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Areej Nazir March 7, 2025

Nutrient Dynamics in Schistosomiasis Transmission: Examining Snail Ingestion Rates Under Varying Resource Conditions

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Abstract

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By Areej Nazir

Schistosomiasis is a parasitic disease caused by trematode worms that relies on an intermediate aquatic snail host for transmission to humans. Infected snails release cercariae, the free-living larval stage of the parasite, which penetrates human skin to initiate infection. With no vaccine and limited treatment options, mitigating the burden of schistosomiasis may be more effectively achieved by through directly modifying the intermediate snails host communities. Traditional epidemiological models have assumed that infection risk is directly correlated with the density of infected snails, but recent research has suggested that cercarial production is significantly impacted by resource availability. Endemic regions of schistosomiasis frequently face fluctuations in resource availability, driven by both natural causes and human activities, which contribute to an excess of nutrients in the environment. If snail resource availability drives human infection risk, it is important to integrate ecological factors into current schistosomiasis control strategies. This study examined whether snails modify their ingestion based on the quality of food that is provided with the hypothesis that snails would ingest lesser quantities of nutrient-rich foods. We fed uninfected *Biomphalaria glabrata* snails varying nutrient enriched foods through a randomized block feeding design. Data was collected on their size, survival, and ingestion. We then created a model for ingestion that incorporated size, ingestion rate, food dissolution, and food availability. Fitting this model to our data indicated that uninfected snails had similar ingestion rates across all food treatments. This study seeks to determine how snail ingestion is modified in response to nutrient-enriched foods, to better understand how nutrient availability influences feeding patterns and potential implications for schistosomiasis control.

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INTRODUCTION

Schistosomiasis is a parasitic disease that serves as a significant public health issue in tropical and sub-tropical regions. Present in 78 countries and endemic in 51, it affects an estimated 240 million people per year (WHO, 2023). Infections in humans cause chronic inflammation as schistosome eggs become trapped in tissues — resulting in damage to organs such as the liver, intestines, urinary tract, and central nervous system (Verjee, 2019). Schistosomiasis is also a preventable disease; the World Health Organization categorizes schistosomiasis as a Neglected Tropical Disease (NTD), a category of diseases that disproportionately affect underserved populations in tropical and subtropical regions. The consequences of schistosomiasis range from significant economic hardship to disability, damage to internal organs, and severe illness (WHO, 2023). This includes poor and rural populations who rely on and come into contact with parasite-infected freshwater during bathing, domestic labor, and agriculture (WHO, 2023). Children are uniquely harmed by infection with 70.9 million of the 90 million treated for the disease in 2016 being school-aged children (Verjee, 2019). In children, the damage of the infection may result in anemia, malnutrition, and a lack of growth (Osakunor et al., 2018). Given the widespread impact of schistosomiasis on vulnerable populations and the intricate relationship between the parasite, freshwater snails, and the environment, schistosomiasis is both a significant public health issue and an important topic of ecological study.

There are two major forms of schistosomiasis, intestinal and urogenital, which are caused by six species of *Schistosoma* trematode worms. Intestinal schistosomiasis is caused by the species *Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma guineensis*, *S. intercalatum*, and *Schistosoma mekongi*; Urogenital Schistosomiasis is caused by *Schistosoma haematobium*

(WHO, 2023). This research focuses on the strain *Schistosoma mansoni*, which is distributed through sub-Saharan Africa, parts of South America, and the Caribbean (CDC, 2024b).

Schistosomiasis is transmitted through a trematode parasite that has an intermediate aquatic snail host. Humans contract schistosomiasis through contact with water that contains cercariae, free-living schistosome life stages which emerge directly from infected snails. Parasite transmission occurs through a complex life cycle, as shown in Figure 1. Adult schistosome worms reproduce in the bloodstream of infected humans. These eggs are excreted through human urine or feces into freshwater sources, where they may hatch under appropriate conditions and release miracidia (CDC, 2024a). Miracidia infect intermediate snail hosts, where they asexually develop into cercariae (CDC, 2024a). Cercariae break through snail tissue and swim freely in the water, and upon contact with humans in infected water, burrow through exposed skin, and the cycle begins again (CDC, 2024a).

Treatment options for schistosomiasis are limited; with no vaccine developed, the main form of disease control is mass drug administrations (MDA) of praziquantel. However, MDA faces several challenges, with the availability and cost of the drug posing a significant barrier to effectively implement the drug into national control programs (Secor, 2015). MDA alone has not shown to be a promising treatment in completely killing all the parasites, especially in individuals with a high infection intensity. Because medical treatments in addressing schistosomiasis have been limited, a multi-faceted approach that focuses on prevention of the disease through sanitation efforts and snail control may be necessary to break the parasite's life cycle and effectively reduce the disease transmission. Control of schistosome transmission through snail control is complicated as intermediate snail hosts are hermaphroditic and have rapid population growth. Furthermore, previous research has shown a close and sensitive

relationship between environmental conditions and the release of cercariae (Civitello et al., 2022). A variety of factors such as natural seasonal changes, climate change, agriculture, and infrastructure development may influence transmission of the disease by affecting environmental conditions of the intermediate snail hosts (De Leo et al., 2020).

In support of this ecological perspective, Woolhouse and Chandiwana demonstrated that schistosome infection in snails varies significantly across short distances and time periods, shaped by fluctuations in temperature, rainfall, water flow, and human contact behavior (Woolhouse & Chandiwana, 1990). Their study used catalytic modeling and field data to show how environmental conditions can influence patterns of snail infection. These finding emphasize the need to study schistosomiasis not only as a medical issue, but also an ecological one that is influenced by environmental conditions and human activity. Disease ecologists support these efforts by studying the interaction between the environment, parasites, humans, and animals in the context of schistosomiasis transmission (Johnson et al., 2007).

Traditional epidemiological models have predicted that infection risk, through cercarial density, is directly correlated with the quantity of infected snails. This assumes that all infected snails are equally infectious (Civitello et al., 2022). Recent research, based on a bioenergetics-based model, has found that resource density is an important determinant of cercarial production, and therefore studying transmission potential requires looking at the availability of food resources for infected snails (Civitello et al., 2022). Key findings from this research have emphasized the role that resource competition plays in infection risk. In both mesocosm experiments and field sites in Mwanza, Tanzania, cercarial production was greater when infected snails had fewer competitors and the greatest access to resource availability (Civitello et al.,

2022). This emphasizes the need for a resource focused approach for schistosomiasis, understanding how the nutrient landscape drives schistosomiasis transmission.

Snail hosts inhabit ecosystems that are constantly changing. Ephemeral water bodies that are formed during rainy seasons offer temporary habitats to support snail survival and schistosomiasis transmission (Starkloff & Civitello, 2022). Fluctuations in the environment and resource availability impacts snail populations and disease dynamics. In endemic areas, snails can sustain themselves for prolonged periods of time in a variable nutrient landscape. However, this limitation can suddenly be released through environmental changes, particularly during the rainy season when nutrient runoff occurs from livestock and agricultural practices. These nutrients eventually enter snail habitats, where they become high quantity and high-quality food sources available for snail hosts. This environmental disturbance, where excess nutrients infiltrate a body of water, is called nutrient pollution. Nutrient pollution has been previously implicated in parasitic disease transmission through resource pulses (Johnson et al., 2007). During resource pulses, limitation of resources due to competition is released as the fertilization of crops and livestock manures contributes to elevated nitrogen and phosphorus levels in the environment. Since nutrient pollution particularly affects agricultural regions where schistosomiasis is prevalent, studying the influence of resource pulses on cercariae production and the ecosystem as a whole is a very relevant way to understand how disease can be mitigated through ecological interventions.

Living organisms have adapted to require a homeostasis of elements that support their growth and reproduction, with most organisms maintaining a homeostatic ratio of at least nitrogen (N) and phosphorus (P) given their biochemical importance (Bernot & Poulin, 2018). Nitrogen is an essential component for amino acids, RNA, and DNA, however it is often a

limiting nutrient despite its significant abundance on Earth (Todd, 2022). Phosphorus is similarly important in living organisms, providing energy and acting as a component of DNA and cell membranes (Todd, 2022). While snail hosts must receive these nutrients through their food, parasites remove nutrients directly from their intermediate hosts and have different elemental requirements for these nutrients as shown in Figure 2 (Bernot & Poulin, 2018). The differing homeostatic needs of the host and the parasite can result in an elemental imbalance that contributes to the virulence of the parasite, especially if the parasite steals nutrients before the host can utilize them (Bernot & Poulin, 2018). If a nutrient limitation exists, both growth and fitness are impaired. Snail hosts respond by modifying their excretion of nutrients by conserving the limiting nutrient and removing the nonlimiting nutrient (Bernot & Poulin, 2018). Thus, while N and P often appear together in the ecosystem, organisms do not necessarily use them in equal proportions — leading to one nutrient becoming limiting, even if both are present (Bernot & Poulin, 2018). In the case of a nutrient limitation, snail hosts may modify their excretion and egestion which further affects nutrient availability in the environment (Bernot & Poulin, 2018). A previous study examining N and P ingestion and excretion in infected snails found that parasites absorbed P faster than the host, resulting in infection snails excreting P at a slower rate and exacerbating P limitation in the broader ecosystem (Bernot, 2013). Another study on trematode parasites in snails found that parasite infection resulted in the host excreting more N, thereby increasing N cycling throughout the environment (Mischler et al., 2016). These interactions at the host-parasite level have larger-scale effects by influencing disease feedback in the environment. For example, a study of freshwater snails in a stream found that at the individual levels, snails were excreting less P per unit of N, but this exacerbated phosphorus limitation, showing how individual elemental use is linked to ecosystem environmental cycling

(Griffiths & Hill, 2014). A parasite can affect a host's behavior and life history traits in a way that cascades throughout the ecosystem. Therefore, a better understanding of whether snails and schistosome are limited by nitrogen, phosphorus, or both is crucial because nutrient limitations influence their survival, growth, reproduction, and cercarial output.

Given the importance of how disease transmission is specifically influenced by N or P limitation, this course of study looks at how snails change their ingestion of food with nutrient availability. Our research focuses on the freshwater snail *Biomphalaria glabrata*, a key intermediate host in the transmission of *S. mansoni*. The genus *Biomphalaria* includes several species that are involved in the transmission of different strains of schistosomiasis. *B. glabrata*, a species within the *Biomphalaria* genus, is relevant for our course of study as it's a well-documented host for *S. mansoni*, our strain of interest. When *S. mansoni* miracidia penetrates *B. glabrata*, how the resulting infection alters the metabolism, physiology, and immunity of the snail host has been studied (Adema et al., 2017). Genomic studies into *B. glabrata* suggest that it is a good representation of the *Biomphalaria* genus due to a shared evolutionary background and similar genomic sequences (Adema et al., 2017). Furthermore, the distribution of *S. mansoni* is strongly linked to the presence of susceptible *Biomphalaria* hosts that support its development into infectious forms (Habib et al., 2021). Notably, *B. glabrata* is remarkably adaptive and has strong invasive potential, characterized by its ability to survive and reproduce in a variety of environmental conditions. In Brazil, for example, *B. glabrata* was found to be present in 10 out of 11 aquatic habitats (Kloos et al., 2001).The adaptability of *B. glabrata* makes it a relevant model for investigation host-parasite interactions in schistosomiasis, especially in the context of varying resources.

In a previous experiment, we broadly examined how nutrient enrichment affected life history traits of *Biomphalaria* snails. We conducted a 10 x2 factorial lifetable, in which snails (n=390) were either exposed (n=120) or unexposed (n=270) to *S. mansoni*. Snails were either fed a baseline food, or food enriched with nitrogen (+N), phosphorus (+P), or both (+NP), with each enrichment being provided at either low, medium, or high levels of nutrient supplementation. We conducted weekly data on snail size, cercarial production in exposed snails, and egg production in unexposed snail. The results, shown in Supplementary Figure 1, indicate that phosphorus supplementation only produced statistically significant results at the highest level of supplementation, and only for cercarial production. In contrast, +N and +NP led to significant increases in snail growth and cercarial production, even at lower levels. Notably, +NP supplementation showed consistent robust increases across all measured traits.

These findings raise the question of whether these observed changes in growth and parasite production are the result of the nutrient content of the food itself, or by changes in feeding behavior, such as increased ingestion due to altered food composition. Previous studies have observed a phenomenon called active compensatory feeding, where organisms consume greater quantities of food in order to compensate for the poor nutritional quality of their food source (Fink & Von Elert, 2006). To better understand how snail hosts required and utilize nutrients, in this experiment we now ask: Does enrichment in snail food sources affect the amount food ingested? We provide nutrient enhanced food through randomized block feeding schedule and collect data on the effect of life history traits of snails such as survival, growth, and food ingested. This study aims to provide insight on how resource fluctuation impacts hosts nutrient needs, to better inform ecological and epidemiological models of schistosomiasis transmission. We hypothesize that snails will exhibit the greatest ingestion rates with nutrient

poor foods, and the least ingestion with nutrient rich foods. The hypothesis follows the idea that snails expend energy to eat, only as is required to reach a certain level of fitness. Higher quality food allows the snails to consume less, while still receiving the necessary nutrients. By studying how nutrient enrichment influences snail ingestion rates, this study seeks to enhance our understanding of the role N and P play in host-parasite dynamics, ultimately contributing to broader ecological and epidemiological models of disease transmission.

METHODS

Snail Selection, Maintenance, and Feeding Schedule

This experiment was conducted using *Biomphalaria glabrata* (n=100) snails of sizes varying from 2-16mm, which were fed weekly a treatment of either baseline food N-enriched, P-enriched, or N+P-enriched (Table 2). Snails were evenly divided into four groups with each group having approximately the same average snail size (Table 1). Snails were maintained in a 12:12 light:dark cycle at 26 degrees Celsius in COMBO, an artificial lake water that consists of 1X Algal Trace Elements (ATE), Animal Trace Elements (ANIMATE), and a B-vitamin complex (VIM), to ensure micronutrients were not limiting (Baer et al., 1999).

This experiment was conducted over the course of 4 weeks. Snails were fed three times a week: an experimental food treatment once a week, and two additional maintenance feedings of known high-quality laboratory food (Civitello et al., 2020). Weekly changes of the COMBO water were conducted for maintenance and to control algae growth. The experimental food treatments were designed to limit snails in N, P or both; food recipes are shown below in Table 2. Additionally, all foods were suspended to agar media to provide a precise way to measure the food quantity. Feeding occurred through a randomized block design feeding schedule so that by the conclusion of the four weeks, each group had been fed each food treatment once (Table 1).

Data Collection

Snail length was measured and recorded in millimeters (mm) for individual snails. Length was measured weekly after a full 3-day period of eating the experimental food. Snails were photographed and their shell length was digitally measured on ImageJ, an image analysis software (Schindelin et al., 2012). Length was recorded as the largest distance across the snail

shell. Snail survival was recorded on a weekly basis. Dead snails were removed from the experiment.

Ingestion was measured by feeding all snails a set quantity of food and removing any remaining food after ~72 hours. The unconsumed food was carefully removed and placed onto microscope slides, labeled with the corresponding snail ID. The slides were then dried at ~55 °C for ~48 hours to remove any fluctuations in measurement as a result of water. Dried food was scraped off the microscope slides and weighed in milligrams on a weigh boat as a measurement of unconsumed food. A control experiment, in which the full feeding amount underwent this process in snail-free cups, provided the original mass of the food. Subtracting unconsumed food from the original mass allowed us to determine the ingested mass of food for each snail.

Control Experiment

A control experiment was conducted to determine the weight of the set serving of experimental food and whether the experimental foods dissolved to the same extent in the COMBO. This control used the same as the experimental feeding and drying procedure, however, in cups of COMBO without snails that would ingest the food. Additional cups were used to increase the control sample size. As ingestion data depends on the amount of unconsumed food removed from the total food provided and each experimental food type has a different nutrient makeup, running this control experiment is important to determine whether dissolution is a factor that influences the results.

Data Analysis

Snails are not capable of infinite amounts of food; instead, their food intake is constrained by their physiological capacity. Therefore, while food availability does influence ingestion, this relationship is not linear. Snails face biological limitations that cause their ingestion rate to eventually plateau, such as their size and the rate at which they can consume food — regardless of food availability. This pattern follows a Holling Type II functional response, which describes how the rate an organism consumes food changes as the food becomes more or less abundant; the relationship is initially linear, but plateaus when the consumer reaches a saturation point and is physiologically unable to consume food at a faster rate.

While this experiment collected data on the initial and remaining quantity of food, there are two different factors that this loss of food mass can primarily be attributed to: dissolution and ingestion. The Ordinary Differential Equation (ODE) below describes this relationship, where F_i represents the amount of food of food treatment *i*.

Equation 1: $\frac{dF_i}{dt} = dissolution - ingestion$

We then tested three different hypotheses: 1) both the rate of dissolution and maximum ingestion vary with each food treatment; 2) only the maximum ingestion rate varies; 3) only the dissolution rate varies.

Equation 2: $\frac{dF_i}{dt} = -d_i F_i - i_m L^2 (\frac{F_i}{F_h + F_i})$

Equation 2 shows the ODE for the first hypothesis with all terms substituted in. In this first hypothesis, the dissolution rate (d_i) is different for each experimental food treatment i. Dissolution was modeled as a first-order process, meaning that food dissolved at a constant rate over time. According to bioenergetic theory and empirical data across hundreds of species (SOURCE), maximum ingestion rate scales with surface area (L^2). As opposed to other shell

measurements such as length of volume, surface area is more directly related to physiological processes like feeding. The maximum ingestion rate per unit of surface area is represented by i_m. The quantity of food available for ingestion is represented by F, while F_h the food concentration at which ingestion has reached half of its maximum rate.

Equation 3: $\frac{dF_i}{dt} = -dF_i - i_m L^2(\frac{F_i}{F_h+F_i})$

Equation 3 represents the ODE for the second hypothesis, where dissolution is held constant (d) and any variation between treatment groups is the result of a different maximum ingestion rate.

Equation 4: $\frac{dF_i}{dt} = -d_i F_i - iL^2(\frac{F_i}{F_h+F_i})$

Equation 4 represents the ODE for the third hypothesis, where maximum ingestion is held constant (i) between all treatment groups while the dissolution rate changes.

Model Fitting

Our hypotheses specify two potential mechanisms by which food is removed during the experiment: ingestion by the snail host and dissolution in the COMBO. The feeding experiment provided information on the snail's ingestion while the control experiment provided data on the background decomposition of the foods. In order to analyze these results, we sought to estimate the rates of the key parameters describing these processes using maximum likelihood estimation (MLE). Because analytical solutions cannot be obtained for ingestion models containing the type II functional response, we numerically simulated models with given parameters and calculated the predicted amount of food remaining after the 72-hour trial given the initial food mass and the size of the focal snail, both factors which we controlled experimentally. In these simulations, a

gradient of small to large snails were studied under three different model assumptions by testing the data against the model using the ingestion equation derived above. The models are described below.

1. Both the decomposition rate and maximum ingestion rate varies by food identity — neither d_i nor i_m is held constant (Equation 2).
2. All foods decompose at the same rate but varying maximum ingestion rates — d_i is held constant (Equation 3)
3. All foods exhibit different decomposition rates, but the same maximum ingestion rates — i_m is held constant (Equation 4).

Following the generation of this simulated data, we compared these predictions to the observed data in R version 4.4.1 using the deSolve package and 'ode()' function. MLE was used to estimate parameter values, using the 'mle2()' function in the 'bbmle' package. The Akaike Information Criterion (AIC) for each model was then computed, and the model with the lowest AIC value was determined to be the best fit using 'aic()' function.

RESULTS

Ingestion and Control Masses

Because our ingestion data required subtracting the remaining food from the amount initially fed, we ran a control experiment to determine the mass of the initial food provided to snails. The control experiment included food that was dried and weighed immediately versus exposed to experimental conditions (soaked) before being dried and weighed. The results indicated a loss in experimental food loss mass due to soaking in the COMBO (Figure 2). However, the degree of mass loss varied across the different experimental food treatments, suggesting potentially significant variability in the rate of dissolution. This indicated to us that our collected ingestion data may be influenced by the extent to which each unique food type has dissolved in the COMBO.

Generalized Linear Mixed Model

To compare ingestion data across food treatments while accounting for repeated measurements (weekly snail size) and group identities, we used a GLMM. The model included snail size and food treatment as fixed effects, and snail ID and group as random effects. The model results are shown in in Figure 3, with Supplementary Figure 2 providing the full statistical output. Compared to the unenriched baseline, we found that the +N and +NP food treatments to significantly influence ingestion rates ($p < 0.001$) while +P significantly did not. These results suggest that snails alter their food ingestion based on N availability. However, because our control experiment indicated variation in the dissolution rate by food treatment, we pursued additional modeling to account for the potential influence of dissolution on our ingestion results.

Model Fitting

We conducted simulations to test whether variation in food dissolution rates could explain differences in measured ingestion. Although we initially began with three hypotheses regarding ingestion and dissolution, we found a clear reduction in food mass due to dissolution (Figure 2). Therefore, we only ran two models to determine whether dissolution, or dissolution and ingestion were altered by the nutrient makeup of experimental foods. Model 1 represents the hypothesis that all foods exhibit different decomposition rates, but the same ingestion; Model 2 represents the hypothesis in which both ingestion and dissolution rates vary by food treatment. After fitting these three models to the data, we determined which model best fit the data by finding the lowest AIC. The results of the model fitting found that Model 1 was the best fit for the data (Table 3), where maximum ingestion rates were held constant across all feeding groups, but the dissolution of food varied by nutritional content (Equation 4).

DISCUSSION

In this experiment we created experimental food treatments that manipulated N:P ratios to study if food enrichment affected ingested quantity of food in uninfected *B. glabrata* snails. This work was motivated by findings from a previous experiment, which showed significant effects on snail life history traits following +N and +NP supplementation. Our goal was to investigate how nutrient dynamics influence a snail's feeding behavior. This question is important for interpreting our previous results — specifically, whether the observed effects were truly due to nutrient content — but also because previous research has found resource availability to be heavily implicated in disease dynamics (Civitello et al., 2022). We hypothesized that snails would exhibit lower ingestion rates when consuming nutrient rich food, as they would expend less energy to meet their nutritional needs.

After concluding the 4-week feeding schedule, we initially found similar ingestion rates for host snails across all food treatment groups despite differences in N:P availability. This would suggest that snails are eating the same amount of food, regardless of what the identity of the food itself is. However, we also conducted a control experiment, in which we sought to understand if foods dissolved at different rates, thereby influencing our expectations of ingestion. We found that dissolution contributed to the loss of food mass during the experiment (Figure 2). This data highlighted to us that both dissolution and ingestion contribute to the loss of mass that was found during this experiment. Our analysis then required us to determine to which extent each of these factors were influencing the data, especially as our hypothesis assumed that ingestion was different amongst each food group. We conducted simulations and model fitting to determine whether variations in food ingestion across experimental groups could truly be exhibited to changes in feeding behavior. We created ODEs for three different hypotheses: 1)

maximum ingestion and food dissolution vary by food treatment (Equation 2); 2) maximum ingestion varies by food treatment, but dissolution does not (Equation 3); and 3) dissolution varies by food treatment, but maximum ingestion does not (Equation 4). Our experimental data best fit the hypothesis that dissolution varies by food treatment, but maximum ingestion does not. Contrary to our initial hypothesis, we found that uninfected *B. glabrata* snails maintained a constant ingestion rate, regardless of the N:P ratio of their food source.

One possible explanation for these results is that *B. glabrata* prioritize consistent grazing, rather than adjusting their intake based on nutrient availability. Alternatively, snails may regulate feeding based on food energy content rather than specific nutrient ratios. A study on freshwater *Elima* snails found that despite the snails increasing consumption when food is available in larger quantities, they did not increase consumption rates specifically when food quality is lower (Stelzer & Lamberti, 2002). The researchers suggest that the snails may be prohibited from greater ingestion of low-quality food due to a high content of inorganic materials or a lack of ability to adjust their feeding behavior (Stelzer & Lamberti, 2002). Because *B. glabrata* are important grazers in freshwater ecosystems, stable ingestion rates may suggest that they exert consistent grazing pressure on food sources, independent of environmental fluctuations in nutrient composition. This behavior can indirectly affect other consumers in the ecosystem.

The composition of experimental foods may also help explain our findings. If N and P levels in the food were insufficient to alleviate nutrient limitations, snails would not be expected to adjust their ingestion of experimental foods. Similarly, if snails were not facing N and P limitations prior to receiving experimental food treatments, nutrient enrichment would not lead to differences in the ingested food quantity. Additionally, since snails were provided with a known high quality laboratory food prior to the experiment and on non-experimental feeding

days, they were not tested under starving conditions. Given that food quality did not influence the quantity of food ingested, these results allow us to study snails' feeding behavior in the future in response to varying food quality without food quantity acting as a confounding factor.

Challenging the assumption that nutrient enrichment will directly alter ingestion behaviors in aquatic snail hosts, we show that uninfected snails maintain stable feeding rates regardless of the nutrient content of their food. This emphasizes the need to further study the feeding behaviors of *B. glabrata* snails— especially given their important roles in freshwater ecosystems and as a host for schistosome parasites. Understanding the feeding ecology of these organisms could have broader implications for disease transmission and ecosystem-level feedback effects. In the future, it would be necessary to conduct this same experiment again, however with snails that have been exposed to and infected with the *Schistosoma* parasite. We would collect ingestion data in a similar manner to determine if the ingestion observed in this experiment is altered by the parasite, and how disease transmission is affected by these experimental food treatments of varying nutrient availability. Furthermore, with the knowledge that food quantity would not serve as a confounding variable to study food quality, we would test snails' feeding behavior under starving conditions to determine if snails exhibit a preference for either N or P nutrients given their nutritional needs and limitations.

FIGURES

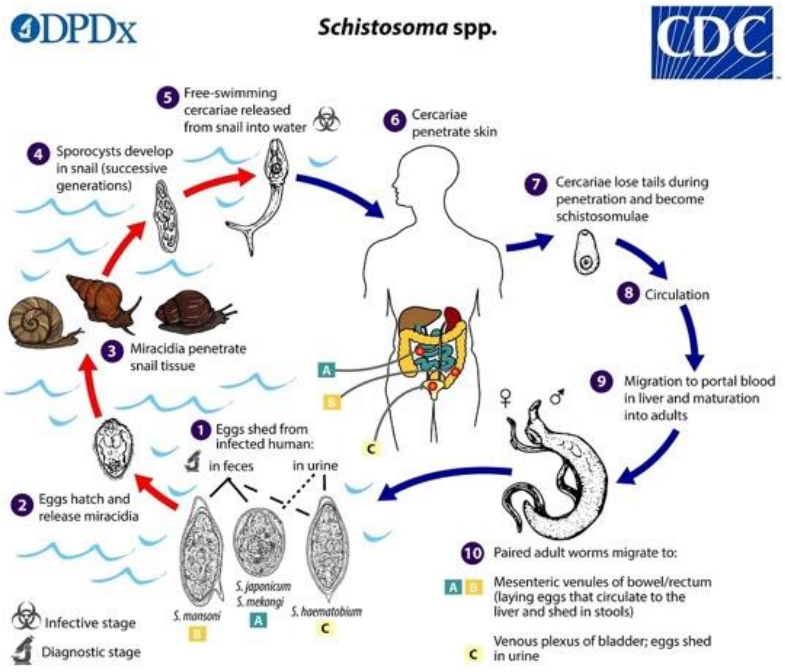

Figure 1: The life cycle of the *Schistosoma* parasite. The *Schistosoma* parasite is transmitted through a multi-host complex lifecycle. The cycle begins when infected humans release *Schistosoma* eggs either through feces or urine. In freshwater environments, these eggs are hatched into miracidia, a free-swimming larvae stage of the parasite. These miracidia must find an intermediate snail host to infect for resources in order to continue their survival and reproduction. These newly infected snails serve as the host for miracidia to undergo several stages of asexual reproduction, with cercariae eventually being released back into the freshwater environment to seek out human hosts. Cercariae infect humans upon coming in contract with skin; they travel throughout the bloodstream and penetrate different tissues depending on the schistosome species. In the human host, they mature into adult worms that are capable of sexual reproduction and restarting the cycle of infection by releasing eggs (CDC, 2024a).

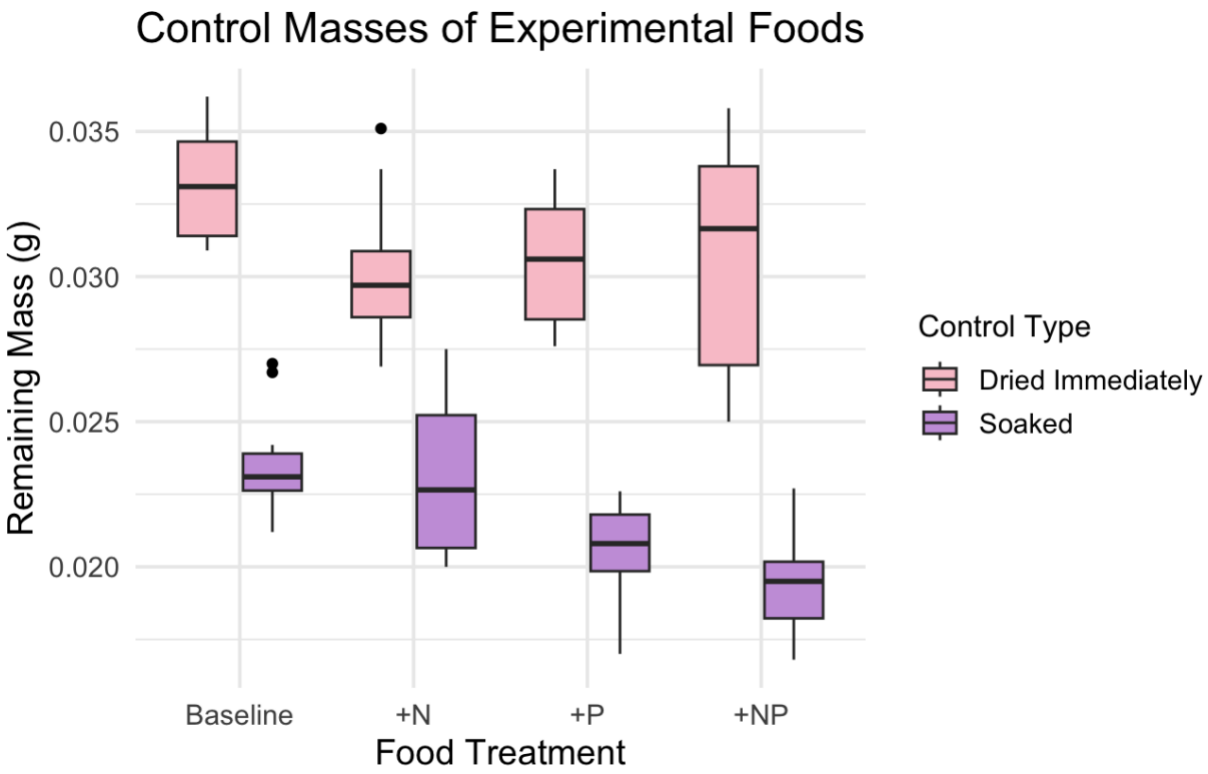

Figure 2: Control masses of experimental foods in soaked and unsoaked conditions. The boxplot represents the average mass of each food treatment following a ~48 hours drying period to remove water weight. The two control conditions are separated by color: the orange represents food that was unsoaked and dried immediately, while the blue represents food that was soaked first in water. The central line of the box plot represents the median, with the interquartile range (IQR) showing the middle 50% of the data. The whiskers extend 1.5x the IQR and black dots represent any outliers. The data suggests that soaking food in combo, as is done in the snail ingestion experiment, may lead to a reduction in residual food mass due to dissolution. This finding is relevant for interpreting the ingestion data, as it may explain a potential confounding factor in assessing ingestion by snails.

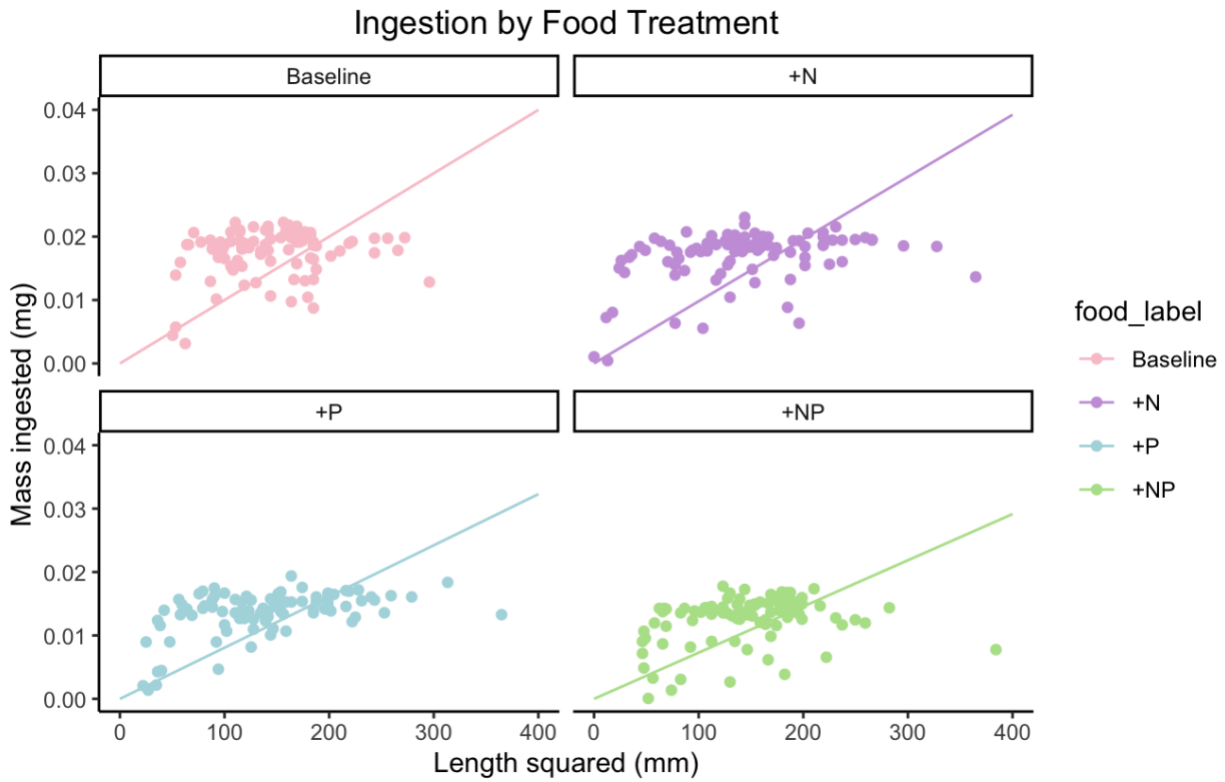

Figure 3: Nutrient enrichment with +N and +NP increases food ingestion. Each panel shows the food ingestion (mg) as a function of snail surface area (mm^2) across four food treatments: unenriched, +P only, +N only, and +NP. Points represent ingestion values that have been corrected by subtracting remaining food from soaked control mass. Lines show predicted values from a generalized linear mixed model (GLMM). The model included fixed effects for surface area, food treatment, and their interaction with random effects for snail ID and group assignment. Ingestion was significantly higher in the +N and +NP treatments compared to the unenriched control, suggesting that snails being fed these nutrient-enriched diets ingest more food overall than the control. No significant interactions between fixed effects were found, meaning that the increase in ingestion with snail size is consistent across all diets. See Supplementary Figure 2 for model results.

	Week 1	Week 2	Week 3	Week 4
Group 1	A	B	C	D
Group 2	D	A	B	C
Group 3	C	D	A	B
Group 4	B	C	D	A

Table 1: Randomized block design feeding schedule. This feeding schedule was implemented to control for potential biases introduced by the order in which experimental foods were presented to the snails. By dividing the snails into four groups which each received the experimental foods in a different sequence, the study ensures that ingestion outcomes were not disproportionately affected by the sequence in which food was presented.

Label	Nutrients	Pleco Wafers	Potato Starch	Marine Collagen	Phytin
A	Baseline	0.600g	5.400g	0.000g	0.000g
B	P	0.600g	3.890g	1.520g	0.000g
C	N	0.600g	5.269g	0.000g	0.131g
D	P + N	0.600g	3.754g	1.520g	0.131g

Table 2: Experimental food recipes. The experimental food recipes were formulated by manipulating known amounts of of high- and low-quality C, N, and P sources. Pleco wafers provided high quality, bioavailable N, P, an C sources. N is readily available for consumption in Marine collagen, and P is similarly available in Phytin. Potato starch is a rich source of Carbon, however poor in other micronutrients. Despite variations in nutrient composition, all experimental foods were standardized to the same ultimate weight (~6.000g).

	Number of Parameters	Negative Log Likelihood	AIC Value	ΔAIC
Model 1	7	287.0134	588.7525	0
Model 2	10	287.3763	594.0267	5.2742

Table 3: Calculated AIC Values resulting from the two models run. Because the experimental data found that dissolution reduced food mass, we only tested the following hypothesis: differences in mass were due solely to food dissolution (Model 1), and differences in mass were due to both dissolution and ingestion (Model 2). We compared these models using Akaike's Information Criterion (AIC), where the lowest AIC value represents the best fit to the data. The analysis found that Model 1 had the lowest AIC value, indicating the data best represents a change in mass due to the dissolution of experimental foods.

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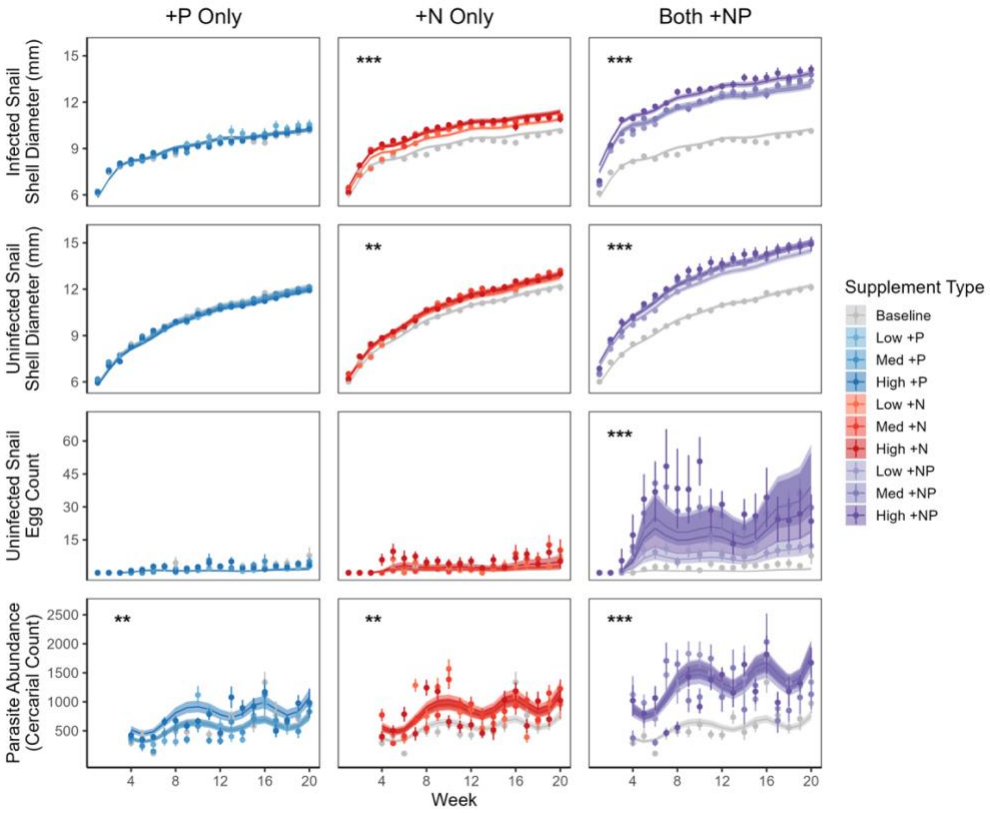
SUPPLEMENTARY FIGURES



Supp. Figure 1: Nutrient supplementation alters snail growth, reproduction, and parasite output. Each panel shows the effect of phosphorus (+P), nitrogen (+N) or both nutrients (N+P) on snail growth, reproduction, and cercarial output over time. Snails were fed diets supplemented with low, medium, or high nutrient levels over a 20-week period. The top two rows display mean shell diameter (mm) for infected (top) and uninfected (bottom) snails. The third row displays data on uninfected snail mean egg counts. The bottom row shows average parasite abundance (cercarial counts) of infected snails. Colors represent food treatment group, while the gray represents data from control groups that were fed a baseline food. The asterisks denote statistically significant differences from the baseline at the corresponding time point. +N and +NP supplementation significantly increased snail growth and reproduction, even at low and

medium levels. +P supplementation showed minimal effects, with significant results only observed in parasite production at the highest level of supplementation.

Main Fixed Effects	P Value
Snail surface area	0.000652***
Food B (+P only)	0.684090
Food C (+N only)	5.99e-05***
Food D (+NP)	6.02e-05***

Interactions between main effects	P Value
L^2:foodB	0.858344
L^2:foodC	0.197150
L^2:foodD	0.682302

Supp. Figure 2: Statistical output from a generalized linear mixed model (GLMM). This table presents the p-value for main fixed effects and their interactions from the GLMM analyzing snail ingestion (corrected using soaked control mass) as a function of snail surface area (length^2). The model included fixed main effects for snail surface area and the three supplemented food treatments and random effects of snail ID and group assignment. Snail surface area was a significant predictor of ingestion ($p = 0.000652$), and ingestion was significantly higher in the +N and +NP food treatments compared to the unenriched baseline ($p < 0.001$). The +P only treatment was not statistically significant ($p = 0.684$). No interactions between main effects were significant, indicating that the relationship between surface area and food ingestion did not vary across treatment groups.