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Opportunity or catastrophe? Effect of sea salt on host-parasite survival and reproduction — Source link

Annabelle O. Yu, J T Vannatta, Stephanie O Gutierrez, Dennis J. Minchella

Institutions: University of California, Berkeley, Purdue University

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Short title: Effect of sea salt on host-parasite interactions

Ao Yu[1], J. Trevor. Vannatta[1], Stephanie O. Gutierrez[1], Dennis J. Minchella[1]

1 Department of Biological Sciences, Purdue University,

915 West State Street, West Lafayette, IN 47907, USA.

* yu692@purdue.edu (AY); ao.yu@berkeley.edu (AY)

jvannat@purdue.edu (JTV); vanna006@umn.edu (JTV)

Author contributions

AY, JTV, SOG, and DJM conceived and designed the study. AY, JTV, and SOG conducted the study. AY and JTV analyzed the data. AY, JTV, SOG, and DJM contributed to writing and revising the manuscript.

Data availability:

All data and code used for this study are available at **https://github.com/vanna006/sea-salt-schisto**

Abstract

Seawater intrusion caused by anthropogenic climate change may affect freshwater species and their parasites. While brackish water certainly impacts freshwater systems globally, its impact on disease transmission is largely unknown. This study examined the effect of artificial seawater on host-parasite interactions using a freshwater snail host, *Biomphalaria alexandrina,* and the human trematode parasite *Schistosoma mansoni*. Four components were analyzed to evaluate the impact of increasing salinity on disease transmission: snail survival, snail reproduction, infection prevalence, and the survival of the parasite infective stage (cercariae). We found a decrease in snail survival, snail egg mass production, and snail infection prevalence as salinity increases. However, cercarial survival peaked at an intermediate salinity value. Our results suggest that seawater intrusion into freshwaters has the potential to decrease schistosome transmission to humans.

Author Summary

Climate change will have numerous impacts on many systems, including host-parasite systems. One mechanism by which climate change with impact host-parasite interactions is by rising sea levels flooding coastal regions, increasing salinity in many freshwaters. Host-parasite interactions are a key component of freshwater ecosystems, but the effects of sea water intrusion on host-parasite dynamics are largely unknown. In this study, we quantify the effects of sea salt concentration on the model host-parasite system,

Biomphalaria alexandrina and *Schistosoma mansoni*. We demonstrate a significant, negative relationship between sea salt concentration and host-parasite survival and reproduction. The increase in freshwater salinity associated with sea level rise has the potential to decrease parasite transmission and disease burden in humans and wildlife.

Introduction

Anthropogenic climate change, associated with increasing greenhouse gases, can change many environmental factors including temperature, pH, precipitation, as well as salinity in both terrestrial and aquatic systems. Increasing salinity in freshwater systems is often caused by rising sea levels and seawater intrusion [1,2]. Salt concentration (salinity) is a crucial abiotic factor that impacts many aspects of biotic interactions [3]. Increases in salinity of freshwaters will undoubtedly result in changes in organismal growth, reproduction, and survival, impacting entire food webs, and thus host-parasite interactions.

Parasites play a key role in freshwater communities [4], and they are commonly recognized for their ability to modify the growth, reproduction, and survival of their hosts [5]. They are also the etiologic agents of human disease. Therefore, understanding how abiotic factors and parasitic diseases interact in the context of climate change, more specifically increasing salinity, is critical in order to understand human disease transmission.

Rising seawater levels and seawater intrusion are affecting numerous bodies of water including the Nile River Delta. The salinization of coastal land in the Nile Delta is caused by the decrease in the Nile River's freshwater levels due to human activity and the increasing sea levels in the Mediterranean Sea [6]. Seawater will submerge large areas in the coastal zone of the Nile Delta in the near future, impacting host-parasite interactions in the region [7].One parasitic disease of humans acquired in the Nile Delta of Egypt is schistosomiasis, which is caused by the trematode *Schistosoma mansoni* [8]. Approximately, 12.7 million infected people are clustered in the Middle East and North Africa region, and Egypt's share of the burden is about 7.2 million [9]. Parasite transmission occurs when humans come in contact with secondary parasite larvae, called cercariae, which are released from infected snails in freshwaters. In areas with perennial irrigation such as the Nile Delta and the Nile River Valley, schistosomiasis prevalence is high (60% infection rate). In contrast, the infection rate is relatively low (6%) in districts of basin irrigation (commonly known as annual flooding) [10]. Development and shift from basin irrigation to perennial irrigation in Egypt has resulted in year-round availability of water in many districts making the likelihood of infection high [11], but the impact of seawater intrusion on parasite transmission in this system is unclear.

This laboratory study explores the effect of increasing salinity (as artificial seawater) on host and parasite success using the Egyptian strain of *Schistosoma mansoni* and its snail intermediate host, *Biomphalaria alexandrina*, to simulate the conditions in

the Nile Delta of Egypt. In order to assess the success of parasite transmission, we exposed snail hosts to the trematode parasite and monitored snail survival, snail egg mass production, infection prevalence, and parasite (cercarial) survival. We predicted lower snail survival and egg mass production as snails become stressed by osmoregulation and allocate less energy to reproduction [12,13]. We further expected to observe lower parasite infection prevalence in snails as salt concentration increased, since the parasite larvae that infect snails (miracidia) may be less successful due to increased energy expenditures required maintain osmolarity [14,15]. Finally, we predicted lower cercarial (the parasite larvae which infects humans) survival as salt concentration increased due to increases in energy expenditures on osmoregulation [16] and fewer available resources from stressed hosts [17].

Materials and Methods

Experimental reagents

Various sea salt brands were assessed to determine which most closely resembled the mineral concentrations of seawater (supplement **Figure S1**; **Table S1**). Based on these comparisons, Instant Ocean® Sea Salt was used to replicate seawater.

Salinity of the Mediterranean Sea is approximately 38 parts per thousand (ppt) [18], which we considered saturated seawater (100% salt solution) in this experiment. Salinity treatment groups consisted of 1% (0.38 ppt), 5% (1.9 ppt), 10% (3.8 ppt), and

15% (5.7 ppt) seawater solutions, which were chosen based on pilot data suggesting seawater concentrations of 20% and higher rapidly killed the snail hosts. The four salinity treatments were prepared from 100% stock solution (38 ppt) diluted with well water (the 1% solution), and the salinity of the final solutions were verified by using a handheld refractometer (Premium Aquatics). The 1% (0.38 ppt) salinity treatment was designated as the control for this experiment as this was the salinity of well water used for dilutions.

Experimental strain maintenance

We used *Biomphalaria alexandrina* snails and *Schistosoma mansoni* parasites, which both originated from Egypt. *B. alexandrina snails* were raised under controlled laboratory conditions (~25 °C, 12 hour light/12 hour dark*)*. The *S. mansoni* life cycle was maintained for the experiment using *B. alexandrina* snails and male Balb/c mice [19].

In order to limit the shock induced by sudden seawater exposure, 60 snails were acclimated for five days in each salinity treatment (total of 240 snails) before inclusion in this study and subsequent *Schistosoma* infections. In a pilot study, five days was shown to sufficiently control for initial mortality. During the experiment, snails were housed in individual 225 mL jars with Styrofoam (for egg laying) and were fed romaine lettuce *ad libitum.* Water in each jar was changed weekly to maintain salinity levels. Egg masses were counted weekly and removed from jars during cleaning.

122 ***Snail infections***

123 Mice were euthanized in accordance with Purdue Animal Care and Use
124 Committee Protocol # 1111000225 to isolate *Schistosoma* eggs. The collected eggs were
125 placed in freshwater for approximately 60 minutes to allow the miracidia (the infective
126 stage for the snails) to hatch. The resulting miracidia were transferred to well plates
127 containing snails and 10 mL of their respective salinity treatment. Each snail was exposed
128 individually using eight miracidia in the corresponding salinity treatment and left
129 overnight.

130

131 ***Data collection***

132 Snail survival and egg masses laid were recorded weekly for eight weeks. In
133 weeks four to seven post parasite exposure, snails from each group were transferred into
134 well plates filled with 10 mL of corresponding salinity treatment solution and cercarial
135 release was observed. After one hour under artificial light, 1 mL of mixed solution was
136 drawn from each well, and the number of cercariae was counted for each snail [20].
137 Snails in which at least 10 cercariae were detected from the randomized 1 mL out of 10
138 mL were designated as infected. Only 13 out of 948 observations (1.37%) resulted in the
139 snail being assigned as uninfected while having countable cercariae from the randomized
140 1 mL sample. Thus, cross-contamination was infrequent, and likely had little impact on
141 our results.

Eight weeks after parasite exposure, cercariae from the 0.38 ppt salinity (control) treatment were collected and were used to examine cercarial survival in various salt solutions. Cercarial survival was assessed using only cercariae from the 0.38 ppt salinity treatment due to high snail mortality and low infection prevalence in the other treatment groups. Approximately 20 cercariae were placed into individual wells with 1ml of the four salinity treatment solutions. Cercariae survival was checked at 4h, 8h, 12h, and 24h after release from the snail, and cercariae were removed if little to no movement was detected. Following the 24h check, all cercariae were euthanized with ethanol and counted to determine the exact number of cercariae in each trial.

Statistical analyses

Snail survival was analyzed with a proportional hazard regression model using the survminer and survival packages in R [21,22]. Snail egg mass production was only compared between uninfected snails to remove the confounding influence of parasitic castration. Even after removal of infected snails, the data had considerable zero-inflation. To account for this, we used a zero-inflated, negative binomial, mixed effects model created in the glmmTMB package to analyze snail reproduction [23]. Differences in infection prevalence between salinity treatments were examined using a binomial General Linear Model. Cercarial survival was analyzed with the coxme package using a mixed effects proportional hazard model[24]. This was done to control for non-independence as

multiple cercariae were taken from the same snail. In these models, salinity treatment was used as a fixed factor and individual snail ID was used as a random factor. All pairwise comparisons were made using the emmeans package with the multivariate t distribution for p value correction[25]. All analyses were performed in R version 3.6.3 [26].

Results

Effect of seawater on snail survival

Survival probability of infected and uninfected snails in the 0.38 ppt control treatment did not differ significantly over the 8-week experiment ($P > 0.05$). Therefore, we compared snail survival probability across all salinity treatments regardless of infection status (**Figure 1**). All salinity treatments were significantly different from each other except for 3.8 ppt and 5.7 ppt ($P > 0.05$). Snails in the 0.38 ppt control treatment had the highest survival probability, while the 3.8 ppt and 5.7 ppt salinity treatments had lower survival than the 1.9 ppt salinity treatment (**Figure 1;** For coefficients and pairwise comparisons see supplement **Table S2**).

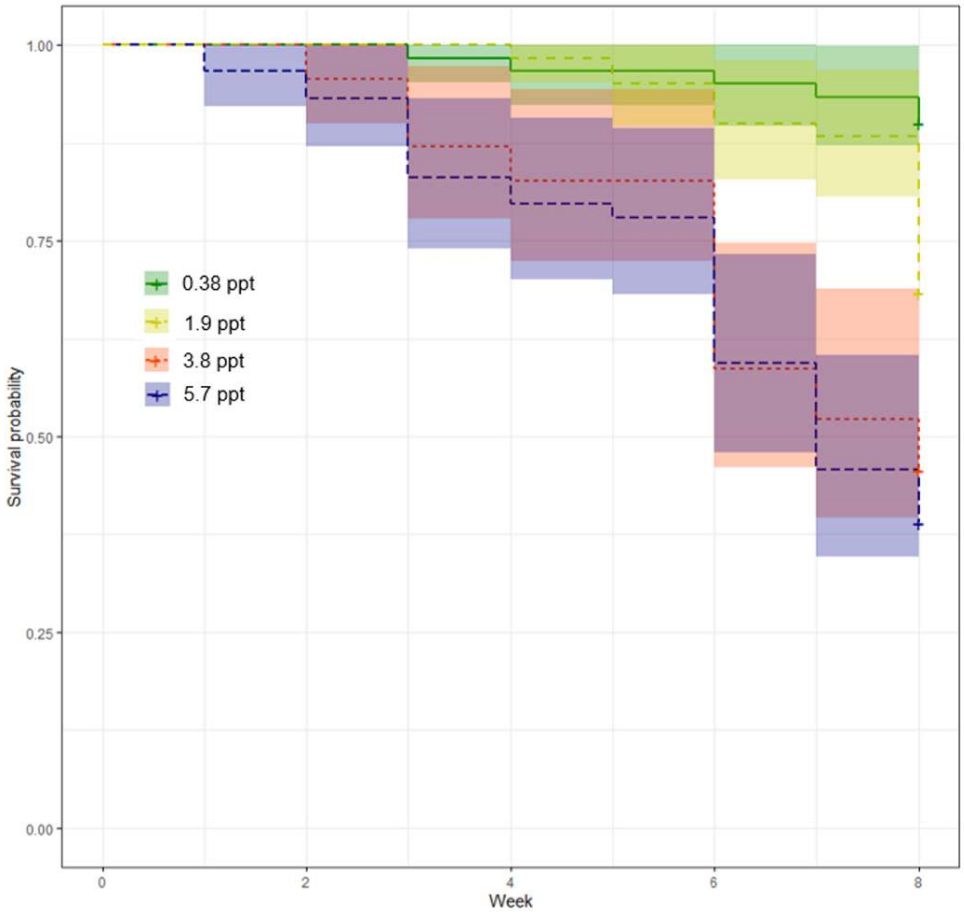

Figure 1. Probability of survival for snails in treatment groups of 0.38 ppt, 1.9 ppt, 3.8 ppt, 5.7 ppt salinity treatments. Snail survival in 0.38 ppt is significantly higher than 1.9 ppt ($P < 0.05$), 3.8 ppt ($P < 0.0001$), and 5.7 ppt ($P < 0.0001$). The survival probability of 1.9 ppt is significantly higher than 3.8 ppt and 5.7 ppt salinity treatments ($P < 0.05$). For coefficients and pairwise comparisons see supplement **Table S2**.

Effect of seawater on snail egg production

Snails infected with *Schistosoma mansoni* eventually become castrated and cease producing egg masses [27]. However, since infection prevalence was lower in the 1.9 ppt, 3.8 ppt, and 5.7 ppt treatments, the decrease in egg mass production was likely in response to increases in salinity (**Figure 2**). Snails from the 0.38 ppt control treatment

had higher egg output than snails in the 3.8 ppt ($P < 0.05$) and 5.7 ppt ($P < 0.0001$) salinity treatments. The 1.9 ppt treatment had a higher output than 5.7 ppt ($P < 0.0001$), and 3.8 ppt has a higher output than 5.7 ppt ($P < 0.05$). For coefficients and pairwise comparisons see supplement **Table S3**.

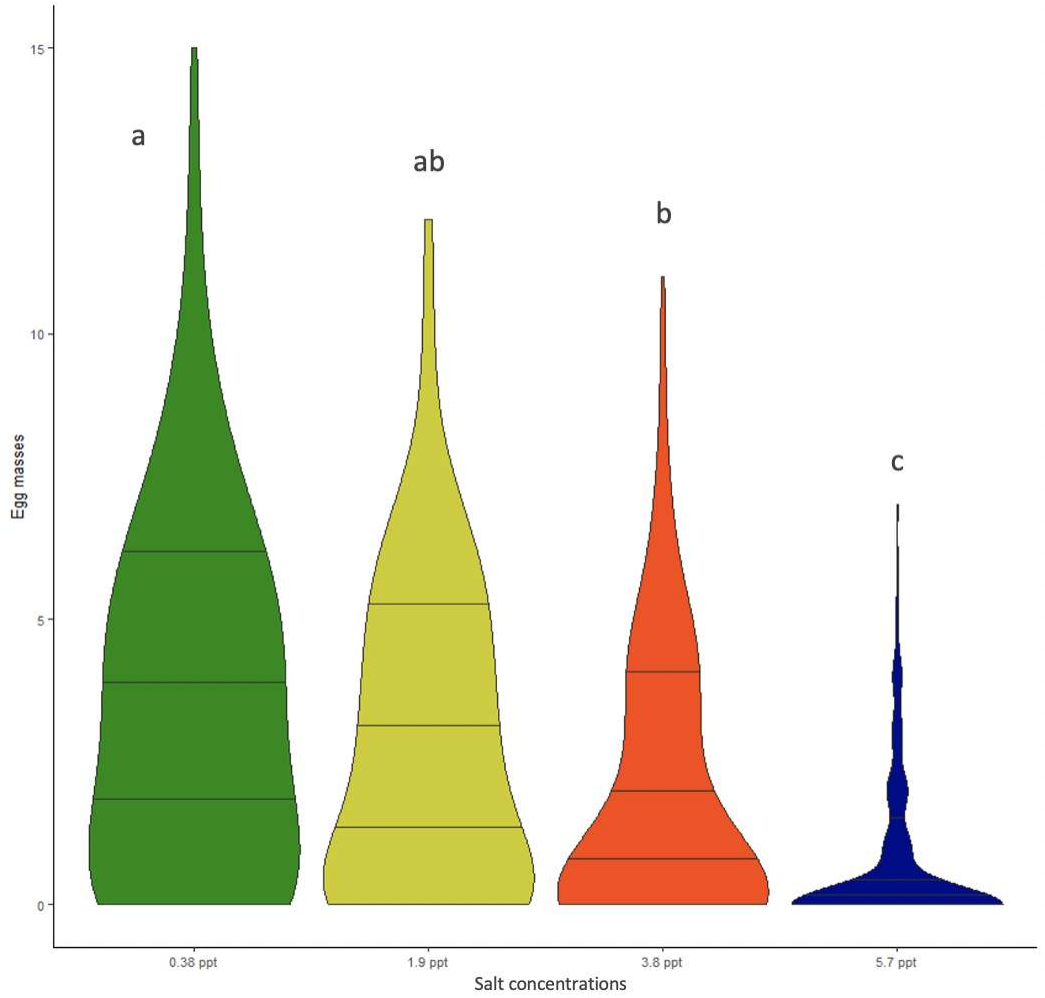

Figure 2. Egg mass output for uninfected snails in treatment groups 0.38 ppt, 1.9 ppt, 3.8 ppt, and 5.7 ppt. Snails in the 0.38 ppt salinity treatment had significantly higher egg mass output than 3.8 ppt ($P < 0.05$) or 5.7 ppt ($P < 0.0001$). Snails in the 1.9 ppt and 3.8 ppt treatments also had a higher output compared to the 5.7 ppt treatment ($P < 0.0001$ and $P < 0.05$, respectively). The horizontal lines on the violin plot represent data quantiles of 25%, 50%, and 75%. Letters above bars indicate significant differences in egg mass production, with different letters representing significant differences. For pairwise comparisons see supplement **Table S3**.

Effect of seawater on infection prevalence

Infection prevalence differed among the salinity treatments with a 70% prevalence in the 0.38 ppt treatment, 16% for 1.9 ppt treatment, 13% for 3.8 ppt treatment, and 9% for 5.7 ppt treatment. Snail infection prevalence in the 0.38 ppt control treatment had significantly higher prevalence than the other 3 treatments ($P < 0.0001$; For coefficients and pairwise comparisons see supplement **Table S4**). However, the prevalence levels in the 1.9 ppt, 3.8 ppt, and 5.7 ppt salinity treatments were not significantly different from each other ($P > 0.05$) (**Figure 3**).

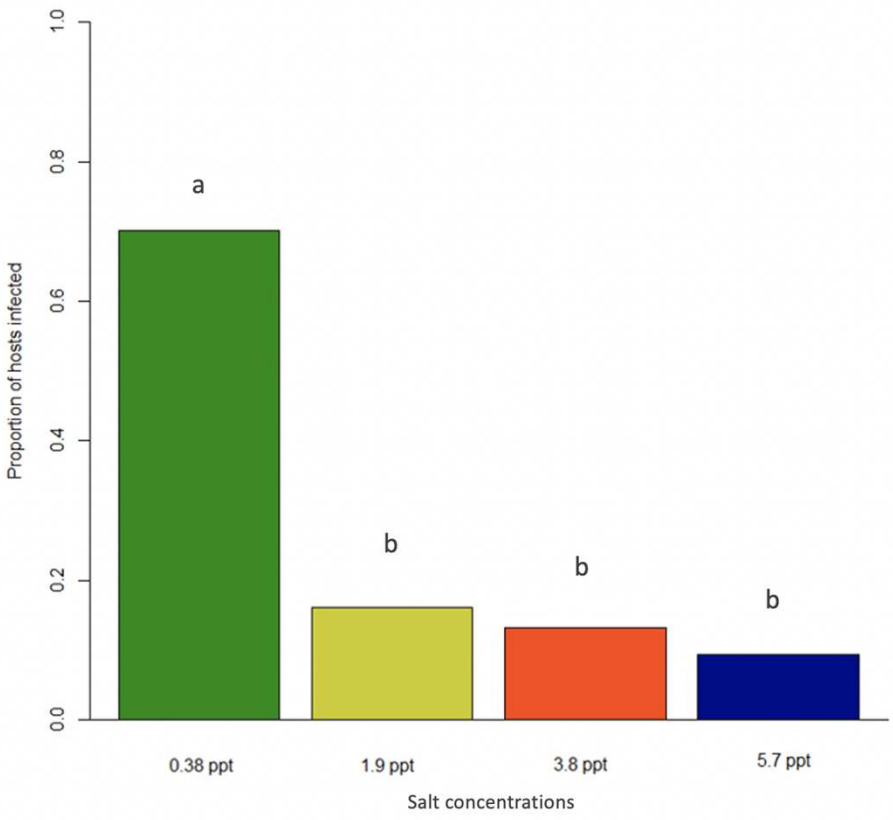

Figure 3. Snail infection prevalence in 0.38 ppt, 1.9 ppt, 3.8 ppt, and 5.7 ppt salinity treatments after exposure to *Schistosoma mansoni* miracidia. Different letters above bars indicate significant differences in infection prevalence among the treatments. For pairwise comparisons see supplement **Table S4**.

Effect of seawater on cercarial survival

Cercariae in the 3.8 ppt salinity treatment had the highest survival rate. The 5.7 ppt salinity treatment had the next highest survival rate, followed by the 1.9 ppt treatment (**Figure 4**). Interestingly, the cercarial survival was lowest in the 0.38 ppt (control) treatment with this treatment having significantly lower survival than the 1.9 ppt ($P < 0.05$), 3.8 ppt ($P < 0.0001$), and 5.7 ppt treatments ($P = 0.0001$; For coefficients and pairwise comparisons see supplement **Table S5**). The 1.9 ppt salinity treatment was significantly lower than the 3.8 ppt treatment ($P < 0.05$) but was not different from the 5.7 ppt treatment ($P > 0.05$). The 3.8 ppt salinity treatment was significantly higher than the 5.7 ppt treatment ($P < 0.05$).

Discussion

Seawater intrusion caused by climate change is an on-going issue in the Nile Delta of Egypt. How seawater intrusion will impact freshwater parasite-host interactions and disease prevalence is of importance, especially in the transmission of human schistosomiasis. In this study, we explored the effect of seawater on *Schistosoma mansoni* infection success in the freshwater snail *Biomphalaria alexandrina*. Experimental conditions were designed to mimic seawater intrusion as it occurs in the Nile Delta of Egypt. We investigated crucial factors that contribute to host and parasite

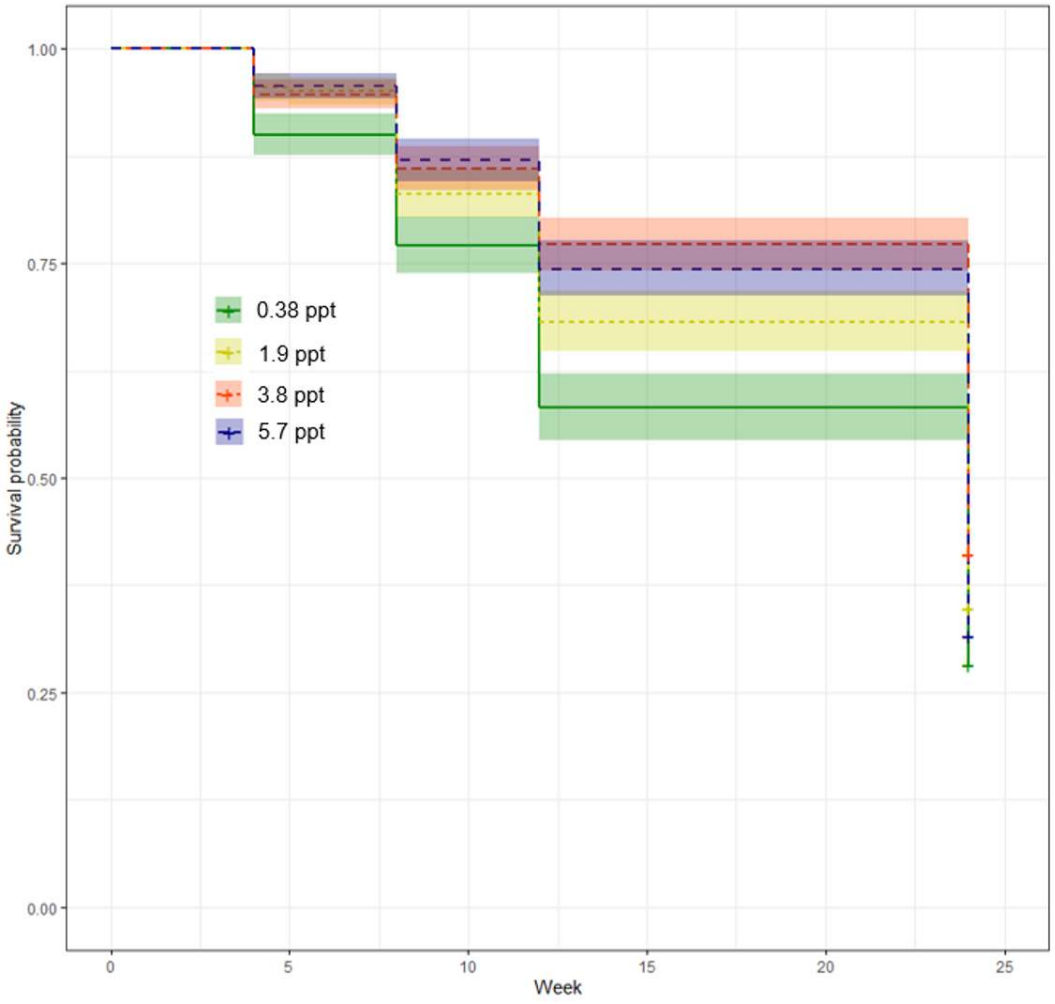

Figure 4. Survival probability of cercariae in 0.38 ppt, 1.9 ppt, 3.8 ppt, and 5.7 ppt salinity treatments over a 24-hour period. All four treatment groups are significantly different from one another except for 1.9 ppt and 5.7 ppt ($P > 0.05$) For pairwise comparisons see supplement **Table S5**.

interactions such as snail survival, snail egg mass production, infection prevalence, as well as parasite (cercarial) survival. Our results demonstrate that snail survival (**Figure 1),** snail reproduction (**Figure 2**), and snail infection prevalence (**Figure 3**) decreased as seawater concentrations increased across treatment groups. Additionally, cercarial

survival showed a nonlinear response to seawater concentrations with the 0.38 ppt treatment having the lowest survival while cercariae in 3.8 ppt had higher survival than those in 0.38, 1.9, and 5.7 ppt treatments (**Figure 4**).

To our knowledge, this study is the first to investigate the impact of seawater on the *Biomphalaria alexandrina – Schistosoma mansoni* host-parasite interaction using environmentally realistic seawater solutions [28]. Previous studies have shown that the fecundity and survival of snails is adversely affected by salinities as low as 1 ppt, with significant reductions occurring between 3.5 and 4.5 ppt resulting in progressive elimination of snails [14,29]. Additionally, our result demonstrating higher cercarial survival rates at intermediate seawater concentrations is supported by previous studies, however, the salinity at which survival peaks varies among different host and parasite species and strains [30–32]. Despite the observed decreases in snail fitness and parasite transmission at higher seawater concentrations, production of infective cercariae proceeded successfully in concentrations of seawater up to 5.7 ppt suggesting that although parasite burden may be lessened, infections could still occur as sea levels rise [15 and the current study]. Of course, cercarial survival is not necessarily predictive of cercarial infectivity. This limits our ability to fully quantify the impact of rising sea levels may have on parasite transmission to humans.

We speculate that these results are caused primarily by osmotic stress and energy allocation to osmoregulation. Osmoregulation is a critical, energy-costly function of a

normal cell to maintain fitness [33]. Organisms under osmotic pressure will have less energy to allocate to growth and reproduction. In our experiment, this likely led to the decrease in snail survival and snail egg mass production. Parasite larvae, such as miracidia that infect snails, are also affected by osmotic stress in the process of finding and infecting hosts, causing lower infection prevalence as salinity increased. Some authors have suggested that in contrast to miracidia, cercariae, which infect humans, require less energy for osmoregulation due to the lower difference between external and internal salinity to a certain threshold [34]. Therefore, cercariae seem to possess a higher tolerance than their snail hosts to increasing salinity, which may drive the non-linear relationship between cercarial survival and seawater concentration [34 and references therein].

Conclusions

Taken together, reduced survival, reproduction, and infection prevalence in snail hosts with increasing salinity will lead to lower snail population sizes and potentially fewer schistosomiasis infections in areas with seawater intrusion. However, the ability of organisms to rapidly adapt to changing conditions cannot be overlooked. Although a decrease in human schistosomiasis might be expected, higher salt concentrations did not completely halt snail reproduction, snail infection, or cercarial release, suggesting parasites could still potentially infect humans, continue the life cycle, and adapt to alterations in salinity.

We have shown that increasing concentrations of seawater in freshwater systems (such as those which will occur with rising sea levels) can have a significant impact on host-parasite interactions. Here, we have focused solely on the interactions between host and parasite, but these interactions are nested within complex food webs. Parasites and their hosts can also function as prey in an ecosystem, and the changing salinity can affect these relationships and parasite transmission [36,37]. Certainly, additional factors should be evaluated to accurately assess the future trend of schistosomiasis transmission in areas with rising sea levels. Besides salinity, climate change will also influence temperature, pH, rainfall, flooding, and drought [35]. Snail fitness is likely impacted by temperature alterations and drought, and these alterations certainly impact snail mortality and parasite production [20,38–43]. Our study reveals how a single abiotic factor, salinity, can play a significant role in disease transmission. Further investigation of the role of multiple environmental factors, food web interactions, and rapid evolutionary responses of hosts and parasites to sea level rise will be needed to more accurately evaluate the future of disease transmission in these altered ecosystems.

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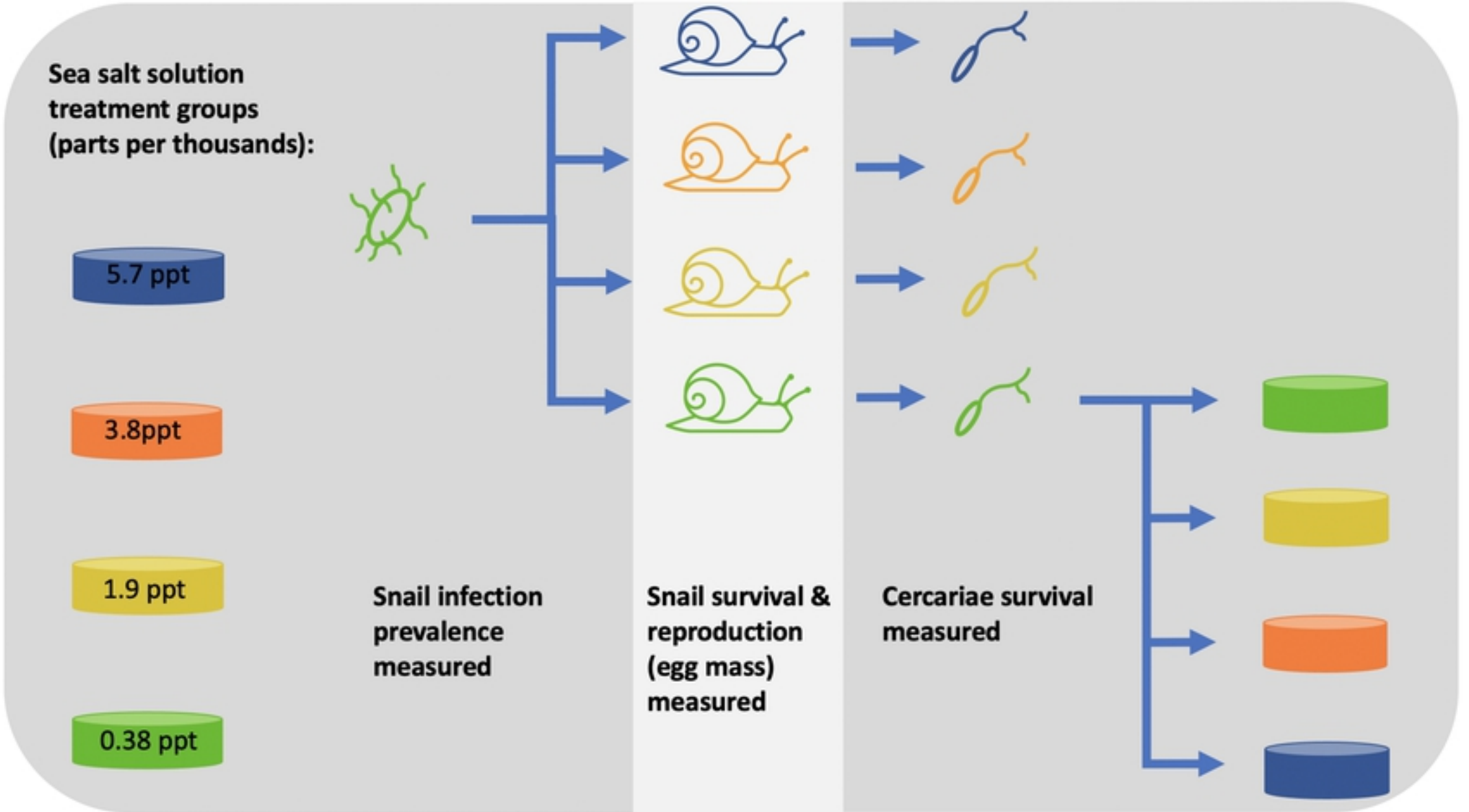

Graphical abstract