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Testing the effects of drought, parasite infection, and their interaction on freshwater snails

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Abstract

Increased drought intensity and frequency pose a risk to freshwater ecosystems around the globe. We investigated the effects of water loss on snail–trematode interactions to determine how *Helisoma trivolvis* (Say, 1816) might respond to anticipated drought in natural systems and whether parasites might modify this response. Trematode-infected and uninfected *H. trivolvis* snails were experimentally subjected to drought conditions in microcosms over a 6-week (44 day) period. We found that drought-altered snail behavior but not mortality. Snails maintained under desiccated conditions were more likely to bury themselves under the sediment than snails that were maintained with constant water levels. Snails in the drought treatment did not exhibit higher rates of mortality than those in the perennial treatment. Rather, mortality appeared to be driven by an interaction between trematode infection and body size. Infected snails of all sizes and across treatments were more likely to die than uninfected snails, and within uninfected snails, larger individuals had increased odds of mortality. Infection also had pronounced effects on snail behavior, as snails with trematode infections were less likely to bury themselves. Our experiment highlights the potential interplay between drought and parasitism in affecting freshwater snail populations, warranting further investigation through larger-scale experiments and field research.

Key words: *Helisoma trivolvis*, snail, host–parasite interactions, disease ecology, drought, microcosm experiment

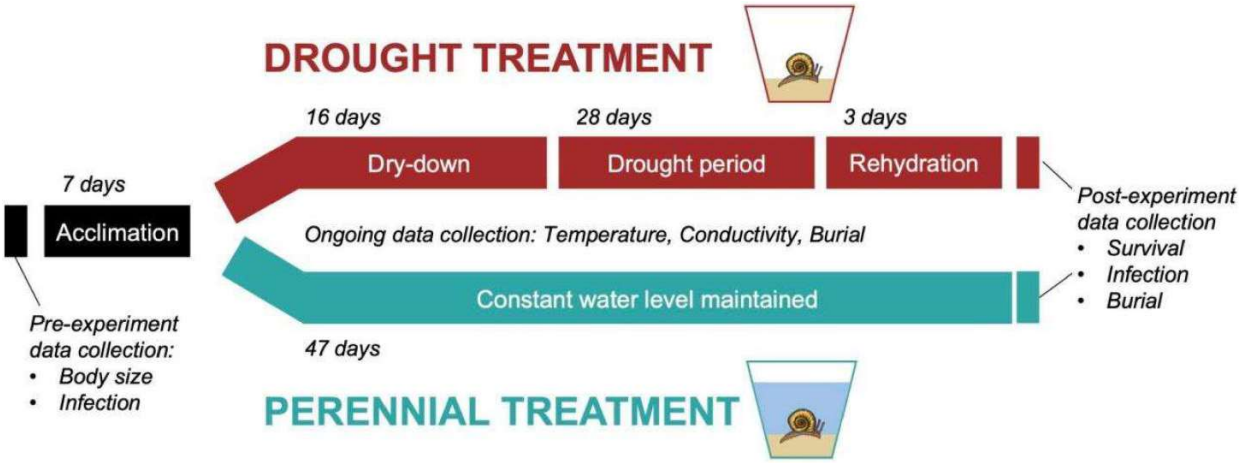
Introduction

Shifting global climate has led to concerns over how changing environmental conditions, most notably temperature, will affect parasites and infectious diseases (Harvell et al. 2002; Lafferty 2009; Rohr and Raffel 2010; Paull et al. 2012; Cohen et al. 2020; Rohr and Cohen 2020). Theoretical models have demonstrated that environmental temperature, by influencing vector physiology and demography, can be a key driver of transmission for mosquito-borne malaria and a suite of arboviruses (Mordecai et al. 2019). Similar models have been extended to snail-borne parasites and have solidified the importance of temperature for shaping invertebrate–parasite interactions and human disease risk (Mangal et al. 2008; Remais 2010; Kalinda et al. 2018; Żbikowska and Marszewska 2018). But climate change involves more than just temperature shifts: within the next century, ecosystems around the world are predicted to experience more frequent and severe drought, with dramatic consequences for aquatic and terrestrial ecosystems (Bradford et al. 2020). Given the reliance of many invertebrate vectors and intermediate hosts on aquatic systems (Johnson and Paull 2011), drought may have considerable impacts on the persistence and spread of parasites.

For parasites with aquatic hosts, the impacts of drought on infection patterns may depend on how vulnerable the host is to desiccation. For instance, if an aquatic host is highly sensitive to water loss, drought-induced mortality can lead to loss of infection (a decline in the parasite's population) (Gérard 2001). Such an impact could be exacerbated if infection itself is stressful and interacts with desiccation to intensify host mortality (Hall et al. 2013). However, hosts that live in habitats with distinct hydroperiods (e.g., semi-permanent ponds and vernal pools) may possess adaptations to contend with temporary water loss. Specifically, some snails can bury in the sediment (Olivier and Simões Barbosa 1956; Richgels et al. 2013), recede into their shells (Li and Graham 2007), and undergo estivation to remain hydrated until conditions improve (White et al. 2008). Drought-tolerant traits in a host could thus provide refuge to parasites during desiccation, allowing a parasite population to persist across hydroperiods.

We addressed the impacts of aquatic habitat dry-down and desiccation on host–parasite interactions, focusing specifically on snail and trematode populations from the California Bay Area region. Current water balance models predict that this region will experience increasingly variable annual and decadal precipitation patterns, leaving the region vulnerable to high frequency and intensity of drought conditions (Flint and Flint 2012; Flint et al. 2018). Several digenean trematode parasites are prominent ecological players in Bay Area ponds

Fig. 1. Experimental timeline. We simulated drought in microcosms containing freshwater snails *Helisoma trivolvis*. Prior to the experiment, we recorded each snail's body size and presence of infection ("Pre-experiment data collection"). All snails were then acclimated to microcosms for 7 days ("Acclimation"). For half of the microcosms (those in the drought treatment, see red timeline above), we simulated drought by removing water from the microcosm over 16 days ("Dry-down") and then maintaining microcosms without water for 28 days ("Drought period"). Drought microcosms were then rehydrated for 3 days at the end of the experiment ("Rehydration"). During the simulated drought, microcosms in the perennial treatment (see teal timeline below) had constant water levels maintained for a total of 47 days. To conclude the study, snails in both treatments were assessed for survival, burial, and trematode infection ("Post-experiment data collection").



(Preston et al. 2013), leading to interest regarding their persistence and dynamics under changing conditions. For instance, some of these trematodes have multihost life cycles, circulating sequentially among (1) snail first intermediate hosts, (2) larval amphibian second intermediate hosts, and (3) vertebrate definitive hosts (Schell 1985). These connections thus create powerful potential for trematodes to modify competitive and trophic interactions both within and among guilds. At the same time, infection by some trematodes can reduce host vital rates. For instance, *Ribeiroia ondatrae* (Price, 1931) can cause direct mortality and limb malformations in amphibians, which decrease amphibian survival due to starvation or increased likelihood of predation (Goodman and Johnson 2011; Wilber et al. 2020). Additionally, trematodes castrate (sterilize) their snail hosts, which may restrict snail foraging and population growth (Hay et al. 2005; Lafferty and Kuris 2009; Brian and Aldridge 2020), with plausible consequences for nutrient cycling and productivity (Wood et al. 2007; Mischler et al. 2016). How snails contend with anticipated drought, and what that means for the trematodes they harbor, thus has relevance for both amphibian health and pond ecology.

In this study, we experimentally simulated drought in microcosms to explore the persistence of uninfected and infected snails (*Helisoma trivolvis* (Say, 1816)) during periods of desiccation. We gradually drew down water levels in experimental drought treatments, while maintaining water levels in perennial controls, and monitored snail burial over the course of 6 weeks (Fig. 1). By combining information on preexisting infections, patterns of burial behavior, and snail survival, we assessed whether snails buried themselves in response to drought, and how the effects of infection and water loss influenced snail mortality. While we observed a higher rate of burial among snails in the drought treatment (suggesting that *H. trivolvis* possess strategies to mitigate desiccation), we found no difference in mortality among the experimental treatments. Rather, trematode infection was a strong predictor of snail mortality regardless of treatment. Taken together, our results highlight key pathways through which drought may affect ubiquitous host–parasite interactions and point to important future work that is required to disentangle mechanisms and extend predictions to field settings.

Materials and methods

Background and collection of experimental animals

All data were collected with the approval of the University of Colorado's Institutional Animal Care and Use Committee (protocols 1002.021302.01) and in accordance with sampling protocols approved by the California Department of Fish and Wildlife (SC-3683 and SC-10560).

We focused here on interactions between freshwater snails, *Helisoma trivolvis*, and four digenetic trematodes that infect *H. trivolvis* as their first intermediate host. The snails and trematodes inhabit natural and constructed freshwater ponds where the trematodes circulate sequentially among snail first intermediate hosts, amphibian second intermediate hosts, and vertebrate definitive hosts (Moss et al. 2020). Thus, whether ponds dry during drought conditions, and how long they remain dry, may have consequences for persistence of trematode infections in snails and their ability to move into amphibian hosts.

Wild *H. trivolvis* snails for use in the experiment were sourced from one pond in the East Bay region of California (37.66176, −121.96478). Snails were collected using a combination of sweeps with dipnets (45.7 cm diameter with

1200 μm mesh size), seine hauls (1.2 × 1.8 m with 3000 μm mesh), and hand collection (for specific details on sampling methods, see Richgels et al. 2013; McCaffrey and Johnson 2017). Following collection, live snails were packaged and shipped from California to the University of Colorado Boulder (CU Boulder).

Experimental set-up

Upon arrival to CU Boulder, snails were non-lethally examined for infection by trematode parasites (Fig. 1, "Pre-experiment data collection"). This process consisted of placing snails individually into 50 mL falcon tubes filled with commercial spring water, then maintaining them for 12 h in both dark and light conditions to stimulate the release of trematode cercariae (Calhoun et al. 2015; Hannon et al. 2018). Each tube was subsequently checked for the presence of cercariae using a stereomicroscope. In cases where cercariae were detected, the taxonomic identity of trematode was determined under a compound microscope (60× objective) using morphological features (e.g., body size, eyespots, tail shape) (Schell 1985; Hannon et al. 2018). Snails were housed in groups based on infection status and trematode identity. We maintained snails in commercial spring water with ad libitum access to food (fish flakes with calcium supplements) in a climate-controlled room (20–25 °C) under a 12 h light–dark cycle. Immediately prior to the experiment, we measured the body length of each snail (in mm) from the aperture to the spire using digital calipers (Fig. 1, "Pre-experiment data collection"). Body length measurements were supplemented with photographs that provided a visual record of each snail and included a scale bar to facilitate future measurements (if needed).

We constructed microcosms to individually house snails for the experiment. These microcosms were round-bottomed Tupperware containers (950 mL volume, 13.7 cm tall) containing 4 cm (250 mL) of substrate and 8 cm (600 mL) of commercial spring water (Sandland and Minchella 2004). The substrate consisted of mud sourced from a pond near where snails were collected. Mud was collected by hand around the margins of the pond where the substrate was moist but not inundated with water, and from areas that had little to no vegetation. The mud was initially hand-sifted in the field and secondarily in the lab to exclude macroinvertebrates (e.g., snails and larval insects), vegetation, and rocks. We did not autoclave the mud to maintain its microbial community and reflect the natural substrate where the snails originated. While naturally derived (and not autoclaved) mud could introduce trematode eggs or miracidia into the microcosms, we attempted to limit this possibility by using mud sourced from a site that had no detectable infections of *H. trivolvis* and that had a multiyear history of low (<0.04) to zero trematode prevalence. Microcosms were prepared 72 h in advance of the experiment to allow the substrate time to settle and reach ambient temperature (20–23 °C).

Snails within each infection status (yes or no) and infection identity (four taxa of trematodes) were randomly assigned to two experimental treatments: drought and perennial. To accomplish the random assignments, all snails were given a unique numeric identifier. We then generated a random number for each snail and sorted snails based on their random number. Those snails within the lower half of random values were assigned to one treatment, and those in the upper half of random values were assigned to the other treatment. We conducted this process within each infection group to ensure that infections were evenly distributed among the treatments. Snails were then relocated to experimental microcosms labeled with the treatment.

Acclimation to microcosms

We allowed snails to acclimate to the experimental microcosms for 3 days indoors (approximately 21 °C) before moving them to an outdoor location, where they acclimated for an additional 4 days (Fig. 1, "Acclimation"). In the outdoor location, the microcosms were kept under polyethylene mesh shade cloth to reduce UV exposure and 1 mm thick clear plastic sheeting to limit precipitation. Blocking precipitation allowed for tight experimental control of the microcosms' water level. The location of the microcosms was randomized (following similar strategies as outlined above) to account for any potential differences in temperature and light among sections of the outdoor space. Microcosms were adjacent to one another in one long band (consisting of columns and rows), such that all were confined to the same space (precluding the need for a block effect in statistical models). Snails were not fed during the acclimation or drought period but relied on the biofilm that developed on the microcosm surfaces.

Simulated drought experiment

At the end of the acclimation period, microcosms in the drought treatment were dried down over 16 days (Fig. 1, "Dry-down"). We allowed dry-down to occur naturally (via evaporation) and manually adjusted water levels as necessary (through water addition or removal) to ensure a decrease of 0.5 cm per day until the water level reached the substrate. This resulted in a cumulative dry-down of 8 cm. After the dry-down was complete, microcosms were maintained in their experimental treatments, drought or perennial, for a 28-day "drought period" (Fig. 1). During this period, 0.5 cm of deionized water (DI) was added to the drought microcosms daily to ensure that the substrate did not entirely desiccate. This better reflected the water retention that occurs naturally in the ponds from which the snails originated. Concurrently, we added DI water to the perennial treatments to maintain a constant water level of 8 cm above the substrate. We used DI water in both treatments to avoid the accumulation of dissolved salts, which would result from continuous addition and evaporation of spring water.

During the dry-down and drought periods, we recorded snail burial and water chemistry parameters three times per week. Burial was assessed visually, and in cases when water clarity was low, we gently examined the walls of the container and surface of the substrate by touch to determine whether an unburied snail could be identified. Due to time limitations, we could not measure water chemistry (temperature and conductivity) for all 260 microcosms in 1 day. We therefore recorded these parameters from $\frac{1}{3}$ of the microcosms

on a given day, such that all microcosms had one weekly measurement of water chemistry. Water temperature (degrees Celsius) and conductivity (microsiemens per cm) were measured using a handheld multiprobe (Hatch Pocket Pro Multi2).

After the simulated drought period, all drought containers were rehydrated (Fig. 1, "Rehydration") to their original water levels (8 cm) and left for 3 days in the outdoor location. We then collected post-experiment data (Fig. 1) for all snails in the two treatments, including burial status, survival, and trematode infection. Rather than shed snails for cercariae, we dissected snails for their final assessment of infection. It is well known that shedding snails for cercariae can yield false negatives (Curtis and Hubbard 1990). The final assessment, via dissection, then allowed us to establish whether any infections were missed during the pre-experiment data collection. Such snails were retroactively labeled as infected and given the appropriate parasite identity based on morphological features of cercariae released during dissection.

Analyses

How do drought and infection influence snail mortality? To explore the impacts of simulated drought and trematode infection on snail survival, we constructed a generalized linear model (GLM) that treated snail mortality as a binomial response (1 = snail mortality; 0 = snail survival). Using a logit link, we statistically explained snail mortality as a function of snail body length (*z*-scaled mm), trematode infection status (infected or uninfected), experimental treatment assignment (drought or perennial), all two-way interactions between pairs of predictors, the three-way interaction among these predictors, and an error term with a binomial distribution. We did not include trematode taxon as a predictor of snail mortality because of sample size limitations.

How do drought and infection influence snail burial? To quantify the effects of simulated drought and trematode infection on snail burial within microcosms, we built further GLMs in which each individual snail's "burial tendency" was considered the response. Burial tendency was simply the proportion of observations in which an individual snail was observed to be buried; it was calculated as the mean of each snail's buried (1) or unburied (0) observations. Because burial tendency ranged from 0 to 1, we specified a model with a beta distribution (with a logit link), which is appropriate for proportional response variables (Bolker et al. 2009). Given that beta distribution models cannot handle exact 0s and 1s, burial tendencies of 0 or 1 were manually adjusted to 0.00000001 and 0.99999999, respectively. We considered how drought and infection affected burial tendency for snails assessed during the 16-day dry-down period (Fig. 1). During this period, snails within different treatments experienced different conditions (i.e., reductions in water volume and any associated changes to water chemistry) that had the potential to affect snail biology and behaviors. Our model statistically explored individual-level burial tendency as a function of experimental treatment assigned (drought or perennial), snail body length (*z*-scaled mm), trematode infection status (infected or uninfected), and a beta error term. To substantiate results from this analysis, we conducted the same analysis on snails assessed during the acclimation period. Because snails in the two treatments experienced identical conditions during acclimation, we expected no difference in their behaviors. A lack of a difference during acclimation could then substantiate that any differences observed during dry-down were a treatment effect.

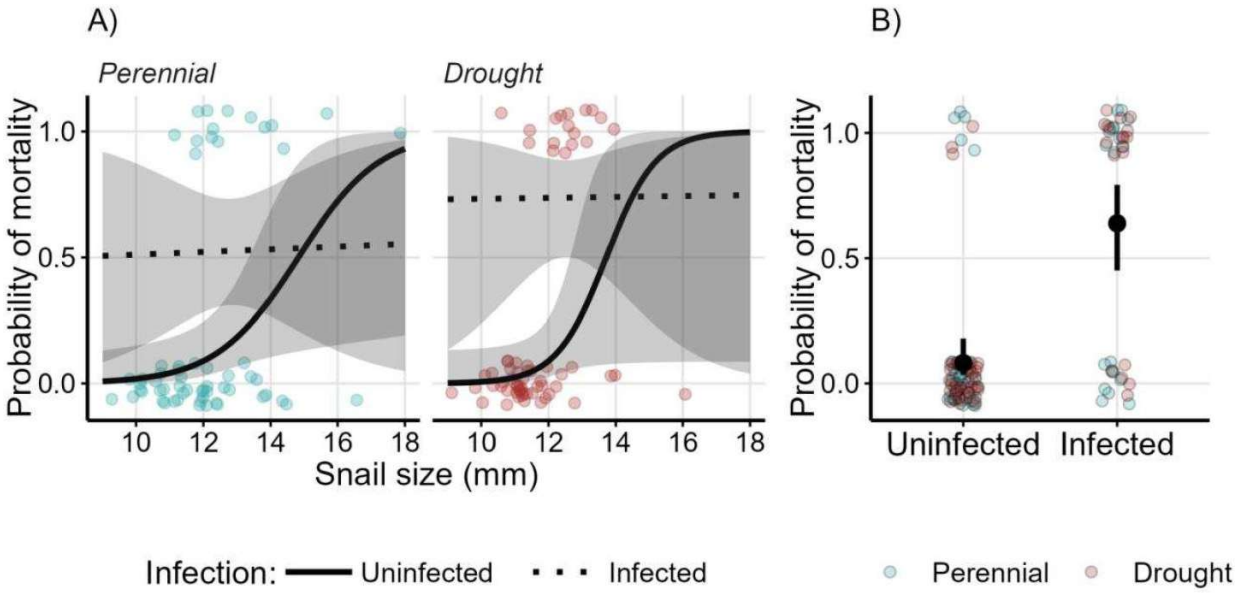
We fit all models using the glmmTMB package (Brooks et al. 2017) within R Statistical Software (version 4.3.1) (R Core Team 2024). We diagnosed model convergence and fit using functions in both the glmmTMB and DHARMa (Hartig 2022) packages. All code and data required to recreate our analysis workflow are available at https://doi.org/10.6084/m9.figshare.25481020.

Results

We collected and transported 124 *Helisoma trivolvis* snails to our experimental facility where they were non-lethally evaluated for trematode infection, subjected to either drought ($n = 64$) or perennial ($n = 60$) treatment conditions, and eventually dissected to obtain final trematode infection status. Among these snails, 31% were infected by trematodes belonging to four taxa (Table S1). These infections were dominated by a single taxon, *Alaria marcinae* (La Rue, 1917) Walton, 1949 ($N = 25$, representing 66% of infections), with additional infections represented by *Ribeiroia ondatrae* ($N = 7$; 18% of infections), *Echinostoma* sp. ($N = 4$; 11% of infections), and *Cephalogonimus americanus* Stafford, 1902 ($N = 2$; 5% of infections). While each of these parasites exhibits similar virulence by castrating the snail host during its parthenita stage (characterized by presence of sporocysts or rediae in the gonad), we note that the four *Echinostoma* sp. infections were at the metacercaria stage, which has a different life history strategy (Zbikowska 2011). Nonetheless, we did not analyze differences among parasite taxa owing to limited statistical power. About 78% of snails buried themselves at least once during the acclimation and dry-down periods, and 26% of snails died by the end of the experiment. During the dry-down period, temperature trends were similar across microcosms and conductivity increased in the drought treatment microcosms as expected (Fig S1).

Snail size and infection by trematodes—but not simulated drought—altered freshwater snail mortality. In a GLM-relating snail size, infection status, and treatment group to the probability of snail mortality, snail size ($\beta_{z\text{-scaled mm size}}$ 95% CI = 0.13–2.20; $p = 0.028$) and trematode infection (β_{infected} 95% CI = 0.87–4.09; $p = 0.003$) had statistical effects on snail mortality in addition to a marginally significant statistical effect of infection status-by-size interaction ($\beta_{\text{size*infection}}$ 95% CI = −2.47–0.20; $p = 0.095$) (Fig. 2A). On average, the odds of an infected snail dying during the experiment was ∼12 times greater than the odds of an uninfected snail dying (Fig. 2B). Among uninfected snails, larger individuals were more likely to die: the odds of an individual dying increased ∼3-fold for every 1.4 mm increase in length (Fig. 2A). Based on this model, there was no clear statistical relationship between the probability of snail mortality and the drought treatment (β_{drought} 95% CI = −1.84–1.74; $p = 0.96$), treatment-

Fig. 2. Snail size and infection by trematodes—but not simulated drought-altered freshwater snail mortality. In a microcosm experiment, the probability of *Helisoma trivolvis* snail mortality increased with body size among uninfected snails (A) and with infection by trematodes (B), but did not vary between drought and perennial treatments (A).



by-size ($\beta_{\text{drought*size}}$ 95% CI = −1.46–2.97; $p = 0.50$), treatment-by-infection ($\beta_{\text{drought*infected}}$ 95% CI = −1.37–3.34; $p = 0.41$), or treatment-by-size-by-infection ($\beta_{\text{drought*size*infected}}$ 95% CI = −3.37–1.83; $p = 0.56$; see Table S2 for the full set of results).

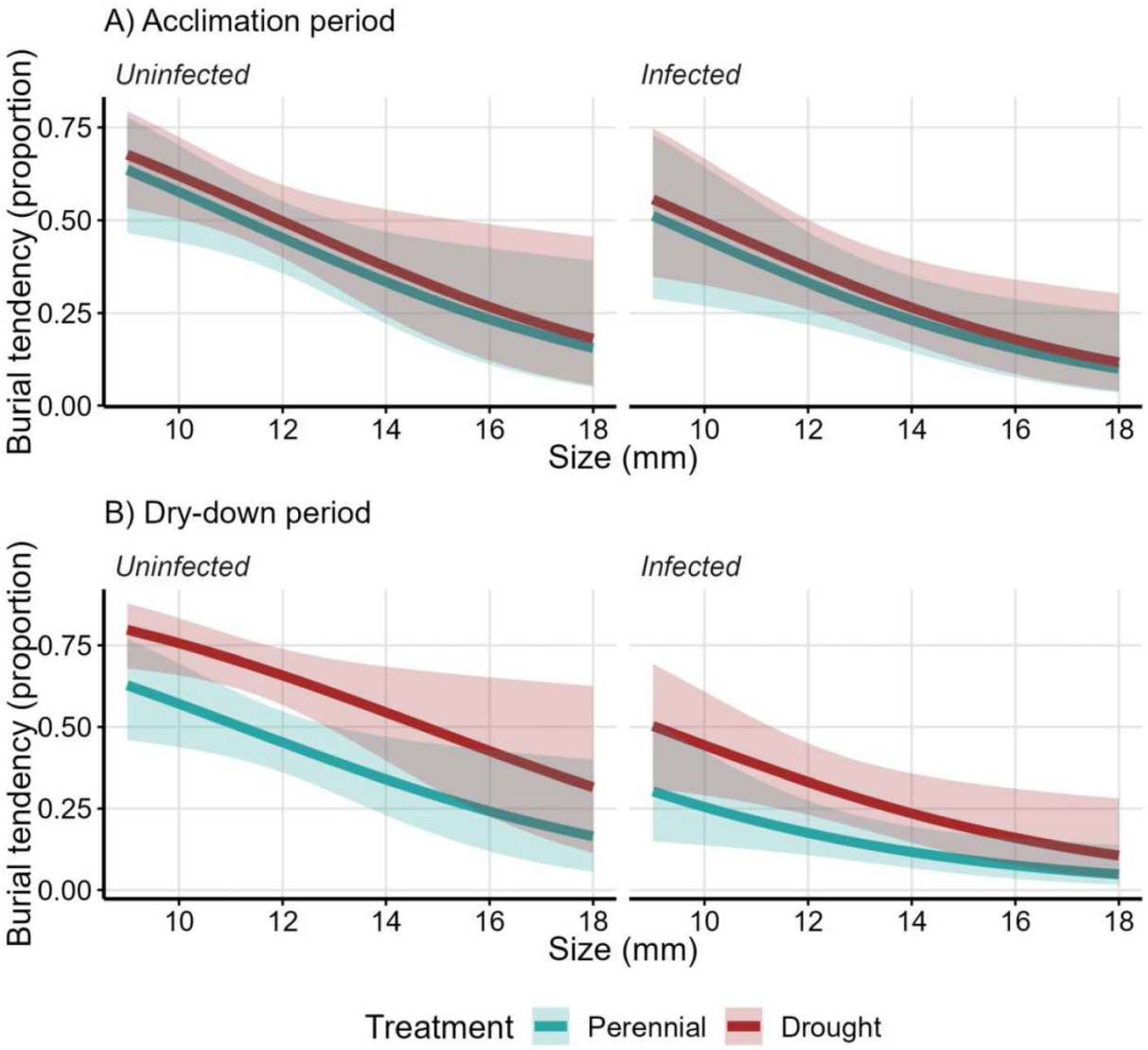
Simulated drought increased the likelihood of burial by freshwater snails. During the acclimation period (Fig. 1), where snails were subject to the same conditions, there was no statistical difference in burial tendency among snails assigned to the perennial treatment versus those assigned to the drought treatment ($\beta_{\text{drought [acclimation]}}$ 95% CI = −0.33–0.69; $p = 0.49$; Fig. 3A). However, during the dry-down period (where snails in the two treatments were experiencing different conditions), snails subjected to experimental drought were more likely to be found buried, relative to those experiencing perennial conditions ($\beta_{\text{drought [dry-down]}}$ 95% CI = 0.34–1.35; $p = 0.001$; Fig. 3B). On average, during the dry-down period, the odds that a snail exposed to experimental drought buried itself were 2.33-fold greater than the odds that a snail exposed to perennial conditions buried itself. Notably during both the acclimation and the dry-down experimental periods, larger snails and those that were infected were less likely to bury (all $p < 0.05$, see Table S3 for the full set of results; Fig. 3). Thus, infection and size may alter drought-associated behaviors of snails with consequences for burial and persistence.

Discussion

Shifting global climate is changing the biology of species in ways that can dramatically shape the ecology of natural systems. Controlled experiments are a valuable tool for isolating these climatic effects and generating predictions for how environmental change will affect species and their contributions to ecosystems. We experimentally assessed the effects of water loss on snail–trematode interactions to determine how freshwater snails might respond to anticipated drought in natural systems and whether parasites might modify this response (Morton and Silliman 2019). Drought, which we simulated within microcosms, altered snail behavior but not mortality. Snails maintained under desiccated conditions (the drought treatment) were more likely to bury themselves under the sediment than snails that were maintained with constant water levels (the perennial treatment). Despite the prolonged period of simulated drought, which included 1 month exposed to late summer temperatures, snails in the drought treatment did not exhibit higher rates of mortality than those in the perennial treatment. Rather, mortality appeared to be driven by an interaction between trematode infection and body size. Infected snails of all sizes and across treatments were more likely to die than uninfected snails; within uninfected snails, larger individuals had increased odds of mortality. Beyond drought effects, infection also had pronounced effects on snail behavior, as snails with trematode infections were less likely to bury themselves. Our experiment cumulatively points to interesting and potentially interactive consequences of drought and parasitism for freshwater snail populations and provides impetus for larger-scale experiments and field-based research.

Infected snails (of all sizes) and large uninfected snails were the most likely to die during the experiment, regardless of treatment. Indeed, infected snails were ~12 times more likely to die than uninfected snails and among uninfected snails, a size increase of 1.4 mm led to a three-fold increase in the odds of dying (Fig. 2). Existing research supports these factors as important drivers of snail biology, behavior, and survival. For instance, in agreement with our observations, a review of 113 field and laboratory studies of snail–trematode interactions reported that negative effects of infection on host survival were exceedingly common (Sorensen and Minchella

Fig. 3. Simulated drought increases the likelihood of burial by freshwater snails *Helisoma trivolvis*. In a microcosm experiment, there was no difference in burial tendency between freshwater snails in treatment and control groups prior to the initiation of treatment (i.e., during the acclimation period; A); in contrast, freshwater snails were more likely to bury themselves in sediment when they were subjected to experimental drying than when water levels were held constant (B). Further, smaller snails and those not infected by trematodes were more likely to bury, independent of treatment status.



2001). Trematodes can alter snail behavior and physiology, but this reduced survival is most likely a direct consequence of tissue damage and energetic demand caused by trematodes infecting and reproducing within snails (which typically also castrates snails). Our observation of greater mortality among larger snails stands in contrast to the results of Kalinda et al. (2018), who conducted a similar experiment and found that large snails were more likely to survive desiccation than small snails. Further, their study revealed that drought had a greater negative effect on survival than did size, whereas we did not detect any effect of drought on snail mortality. This lack of a drought effect was surprising and inconsistent with our a priori hypotheses, but may point to high resilience of *Helisoma* snails to desiccation, as has been noted elsewhere (White et al. 2008).

The drought treatment—where snails were held without water at elevated summer temperatures—did not ultimately result in greater levels of snail mortality than the perennial treatment. Evidence for an effect of desiccation on survival is mixed in the literature (White et al. 2008; Mostafa 2009; Kalinda et al. 2018; Zhang et al. 2023). Within our study, there are both statistical and biological explanations for this result. First, it is possible that the strong effects of snail size and infection on survival outweighed any effects driven by the abiotic environment. Importantly, the size and infection differences in our study were not noise, but represented critical axes of variation for the implications of our work. In considering how parasite transmission might be altered by drought, infection status (whether a parasite is present) and host body size (which shapes parasite reproduction) interact

to influence the total size of the infective parasite population (Johnson et al. 2024). Identifying whether mortality occurs differently depending on these factors can then yield predictions for parasite pressure following drought. But beyond statistical artifacts, another possibility for the similar survival rates is that the snails possessed traits that allowed them to withstand the simulated drought conditions. The snails used in our study were collected from a region in California that experiences a seasonally dry, Mediterranean climate. Our snail source ponds can be subject to dry-down and can have variable hydroperiods (Moss et al. 2021), and snail burial behavior may be an adaptation to contend with limited water availability (Poznańska et al. 2015; Kalinda et al. 2018). If burial buffers against water loss, then snails may have been able to escape desiccation in the drought treatment, decoupling their survival from environmental drying.

In support of burial as an adaptive behavioral response to dry-down, we found that the odds of burial for snails held in drought conditions were 2.3-times greater than for snails held in perennial conditions. This adaptive response has been well studied in aquatic snails that experience temporary water loss (Poznańska et al. 2015; Kalinda et al. 2018; Zhang et al. 2023) and can be triggered by drought-associated environmental cues, such as temperature and food availability (Wada and Yoshida 2000). It is plausible that such cues arose in our microcosms and provided signals for snail burial (Fig. S1). In addition to treatment effects, we also observed that snail body size and infection influenced the likelihood of burial, where large and infected snails were the least likely to bury. That large and infected snails were also the most likely to die raises several interesting questions for future research. Did the stress of infection manifest as a lower ability to bury (eg, Morgan et al. 2012)? Or is it possible that large snails and infected snails succumbed to mortality *because* they failed to bury? By altering surface-to-volume ratios, size may modify how environmental cues are detected, the time period over which desiccation occurs, and the ease with which an individual can bury into the substrate. Each of these processes—cue detection, water homeostasis, and locomotory behaviors—may be influenced by infection. There are thus important connections between size and infection that require clarification as we ask how snails and trematodes will respond to drought.

One of the limitations of our study design was that snails were assessed for survival at a single time point following administration of the treatments. Because we evaluated all snails at the terminus of the experiment, we did not have information on when a snail died. In part, this aspect of our study design arose from constraints inherent to the experimental set-up; we could not assess survival of buried snails without removing the sediment from the microcosm and extracting the snail. But this single time point led to challenges in interpreting the relationship between burial behavior and survival. While we expected that the choice to bury or not bury would have ramifications for survival, our study design meant that we could not statistically assess a causal relationship between these two factors. This is because, for snails that were unburied at the end of the experiment, we could not know whether they died because they did not bury, or whether death precluded their burial. Such confounding of mortality and burial is also why we limited our analysis of the latter to the dry-down period, likely making our results somewhat conservative. By providing precise information on when snails buried versus died, finer-scale temporal data could overcome these challenges and allow for a better understanding of the causal pathway. Future experiments would therefore benefit by collecting aggregate longitudinal data (i.e., taking down a subset of microcosms and assessing burial and survival at regular intervals over the duration of the study) (Kalinda et al. 2018).

The host–pathogen–environment interactions that we observed in this study may have been influenced by methodological factors. We collected specimens from the field and leveraged naturally infected snails in our study design, allowing us to study the representative variation within wild populations. Yet, doing so precluded the ability to control for and randomize parasite load, genetic variation, and prior environmental exposures. Indeed, the naturally occurring infections of our experimental snails were strongly dominated by one particular species (*Alaria marcinae*), which restricted our ability to get parasite-specific responses. While the four parasites in our study share many ecological similarities, there are certainly life history differences that could generate differences in their vulnerability to drought (McDevitt Galles et al. 2021). Future research efforts in which snails are experimentally infected with a diverse and highly replicated array of parasite taxa likely hold considerable value. Such experiments can improve our ability to predict parasite-specific responses to drought, and can enhance theory and understanding of which infections are drought-vulnerable and why.

Whether and how snails survive drought may have broad implications for the diversity and ecological functioning of aquatic systems. As benthic detritivores and periphyton grazers, snails are core consumers in wetlands and mediate connections between primary producers and secondary consumers. And as first intermediate hosts to a suite of trematode parasites, snails play key roles in supporting parasite life cycles. Our study was thus motivated by the idea that drought-driven reductions to snail populations (as well as differential mortality due to infection) could have ramifications for the presence and strength of aquatic interactions, including disease. While our study did not observe a conclusive effect of drought on snail mortality, it pointed toward additional biological drivers of death, namely, snail body size and trematode infection. The importance of these factors for mediating survival points to the relevance of life history and biotic interactions when diagnosing the effects of shifting abiotic conditions on snail populations.

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Data availability

All code and data to recreate data manipulation, model fitting, and creation of figures are publicly available at https://doi.org/10.6084/m9.figshare.25481020.

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Competing interests

The authors declare no conflicts of interest.

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Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/cjz-2024-0194.

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