUD threshold. Coloured dots represent individual snails; black dots and error bars indicate predicted group means and 95% credible interval (CI) from Bayesian multilevel modelling. Annotated probabilities above brackets indicate the posterior probability of directional differences between treatment groups. Probabilities of 0.90–0.95 indicate moderate evidence, 0.95–0.99 indicate strong evidence and >0.99 indicate very strong evidence in favour of the specified directional difference.

there is moderate evidence that snails in the high group moved 28% slower on average than those in the control group (Fig. S7).

Snail behaviour related to water

Overall, more than 80% of snails spent at least half of their time in water. Across treatments, we observed increasing trends from control to high treatments in the proportion of time in water, the average time spent in water per entry and the average distance from the top of the arena, accompanied by a decrease in the number of water entries (Fig. 2). Snails in the high treatment group spent more time in water overall (Table 2), with strong evidence that they spent on average 3.4 h more per day in water compared with control snails (Fig. 2A). Based on the estimated log-odds difference, high treatment snails were more than twice as likely to be in water at any given time. There is also moderate evidence that high snails spent over 2.7 h more in water than those in the low and medium groups. For the number of water entries, the upper bound of the CI for the high group slightly overlapped zero, indicating some uncertainty (Table 2). Nonetheless, there is moderate and strong evidence that snails in the high treatment group entered water less

frequently than those in the low and control groups, respectively (Fig. 2B). This aligns with the average time spent in water per entry, which showed a substantial increase in the high group (Table 2). Snails in this group spent on average 23.9 min more per entry than control snails (131% increase with very strong evidence) and 16.6 min more than those in the low group (65% increase with moderate evidence) (Fig. 2C). Finally, snails in the high treatment group may have spent more time further from the top of the arena, although this effect is uncertain because of the CI overlapping zero (Table 2). Still, moderate evidence suggests that snails in the high treatment group were slightly further from the top than those in the control and low groups (Fig. 2D).

Shell size, shape and crush resistance

Crush resistance visibly increased from the control to high treatment groups (Fig. 3A). In contrast, shell shape and shell size did not exhibit clear treatment-related differences, as shown by largely overlapping PCA convex hulls (Fig. 3B) and similar centroid sizes across treatments (Fig. 3C). The first two principal components of the PCA for shell shape explained 70.9% of the variance in the data. For crush

Table 2. Posterior estimates and 95% credible interval (CI) for Bayesian multilevel models assessing the effects of *P. varius* hairworm larvae exposure (treatment) on *P. acuta* snail behavioural and developmental traits

Model	Treatment	Estimate	95% CI	Iterations (adapt_delta)
Average distance from top (cm)	Low	-0.10	[-0.82, 0.62]	6000 (0.99)
	Medium	0.29	[-0.42, 0.99]	
	High	0.52	[-0.20, 1.23]	
Average time per entry (min)	Low	7.33	[-11.95, 26.81]	4000 (0.99)
	Medium	12.44	[-7.12, 32.10]	
	High	23.89	[3.84, 44.18]	
Centroid size	Low	0.40	[-1.29, 2.08]	4000 (0.80)
	Medium	-0.52	[-2.14, 1.11]	
	High	0.01	[-1.80, 1.83]	
Crush mass (g)	Low	55.35	[-69.90, 179.75]	4000 (0.80)
	Medium	308.36	[182.23, 431.95]	
	High	472.52	[332.25, 609.69]	
Number of entries into water	Low	-0.09	[-0.54, 0.36]	6000 (0.99)
	Medium	-0.19	[-0.65, 0.28]	
	High	-0.41	[-0.89, 0.05]	
Proportion of time in water	Low	0.14	[-0.46, 0.73]	4000 (0.80)
	Medium	0.13	[-0.48, 0.75]	
	High	0.72	[0.06, 1.38]	
Core activity area (cm ² at 50% KUD)	Low	-0.38	[-1.07, 0.32]	4000 (0.99)
	Medium	-0.15	[-0.86, 0.56]	
	High	-0.65	[-1.40, 0.08]	
Total home range (cm ² at 95% KUD)	Low	-1.77	[-4.86, 1.38]	4000 (0.99)
	Medium	-1.40	[-4.54, 1.81]	
	High	-4.26	[-7.58, -1.00]	

Number of iterations and the adapt_delta control parameter are included for each model. Treatments in bold indicate posterior estimates with 95% CI that do not overlap with 0. Note: estimates are reported relative to the control treatment (reference group).

resistance, including centroid size or its interaction with treatment did not improve model fit or predictive accuracy, based on LOO-CV. Therefore, the model containing treatment as the sole predictor was selected for its parsimony. High and medium treatments increased shell crush resistance (Table 2). Very strong evidence suggests shells from high treatment group snails were more crush resistant than those from all other groups (Fig. 3A). For example, shells from this group required 472 g more sand on average to break than those in the control group. Very strong evidence also suggests shells from snails in the medium treatment group were more crush-resistant than those in control and low groups. Based on Procrustes linear model permutations, centroid size explained over two times more variance ($R^2=0.105$, $F=6.94$, $P<0.001$) than treatment ($R^2=0.050$, $F=1.10$, $P=0.348$), indicating that variation in size contributed to differences in shell morphology across individuals, whereas treatment did not. Posterior means showed no clear effect of treatment on centroid size (Table 2, Fig. 3C).

Model diagnostics and cohort effects

All Bayesian multilevel models showed good convergence, with $\hat{R}=1.000$ and effective sample sizes exceeding 3800 for all parameters (Table 1). Posterior predictive checks indicated that the models adequately captured the observed data distributions across treatment groups (Figs S8–S19). Bayesian R^2 values ranged from 0.10 to 0.52, suggesting moderate to high explanatory power depending on the response variable. For models experiencing initial sampling problems, increasing the number of iterations or modifying the adapt_delta parameter improved convergence (Table 2). Snails with a Pareto $k>0.7$ (potentially influential) were reviewed and kept in the analyses because of their biological validity. There was no evidence that behavioural or developmental outcomes were systematically influenced by cohort identity. Posterior estimates of group-level (cohort) intercepts showed minimal deviation from the overall means (Figs S20–S31).

DISCUSSION

Dead-end hosts are traditionally viewed as biological endpoints that stop parasite transmission in their tracks and contribute little beyond limiting parasite fitness. However, our findings challenge this view by demonstrating that exposure to *P. varius* hairworm larvae imposes pronounced sublethal effects on the freshwater snail *P. acuta*, a likely dead-end host in this system. It is important to note that, although *P. acuta* harbours nematomorph cysts, it functions as a paratenic or dead-end host rather than an intermediate host, as no parasite development occurs within the snail. Thus, the effects documented here arise from parasite exposure and encystment rather than from obligate developmental interactions typical of intermediate hosts. While *P. acuta* can, under laboratory conditions, pass cysts to some predators, its predominantly aquatic lifestyle means that encounters with terrestrial definitive hosts are probably rare, making consistent paratenic transmission unlikely. Even in the absence of successful transmission, increasing parasite exposure altered both snail behaviour and physical development, pointing to broader ecological consequences for hosts that function as parasite sinks in host–parasite networks.

Our data show that infection intensity scaled positively with larval dose and correlated with behavioural changes in movement and space use. Snails in the highest infection group exhibited markedly reduced total home range, nearly one-third smaller than that of uninfected snails, and probably smaller core activity areas. Also, snails in this treatment group were probably slower on average than uninfected snails. As there were no cues except water in the behavioural arenas, this suggests a reduction in exploratory behaviours. Behaviour was measured 5 days after exposure, when larvae were migrating through or encysting within host tissues; reduced locomotion may therefore reflect acute physiological disturbance during infection rather than effects of fully matured cysts. While parasitism can decrease locomotor activity and performance in other host–parasite systems, compensatory increases in behaviours such as foraging are also

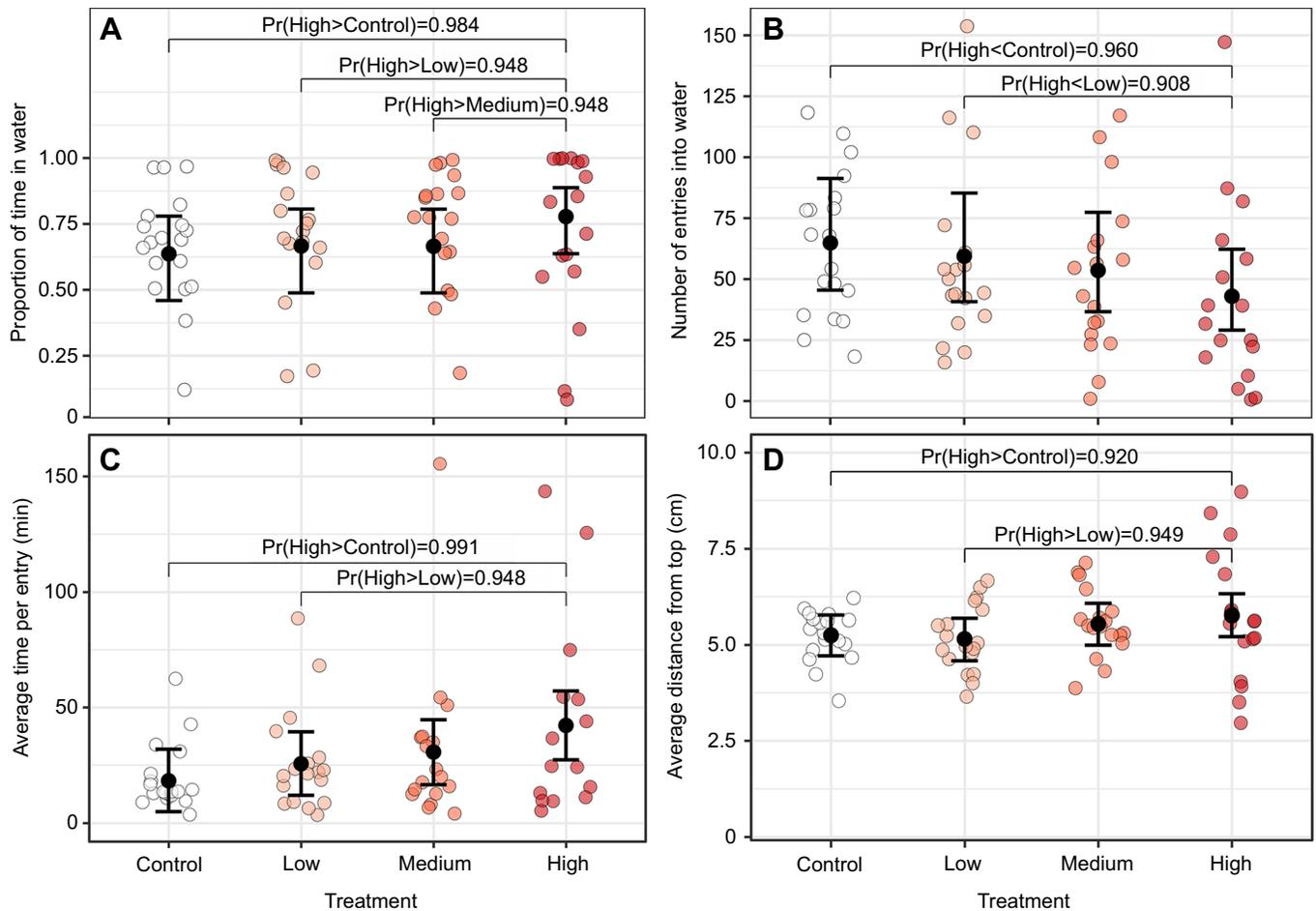


Fig. 2. Effect of *P. varius* hairworm larvae exposure (treatment) on *P. acuta* snail spatial behaviour in relation to water. (A) Proportion of total time spent below the waterline. (B) Number of times snails entered the water. (C) Average duration of time spent in the water per entry. (D) Average distance from the top of the arena. Coloured dots represent individual snails; black dots and error bars indicate predicted group means and 95% CI from Bayesian multilevel modelling. Annotated probabilities above brackets indicate the posterior probability of directional differences between treatment groups. Probabilities of 0.90–0.95 indicate moderate evidence, 0.95–0.99 indicate strong evidence and >0.99 indicate very strong evidence in favour of the specified directional difference.

possible (Binning et al., 2017). Additionally, infected snails spent more time in the water overall, exited and entered the water less frequently, and remained submerged longer per entry, all of which may represent adaptive responses to infection. Given that performance is often a direct target of natural selection (Arnold, 1983; Bennett and Huey, 1990), these behavioural shifts likely reduce ecological plasticity and could increase susceptibility to predation or environmental stress. While it remains unclear whether reproductive success is affected, the capacity for self-fertilisation (Jarne et al., 2000) may buffer *P. acuta* against reduced mate encounters.

We found no evidence that hairworm infection altered shell size or shape. Allometry was confirmed here as shell size impacted its shape. Depending on the system, snails as intermediate hosts of trematodes can show a marked increase in shell size following infection (host gigantism), which may reflect an adaptive response from the host to outlive infection or a direct developmental alteration by the parasite to support its own growth and reproduction (Ballabeni, 1995; Miura and Chiba, 2007; Poulin, 2007). Here, infected snails developed more crush-resistant shells, even without changes in shape or size. This increase may reflect a compensatory thickening or altered shell composition rather than gross morphology. Whether this represents an adaptive host response to physiological damage or an incidental

byproduct of infection remains unclear. Snails in the high and medium groups had stronger shells than those in the low or control groups. As high treatment group snails also spent 131% more time in the water per entry than uninfected snails, they may have ingested more calcium from the substrate, suggesting a potential experimental artifact. For instance, local environmental conditions impacting shell thickness and composition could override the effects from parasitism alone in other molluscs (García-Huidobro et al., 2019, 2020). Testing this hypothesis would require direct measurement of foraging behaviour or calcium uptake. Interestingly, the medium group did not differ statistically in behaviour from the control or low groups yet still showed elevated shell strength, suggesting behavioural or physiological responses not fully captured in the measured variables. While parasites can reduce shell strength in *P. acuta* by depleting host resources when these snails act as true intermediate hosts (Gustafson and Bolek, 2016), hairworm infections may drive behavioural shifts that promote calcium acquisition. Regardless of mechanism, increased shell strength in relation to infection could have costs not measured here, such as reduced reproductive output or altered predator susceptibility. For example, these effects may reduce susceptibility to crushing predators such as crayfish, while offering little protection against predators that rasp soft tissue or consume snails whole. Such possibilities remain

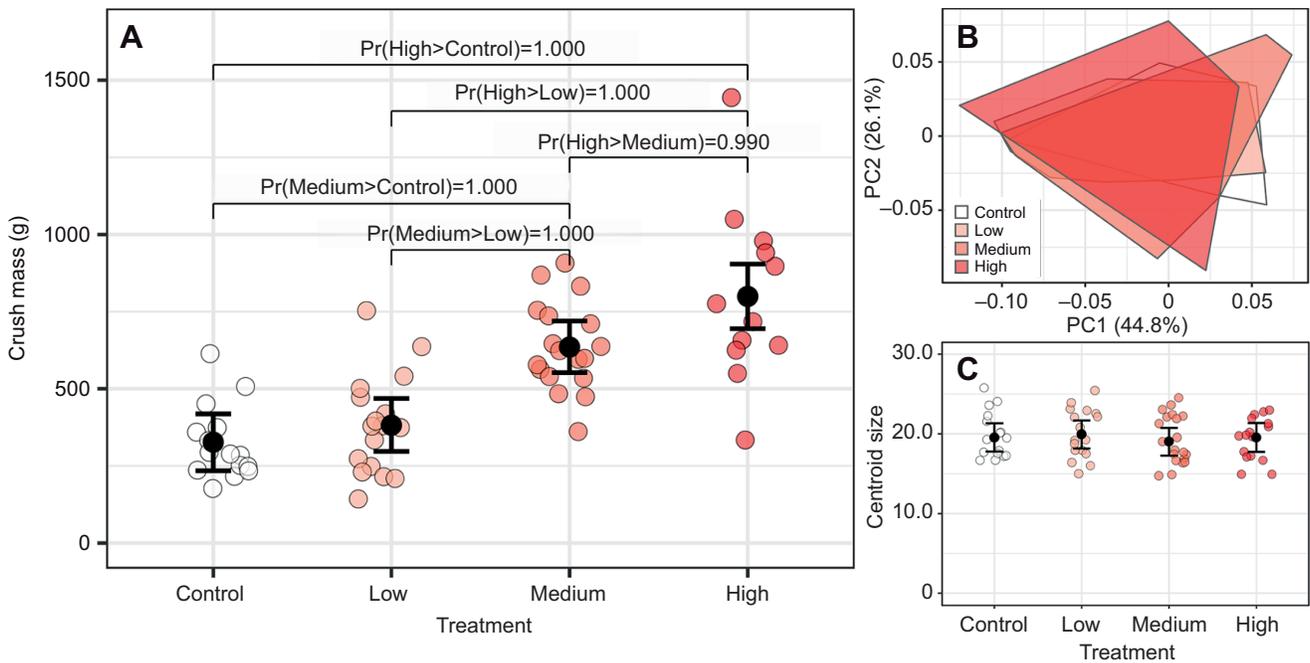


Fig. 3. Effect of *P. varius* hairworm larvae exposure (treatment) on *P. acuta* snail development 15 days post-exposure. (A) Shell strength measured as crush resistance, quantified by the mass of sand required to break the shell. (B) Principal component analysis (PCA) of shell shape derived from generalised Procrustes analysis (GPA) of 22 shell landmarks; shaded convex hulls represent group-level variation. (C) Shell centroid size calculated from the 22 shell landmarks. Coloured dots represent individual snails; black dots and error bars indicate predicted group means and 95% CI from Bayesian multilevel modelling. Annotated probabilities above brackets indicate the posterior probability of directional differences between treatment groups. Probabilities of 0.90–0.95 indicate moderate evidence, 0.95–0.99 indicate strong evidence and >0.99 indicate very strong evidence in favour of the specified directional difference.

speculative but illustrate the need for ecological context when interpreting morphological responses to infection.

These findings extend our understanding of how parasites influence populations of free-living species beyond traditional transmission-focused frameworks. Although *P. acuta* most likely does not support regular *P. varius* transmission, parasite exposure still imposed measurable developmental and behavioural shifts. Given that natural infections can include hundreds of cysts per snail (Hanelt et al., 2001), our single-dose treatments may underestimate impacts from real-world exposure levels. Additional hairworm infections or co-infection with other parasites could intensify the behavioural and physiological impacts measured here. Other low-transmission or dead-end hosts, including aquatic oligochaetes and platyhelminths, harbour large proportions of cysts in aquatic invertebrate communities (Doherty and Poulin, 2022), suggesting this phenomenon may be widespread. As hairworms are globally distributed (Schmidt-Rhaesa, 2013), dead-end host impacts may be common across freshwater ecosystems. Moreover, invasive species such as *P. acuta*, often assumed to benefit from reduced parasite loads in the areas they invade (enemy release hypothesis) (Brian and Catford, 2023), may nonetheless accumulate local parasites, including hairworms. For instance, the North American sister species *Physella gyrina* has been infected with African hairworms in laboratory settings (Bolek et al., 2013a), suggesting cross-regional compatibility is possible. If *P. acuta* functions as a dead-end host for hairworms in invaded habitats, behavioural shifts could influence their broader role as an intermediate host for other parasites such as trematodes. For example, hairworm-induced reductions in home range could concentrate cercarial output, amplifying local transmission risks. These interactions remain speculative and require proper testing, but they underscore the need to study the impacts of non-competent hosts in disease ecology and invasion biology.

This study examined a single host–parasite pair under controlled conditions, and extrapolation to natural settings should be done with caution. The fitness consequences of enhanced shell strength remain unknown, and long-term studies could assess survival, reproduction and competitive ability. Additionally, histological or physiological analyses could clarify whether behavioural changes stem from immune activation, tissue damage or neurochemical disruption. Finally, broader comparative work is needed across parasite taxa and host types to determine how generalisable these effects are. While dead-end hosts do not contribute directly to parasite transmission, they may still be key participants in parasite-driven ecological dynamics.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: J.-F.D., B.R., B.J.M.; Data curation: J.-F.D., B.R.; Formal analysis: J.-F.D.; Funding acquisition: L.J.F., B.J.M.; Investigation: J.-F.D., B.R.; Methodology: J.-F.D., B.R., B.J.M.; Project administration: J.-F.D.; Resources: B.J.M.; Supervision: J.-F.D., L.J.F., B.J.M.; Visualization: J.-F.D., B.R.; Writing – original draft: J.-F.D.; Writing – review & editing: J.-F.D., B.R., L.J.F., B.J.M.

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Data and resource availability

Full datasets are available from the figshare: <https://doi.org/10.6084/m9.figshare.31449361.v1>. All other relevant data and details of resources can be found within the article and its [supplementary information](#).

References

- Anderson, R. M. and May, R. M. (1991). *Infectious Diseases of Humans: Dynamics and Control*. Oxford University Press. doi:10.1093/9780198545996.001.0001
- Anderson, L. C., Loker, E. S. and Wearing, H. J. (2021). Modeling schistosomiasis transmission: the importance of snail population structure. *Parasit. Vectors* **14**, 94. doi:10.1186/s13071-021-04587-8
- Auld, S. K. J. R. and Tinsley, M. C. (2015). The evolutionary ecology of complex lifecycle parasites: linking phenomena with mechanisms. *Heredity* **114**, 125-132. doi:10.1038/hdy.2014.84
- Arnold, S. J. (1983). Morphology, performance and fitness. *Am. Zool.* **23**, 347-361. doi:10.1093/icb/23.2.347
- Baken, E., Collyer, M., Kaliontzopoulou, A. and Adams, D. (2021). geomorph v4.0 and gmShiny: enhanced analytics and a new graphical interface for a comprehensive morphometric experience. *Methods Ecol. Evol.* **12**, 2355-2363. doi:10.1111/2041-210X.13723
- Ballabeni, P. (1995). Parasite-induced gigantism in a snail: a host adaptation? *Funct. Ecol.* **9**, 887-893. doi:10.2307/2389987
- Baptiste, A. J., Kiendrebeogo, T., Kouato, F. and Otchoumou, A. (2024). Growth performance of *Achatina achatina* fed with leaf powder enriched in oyster shell powder. *Asian J. Dairy Food Res.* **44**, 128-133. doi:10.18805/ajdfr.DRF-419
- Benesh, D. P., Parker, G. and Chubb, J. C. (2021). Life-cycle complexity in helminths: what are the benefits? *Evolution* **75**, 1936-1952. doi:10.1111/evo.14299
- Bennett, A. B. and Huey, R. B. (1990). Studying the evolution of physiological performance. In *Oxford Surveys in Evolutionary Biology* (ed. D. Futuyma and J. Antonovics), pp. 251-284. Oxford University Press. doi:10.1093/oso/9780195062892.001.0001
- Bernot, R. J. (2003). Trematode infection alters the antipredator behavior of a pulmonate snail. *J. North. Am. Benthol. Soc.* **22**, 241-248. doi:10.2307/1467995
- Bernot, R. J. and Lambert, G. A. (2008). Indirect effects of a parasite on a benthic community: an experiment with trematodes, snails and periphyton. *Freshw. Biol.* **53**, 322-329. doi:10.1111/j.1365-2427.2007.01896.x
- Binning, S. A., Shaw, A. K. and Roche, D. G. (2017). Parasites and host performance: incorporating infection into our understanding of animal movement. *Integr. Comp. Biol.* **57**, 267-280. doi:10.1093/icb/ixc024
- Bolek, M. G., Szymgiel, C., Kubat, A., Schmidt-Rhaesa, A. and Hanelt, B. (2013a). Novel techniques for biodiversity studies of gordiids and description of a new species of *Chordodes* (Gordiida, Nematomorpha) from Kenya, Africa. *Zootaxa* **3717**, 23-38. doi:10.11646/zootaxa.3717.1.2
- Bolek, M. G., Rogers, E., Szymgiel, C., Shannon, R. P., Doerfert-Schrader, W. E., Schmidt-Rhaesa, A. and Hanelt, B. (2013b). Survival of larval and cyst stages of gordiids (Nematomorpha) after exposure to freezing. *J. Parasitol.* **99**, 397-402. doi:10.1645/12-62.1
- Bolek, M. G., Schmidt-Rhaesa, A., de Villalobos, L. C. and Hanelt, B. (2015). Phylum Nematomorpha. In *Ecology and General Biology: Thorp and Covich's Freshwater Invertebrates* (ed. J. H. Thorp and D. C. Rogers), pp. 303-326. Academic Press. doi:10.1016/B978-0-12-385026-3.00015-2
- Brian, J. I. and Catford, J. A. (2023). A mechanistic framework of enemy release. *Ecol. Lett.* **26**, 2147-2166. doi:10.1111/ele.14329
- Bürkner, P.-C. (2017). brms: an R package for Bayesian multilevel models using Stan. *J. Stat. Softw.* **80**, 1-28. doi:10.18637/jss.v080.i01
- Calenge, C. (2006). The package "adehabitat" for the R software: a tool for the analysis of space and habitat use by animals. *Ecol. Model.* **197**, 516-519. doi:10.1016/j.ecolmodel.2006.03.017
- Chiara, V. and Kim, S.-Y. (2023). AnimalTA: a highly flexible and easy-to-use program for tracking and analysing animal movement in different environments. *Methods Ecol. Evol.* **14**, 1699-1707. doi:10.1111/2041-210X.14115
- Civitello, D. J., Cohen, J., Fatima, H., Halstead, N. T., Liriano, J., McMahon, T. A., Ortega, C. N., Sauer, E. L., Sehgal, T., Young, S. et al. (2015). Biodiversity inhibits parasites: broad evidence for the dilution effect. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 8667-8671. doi:10.1073/pnas.1506279112
- de Villalobos, C., Rumi, A., Núñez, V., Schmidt-Rhaesa, A. and Zanca, F. (2003). Paratenic hosts: larval survival strategy of *Paragordius varius* (Leidy, 1851) (Gordiida, Nematomorpha). *Acta Parasitol.* **42**, 98-102.
- de Villalobos, C., Ronderos, M. M., Greco, N., Zanca, F., Díaz, F. and Achioro, C. (2006). Susceptibility of *Aedes aegypti* larvae to parasitism by *Paragordius varius* under laboratory conditions. *Trans. Am. Entomol. Soc.* **132**, 121-127. doi:10.3157/0002-8320(2006)132[121:SOAALT]2.0.CO;2
- Dobson, M. (2004). Replacement of native freshwater snails by the exotic *Physa acuta* (Gastropoda: Physidae) in southern Mozambique; a possible control mechanism for schistosomiasis. *Ann. Trop. Med. Parasitol.* **98**, 543-548. doi:10.1179/000349803225021334
- Doherty, J.-F. and Poulin, R. (2022). Come with me if you want to live: sympatric parasites follow different transmission routes through aquatic host communities. *Int. J. Parasitol.* **52**, 293-303. doi:10.1016/j.ijpara.2021.11.009
- Doherty, J.-F., Chai, X. and Poulin, R. (2019). Varying levels of melanotic encapsulation of gordioid hairworm cysts (Nematomorpha) by aquatic insect larvae: seasonal and host effects. *J. Invertebr. Pathol.* **168**, 107258. doi:10.1016/j.jip.2019.107258
- Dumida, A., Ardpairin, J., Pansri, S., Homkaew, C., Nichitcharoen, M., Thanwisai, A. and Vitta, A. (2024). Genetic diversity and population structure of *Physella acuta* (Gastropoda: Physidae) in Thailand using mitochondrial gene markers: COI and 16S rDNA. *Sci. Rep.* **14**, 13161. doi:10.1038/s41598-024-64184-4
- Ebbs, E. T., Loker, E. S. and Brant, S. V. (2018). Phylogeography and genetics of the globally invasive snail *Physa acuta* Draparnaud 1805, and its potential to serve as an intermediate host to larval digenetic trematodes. *BMC Evol. Biol.* **18**, 103. doi:10.1186/s12862-018-1208-z
- García-Huidobro, M. R., Varas, O., George-Nascimento, M., Pulgar, J., Aldana, M., Lardies, M. A. and Lagos, N. A. (2019). Role of temperature and carbonate system variability on a host-parasite system: implications for the gigantism hypothesis. *Int. J. Parasitol. Parasites Wildl.* **9**, 7-15. doi:10.1016/j.ijppaw.2019.03.016
- García-Huidobro, M. R., Aldana, M., Varas, O., Pulgar, J., García-Herrera, C., Abarca-Ortega, A., Grenier, C., Rodríguez-Navarro, A. B. and Lagos, N. A. (2020). Geographical variability and parasitism on body size, reproduction and shell characteristics of the keyhole limpet *Fissurella crassa* (Mollusca: Vetigastropoda). *Mar. Environ. Res.* **161**, 105060. doi:10.1016/j.marenvres.2020.105060
- Gulanicz, J. K. and Poznańska-Kakareko, M. (2018). Effects of water level fluctuations and substratum drying on the survival and behaviour of the invasive freshwater snail *Physa acuta* Draparnaud, 1805. *Mar. Freshw. Res.* **69**, 1389-1396. doi:10.1071/MF17349
- Gustafson, K. D. and Bolek, M. G. (2016). Effects of trematode parasitism on the shell morphology of snails from flow and nonflow environments. *J. Morphol.* **277**, 316-325. doi:10.1002/jmor.20497
- Gustafson, K. D., Kensing, B. J., Bolek, M. G. and Luttbeg, B. (2014). Distinct snail (*Physa*) morphotypes from different habitats converge in shell shape and size under common garden conditions. *Evol. Ecol. Res.* **16**, 77-89.
- Hanelt, B. and Janovy, J. J., Jr. (2004). Spanning the gap: experimental determination of paratenic host specificity of horsehair worms (Nematomorpha: Gordiida). *Invertebr. Biol.* **122**, 12-18. doi:10.1111/j.1744-7410.2003.tb00068.x
- Hanelt, B., Grother, L. E. and Janovy, J. J., Jr. (2001). Physid snails as sentinels of freshwater nematophorans. *J. Parasitol.* **87**, 1049-1053. doi:10.1645/0022-3395(2001)087[1049:PSASOF]2.0.CO;2
- Harkins, C., Shannon, R., Papeş, M., Schmidt-Rhaesa, A., Hanelt, B. and Bolek, M. G. (2016). Using Gordiid cysts to discover the hidden diversity, potential distribution, and new species of Gordiids (Phylum Nematomorpha). *Zootaxa* **4088**, 515-530. doi:10.11646/zootaxa.4088.4.3
- Jarne, P., Perdiou, M.-A., Pernot, A.-F., Delay, B. and David, P. (2000). The influence of self-fertilization and grouping on fitness attributes in the freshwater snail *Physa acuta*: population and individual inbreeding depression. *J. Evol. Biol.* **13**, 645-655. doi:10.1046/j.1420-9101.2000.00204.x
- Koprivnikar, J., Thielges, D. W. and Johnson, P. T. J. (2023). Consumption of trematode parasite infectious stages: from conceptual synthesis to future research agenda. *J. Helminthol.* **97**, e33. doi:10.1017/S0022149X23000111
- Lawton, S. P., Allan, F., Hayes, P. M. and Smit, N. J. (2018). DNA barcoding of the medically important freshwater snail *Physa acuta* reveals multiple invasion events into Africa. *Acta Trop.* **188**, 86-92. doi:10.1016/j.actatropica.2018.08.027
- Le Clec'h, W., Fiaz, R., Chevalier, F. D., McDew-White, M. and Anderson, T. J. C. (2019). Striking differences in virulence, transmission and sporocyst growth dynamics between two schistosome populations. *Parasit. Vectors* **12**, 485. doi:10.5281/zenodo.3466005
- Lydeard, C., Campbell, D. and Golz, M. (2016). *Physa acuta* Draparnaud, 1805 should be treated as a native of North America, not Europe. *Malacologia* **59**, 347-350. doi:10.4002/040.059.0213
- Makowski, D., Ben-Shachar, M. S., Annabel Chen, S. H. and Lüdtke, D. (2019). Indices of effect existence and significance in the Bayesian framework. *Front. Psychol.* **10**, 2767. doi:10.3389/fpsyg.2019.02767
- Miura, O. and Chiba, S. (2007). Effects of trematode double infection on the shell size and distribution of snail hosts. *Parasitol. Int.* **56**, 19-22. doi:10.1016/j.parint.2006.10.002
- Ni, S. and Doherty, J.-F. (2022). Species-specific tidal locomotion linked to a parasitic infection in sympatric sea snails. *Mar. Biol.* **169**, 88. doi:10.1007/s00227-022-04075-2
- Núñez, V. (2010). Differences on allocation of available resources, in growth, reproduction, and survival, in an exotic gastropod of Physidae compared to an endemic one. *Iheringia Série Zool.* **100**, 275-279. doi:10.1590/S0073-47212010000300014
- Pebesma, E. (2018). Simple features for R: standardized support for spatial vector data. *R. J.* **10**, 439-446. doi:10.32614/RJ-2018-009
- Poinar, G. O., Jr. and Doelman, J. J. (1974). A reexamination of *Neochordodes occidentalis* (Montg.) comb. n. (Chordodidae: Gordioidea): larval penetration and defense reaction in *Culex pipiens* L. *J. Parasitol.* **60**, 327-335. doi:10.2307/3278476
- Poulin, R. (2007). *Evolutionary Ecology of Parasites*. Princeton University Press.
- Schmidt-Rhaesa, A. (2013). Nematomorpha. In *Handbook of Zoology* (ed. A. Schmidt-Rhaesa), pp. 29-145. De Gruyter Publisher.
- Sures, B., Nachev, M., Selbach, C. and Marcogliese, D. J. (2017). Parasite responses to pollution: what we know and where we go in 'Environmental Parasitology'. *Parasit. Vectors* **10**, 65. doi:10.1186/s13071-017-2001-3
- Stewart Merrill, T. E., Calhoun, D. M. and Johnson, P. T. J. (2022). Beyond single host, single parasite interactions: quantifying competence for complete multi-host, multi-parasite communities. *Funct. Ecol.* **36**, 1845-1857. doi:10.1111/1365-2435.14068

- Thieltges, D. W., Jensen, K. T. and Poulin, R.** (2008). The role of biotic factors in the transmission of free-living endohelminth stages. *Parasitology* **135**, 407-426. doi:10.1017/S0031182007000248
- Venables, W. N. and Ripley, B. D.** (2002). *Modern Applied Statistics with S*. Springer. doi:10.1007/978-0-387-21706-2
- Vehtari, A., Gelman, A. and Gabry, J.** (2017). Practical Bayesian model evaluation using leave-one-out cross-validation and WAIC. *Stat. Comput.* **27**, 1413-1432. doi:10.1007/s11222-016-9696-4
- Verbrugge, L. M., Rainy, J. J., Reimink, R. L. and Blankespoor, H. D.** (2004). Swimmer's itch: incidence and risk factors. *Am. J. Public Health* **94**, 738-741. doi:10.2105/AJPH.94.5.738
- Verocai, G. G., Chaudhry, U. N. and Lejeune, M.** (2020). Diagnostic methods for detecting internal parasites of livestock. *Vet. Clin. North Am. Food Anim. Pract.* **36**, 125-143. doi:10.1016/j.cvfa.2019.12.003
- Welsh, J. E., Markovic, M., van der Meer, J. and Thieltges, D. W.** (2024). Non-linear effects of non-host diversity on the removal of free-living infective stages of parasites. *Oecologia* **204**, 339-349. doi:10.1007/s00442-023-05462-2
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J. et al.** (2019). Welcome to the Tidyverse. *J. Open Source Softw.* **4**, 1686. doi:10.21105/joss.01686