Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Maggie Weber   March 21, 2022

Impacts of water hyacinth detritus on the growth, reproduction, and survival of the snail hosts of human schistosomes

by

Maggie Weber

Dr. David Civitello
Advisor

Biology

Dr. David Civitello

Advisor

Dr. Carolyn Keogh

Committee Member

Dr. Christopher Beck

Committee Member

2022

Impacts of water hyacinth detritus on the growth, reproduction, and survival of the snail hosts of human schistosomes

By

Maggie Weber

Dr. David Civitello

Advisor

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Biology

2022

Abstract

Impacts of water hyacinth detritus on the growth, reproduction, and survival of the snail hosts of human schistosomes
By Maggie Weber

Machines that shred the invasive macrophyte water hyacinth may inadvertently produce resources for the intermediate host of human schistosomes, *Biomphalaria glabrata.* Yet, prior work has shown that shredding water hyacinth in experimental mesocosm tanks resulted in substantial *B. glabrata* egg laying but juvenile recruitment failure. One potential explanation of this phenomenon is that the consumption of water hyacinth detritus may differentially impact juvenile and adult *B. glabrata* individuals. In this 12-week study, we evaluated this hypothesis by characterizing the life history traits of snails of different initial sizes fed either water hyacinth detritus or detritus from another invasive plant, water lettuce, which is a known high-quality resource. Overall, water hyacinth increased mortality for large, adult snails, decreased growth and reproduction for all snails, and resulted in altered growth patterns for snails across size classes. This study contextualizes prior work on the potential effects of shredding machines on human schistosome host ecology by demonstrating that water hyacinth is a low-quality food source for *B. glabrata* of all sizes with significant negative effects on snail life history traits. Thus, population-level effects may have arisen from other factors, such as complementary food sources or changes in egg-hatching rates, that were possible in prior experiments but excluded from this study. More broadly, this work contributes to ecological understanding of the effects of diet on life history traits as well as the role of size in mediating these effects.

Impacts of water hyacinth detritus on the growth, reproduction, and survival of the snail hosts of human schistosomes

By

Maggie Weber

Dr. David Civitello

Advisor

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Biology

2022

Acknowledgments

I am deeply grateful for the many people who made this project possible. First, I would like to thank Dr. David Civitello, whose steadfast support, enthusiasm, and advice were essential for this project and have inspired me to study disease ecology at Emory and beyond. To Lynda Bradley, my research mentor: thank you for being so generous with your time and expertise. You were integral to every part of this project, from its initial conception to final data analysis and writing, and I am lucky to consider you a mentor, a colleague, and a friend. I would also like to thank Dr. Carolyn Keogh and Dr. Christopher Beck for their guidance as committee members and for mentoring me throughout my time as an undergraduate. Thank you to Rachel Hartman for her generous help with data collection, Kelsey Shaw for her advice on experimental design, and Dr. Daniel Desautels for his thought-provoking work on invasive plants and schistosomiasis that formed the basis of this project. You have all taught me what it means to be a scientist, and I could not be more grateful.

Finally, a sincere thank-you to the friends and family who have lovingly and patiently listened to me talk about snails for the past two years. Thank you for your encouragement, for your excellent questions, and for nodding at all the right times.

My appreciation for you all goes beyond words. Thank you.

Table of Contents

INTRODUCTION:

Water hyacinth (*Eichhornia crassipes*) is a highly-successful invasive freshwater macrophyte with significant ecological and socioeconomic impacts worldwide. The plant grows in thick, floating mats that reduce oxygen exchange between the air and the water's surface, monopolize aquatic nutrients, and block light penetration, creating hostile conditions for many aquatic organisms (Villamagna & Murphy 2010). Additionally, the dense physical structure of water hyacinth mats can block human access to water, fishing, boat navigation, hydroelectric power generation, and irrigation (Ayanda et al. 2020).

Water hyacinth is particularly problematic in Lake Victoria, the largest tropical lake in the world and a hotspot for the neglected tropical disease schistosomiasis (Albright et al. 2004, Olsen et al. 2015). Water hyacinth is estimated to cause approximately 350 million USD in economic losses per year in the Lake Victoria basin (Mkumbo & Marshall 2015). To combat water hyacinth invasions, shredding machines may be used to chop water hyacinth into small pieces and return it to the water. While this strategy is effective in removing large sections of water hyacinth mats, it can also indiscriminately destroy other plant life, spread still-living fragments of water hyacinth to new sites within water bodies, and exacerbate anoxic conditions and eutrophication through decomposition of detritus (Hussner et al. 2017, Villamagna and Murphy 2010, Bicudo et al. 2007).

Efforts to control water hyacinth in Lake Victoria with shredding machines may also have unintended impacts on snails that act as intermediate hosts for human schistosomes, the agents of schistosomiasis. Schistosomiasis is a neglected tropical disease that affects over 250 million people and results in an estimated loss of 1.9 million disability-adjusted life years worldwide (CDC 2018, McManus et al. 2018). The disease also reduces economic productivity

in endemic areas due to its effects on child development and adult work efficiency (McManus et al. 2018). Lake Victoria is a particular hotspot for schistosomiasis: one third of the villages along the shore of Lake Victoria were classified as moderately or heavily infected with human schistosomes by a 2015 study (Colley et al. 2014, Olsen et al. 2015).

Schistosomes follow a complex life cycle: the snail-infectious life stage (miracidia) infects host snails of the genus *Biomphalaria* and then reproduces asexually within their tissues, releasing the human-infectious life stage (cercariae) into surrounding waters (CDC 2018). Lake Victoria has high densities of *Biomphalaria* host snails (Olsen et al. 2015), and prior work suggests a link between *Biomphalaria* and water hyacinth: the presence of water hyacinth is associated with higher *Biomphalaria* snail abundance (Mutuku et al. 2019), and the experimental introduction of water hyacinth into lake enclosures results in an increase in *Biomphalaria* populations (Plummer 2005). *Biomphalaria* are primarily detritivores. They are incapable of consuming living water hyacinth tissues, but decaying water hyacinth detritus may serve as a food source, allowing for the growth of snail individuals and populations (Thomas et al. 1985). In turn, the size, abundance, and nutrition of *Biomphalaria* snails all increase the total production of schistosome cercariae, an indicator of human infection risk (Civitello et al. 2022, Coles 1973, Civitello et al. 2018). As such, the use of shredding machines in Lake Victoria -- and subsequent production of water hyacinth detritus, a potential food source -- may have major cascading effects on individual snail traits, snail population dynamics, schistosome cercariae production, and human schistosomiasis infection risk.

Prior work on the effects of water hyacinth shredding on snail hosts has focused on population-level dynamics. In particular, one prior study found that shredding water hyacinth in mesocosm tanks affected *Biomphalaria glabrata* and *Schistosoma mansoni* dynamics in an

unanticipated way (Desautels et al., in prep). Specifically, water hyacinth shredding resulted in an increase in snail biomass and egg production and a transient increase in the production of schistosome cercariae, an indicator of human infection risk. However, an unusual aspect of *Biomphalaria* ecology observed in this experiment merits further study. Despite increased snail biomass and egg production following water hyacinth shredding, snail abundance remained low, suggesting a failure in recruitment of juvenile snails. In other words, large adult snails grew and laid eggs, but the small juvenile snails from those eggs were not detected in the population. This pattern of failed recruitment was not seen in mesocosm tanks where water hyacinth was completely removed or left unmanaged, suggesting that the addition of water hyacinth detritus itself was involved in juvenile recruitment failure. However, the mechanism by which water hyacinth detritus may have promoted snail growth and reproduction while preventing juvenile recruitment is still unknown.

Size-specific or life stage-specific effects of the consumption of water hyacinth detritus may offer an explanation of this phenomenon. Diet is thought to affect life history traits in many taxa (e.g., Richman et al. 2015, Zandonà et al. 2011), and shifts in growth, reproduction, and mortality profoundly impact population dynamics (de Roos et al. 2003). Life history bottlenecks brought about by life-stage-specific dietary demands have been described previously in zooplankton and copepods, and prior work has also shown that juvenile and adult *Biomphalaria* snails have different preferred feeding niches, potentially due to differences in nutritional requirements or digestive abilities (Villar-Argaiz & Sterner 2002, Nakazawa 2011, Thomas et al. 1985, Cedeno-Leon & Thomas 1982). As such, it is possible that variation in the quality of shredded water hyacinth as a food source for *Biomphalaria* across its lifespan could explain the role of water hyacinth in mediating the observed juvenile bottleneck. However, the specific

effects of a water hyacinth diet on *Biomphalaria* individuals have not been described. Here, I studied the effects of water hyacinth detritus on the growth, reproduction, and mortality of *Biomphalaria glabrata* individuals of different size classes. Based on the lack of juvenile recruitment observed by Desautels et al. (in prep), I hypothesized that a water hyacinth diet would have strong negative effects on small, juvenile snails, but not on large, adult snails. Physiologically, this could occur because smaller individuals have shorter digestive tracts, thereby limiting digestive efficiency, especially for digestion-resistant plant material such as water hyacinth. I predicted that juvenile *B. glabrata* fed water hyacinth would experience reduced growth, reduced cumulative reproduction, and heightened mortality compared to a control high-quality diet, while I expected that adult snails would not experience significant differences in growth, reproduction, and mortality.

This study seeks to clarify the effects of a water hyacinth diet on the life history traits of *B. glabrata* snails and the role of size in mediating those effects. In doing so, this work further contextualizes Desautels et al. by exploring size-mediated effects of water hyacinth consumption that could explain observed *B. glabrata* juvenile bottlenecks. More broadly, the results of this study contribute to scientific understanding of the dietary ecology of a critical disease vector and corroborate prior work on the effects of diet quality on life history traits, in addition to exploring the lesser-studied role of individual size variation in mediating dietary effects on life history traits.

METHODS:

Study system:

NMRI strain *Biomphalaria glabrata* individuals were obtained from the Civitello laboratory colony as in Desautels et al. (in prep). Snails were reared on a non-plant food source, chicken feed, and so were not acclimated to a particular detritus type. Snails were selected from each of 6 initial size classes: 1mm, 2mm, 4mm, 8mm, 12mm, 15mm. Size class was determined by the longest distance measured across an individual's shell with calipers (within ± 10% of size class value). Reproductive maturity may partially depend on diet, but snails typically begin laying eggs at around 7mm in size; as such, size classes 1mm, 2mm, and 4mm were considered juveniles, while size classes 8mm, 12mm, and 15mm were considered adults (Pimentel 1957).

Water lettuce, another invasive macrophyte in Lake Victoria, was chosen as a control "high-quality" food to compare to water hyacinth because it is also present in the natural habitat of *Biomphalaria glabrata*, is rich in nitrogen and phosphorous, increased snail abundance when present in a prior mesocosm experiment (Reddy & De Busk 1985, Desautels et al. 2022), and is highly nutritious for snails (Desautels et al. in review). Live water hyacinth and water lettuce plants were purchased from Atlanta Water Gardens, a local garden store. Plants were dried and finely milled. Plant detritus was stored in covered plastic bins in a drying oven at 45-55°C throughout the experiment to reduce effects of moisture on weight measurements.

Life table experiment:

A 2 x 6 factorial life table experiment (n=120) was run for 12 weeks, from August 3rd, 2021 - October 26th, 2021. The two factors crossed in this life table experiment were detritus type (water hyacinth, water lettuce) and initial snail size class (1mm, 2mm, 4mm, 8mm, 12mm,

15mm). We maintained all snails in artificial lake water (HH-COMBO; Kilham et al. 1998) in lidded plastic cups.

The initial dry weight of detritus provided to snails of each size class was determined with a model correlating (snail length)^2 with resource requirements, reflecting an empirically-supported assumption that resource consumption increases with a snail's surface area (Kooijman 2010). In order to continue feeding snails *ad libitum* as they grew, the detritus level in the cups was observed one day prior to data collection each week. If little detritus remained in a cup for any snail, 3 individuals were selected from that treatment group and measured with calipers. Sizes were averaged, and a new required detritus quantity was calculated for the treatment group based on this size using the aforementioned model. If an abundance of detritus remained in all cups for a given treatment, the same detritus quantity was given as the previous week. See Appendix B for weekly detritus amounts provided to each treatment.

One day prior to the start of the experiment, water hyacinth and water lettuce detritus were weighed and added to labeled plastic deli cups. Cups were misted to wet detritus and then filled halfway with HH-COMBO. Cups were stored overnight in an incubator held at a constant 26°C.

The following day, 20 NMRI strain *Biomphalaria glabrata* individuals of each of six size classes (1mm, 2mm, 4mm, 8mm, 12mm, 15mm) were selected from our laboratory colony. Half of the individuals in each size class were assigned to the experimental water hyacinth detritus, and half were assigned to the control water lettuce detritus (n=10 per treatment). For the next 12 weeks, one day prior to data collection, new, labeled cups of HH-COMBO and plant detritus were prepared and incubated overnight at 26°C. The following day, snails were measured, eggs were counted, and each individual was cleaned with a Kim-Wipe and moved to a new cup with a

new stock of the appropriate plant detritus established the previous day. To determine snail size, each individual was photographed on a laminated sheet printed with a grid of known size. Photographs were processed in ImageJ, and size was recorded as the major axis of an ellipse fitted to the snail's shell. Snail death was determined by significant decay of snail tissue within the shell. Presence or absence of plant detritus was determined visually. Number of egg masses was determined visually, and the total number of eggs laid by each snail was counted under a microscope. This process of data collection and passaging to new cups with new detritus was repeated once weekly for 12 weeks. After data collection on the 12th week, all individuals were euthanized in ethanol.

Growth data analysis:

For each detritus type and starting size class treatment (n=10), the size of all surviving individuals was averaged each week. Dead snails were excluded from the week of death onward because the amount of time spent alive (and potentially consuming detritus and growing) in the prior week was unknown. Mean size and standard error were plotted (R package: ggplot2) over the course of the experiment for visual comparison to identify patterns in growth across size classes and detritus types.

For statistical tests, maximum snail size was used to account for potential variation in measurements between weeks. A general linear model was fitted to this data (R package: stats) to test the ability of initial size class and detritus type to predict maximum snail size. Based on this model, estimated marginal means for maximum snail size were calculated, and pairwise contrasts were conducted for each detritus type and starting size class with correction for multiple testing following the Tukey method (R package: emmeans).

Reproduction data analysis:

For each detritus type and starting size class treatment (n=10), the cumulative number of eggs laid by each individual was averaged each week. Dead snails were excluded from the week of death onward because the amount of time spent alive (and potentially consuming detritus and reproducing) in the prior week was unknown. Comparisons were performed on the log10 transformation of cumulative egg production.

The mean and standard error of log-transformed weekly cumulative egg production were plotted (R package: ggplot2) over the course of the experiment for visual comparison to identify patterns in egg production across size classes and detritus types. A general linear model was fitted to this data (R package: stats) to test the ability of initial size class and detritus type to predict total cumulative egg production. Based on this model, estimated marginal means for total cumulative egg production were calculated with the emmeans R package, and pairwise contrasts were conducted for each detritus type and starting size class using the Tukey correction for multiple testing (R package: emmeans).

Mortality data analysis:

Mortality was formally compared at the end of the 12-week experiment, when all snails had been exposed to their assigned detritus diets for as long as possible and some mortality had been observed. Mortality was calculated as the number of dead snails per detritus type and starting size class treatment divided by the initial number of snails in that treatment (n=10). Proportion mortality was visually compared across treatments with a paired bar chart to emphasize differences across detritus type treatments (R package: ggplot2).

RESULTS:

Snail growth:

Within all size classes, the water hyacinth treatment reduced growth compared to the water lettuce treatment. This difference is visually apparent across initial size classes 1mm, 2mm, 4mm, and 8mm as early as week 2 of the experiment (see Figure 1). By the end of the experiment, snails fed water lettuce attained significantly larger maximum sizes than snails fed water hyacinth within all starting size classes ($p < 0.05$ for all contrasts, see Table 1).
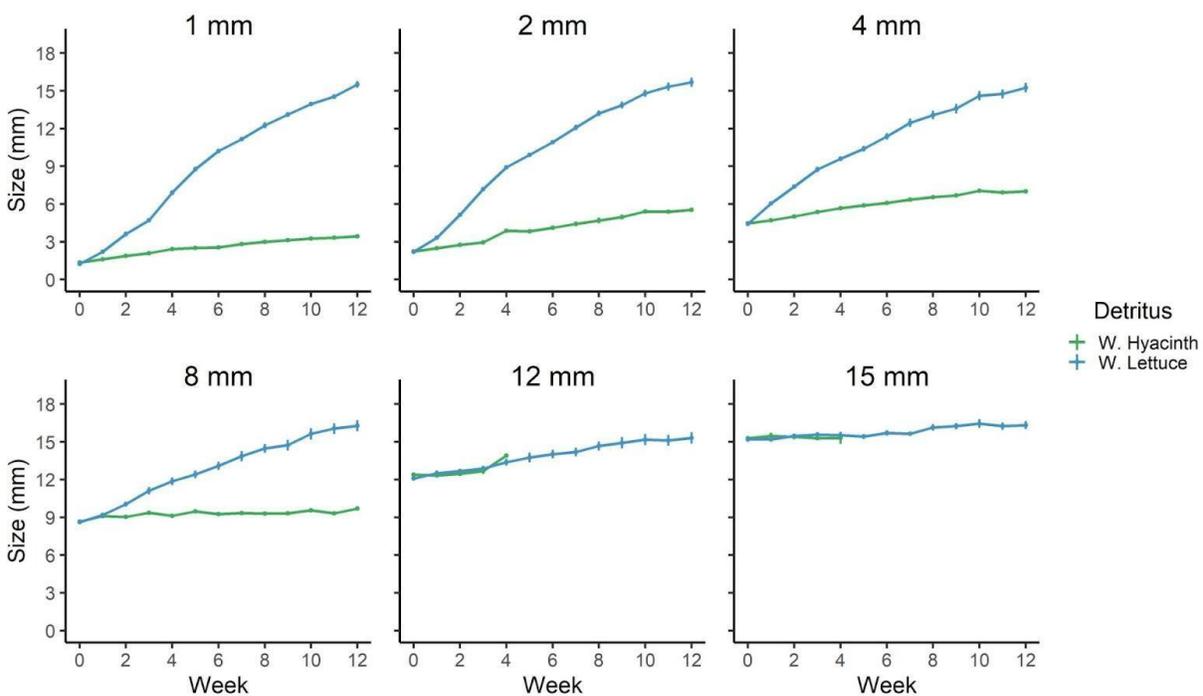
Figure 1. Effect of detritus type on snail growth within each initial size class over 12 weeks. Data points represent mean size of surviving snails of each initial size class and detritus treatment over the 12-week experiment, and error bars represent +/- SE. Snails were provided with either water lettuce or water hyacinth detritus *ad libitum* each week as their sole food source. Line color indicates detritus type (blue: water lettuce, green: water lettuce, n=10 individuals per treatment). Lines that terminate prior to week 12 indicate the death of all snails of a given initial size class and detritus treatment. Overall, regardless of initial size class, snails fed water hyacinth grew less than snails fed water lettuce.

In addition to its effects on relative snail growth within size classes, detritus treatment resulted in different patterns of growth across size classes. Regardless of initial size class, most size classes of snails fed water lettuce converged on a similar maximum size by the end of the experiment (per Table 2, only 15mm snails were significantly different in final size from 1mm/4mm/12mm snails). In contrast, snails of each initial size class in the water hyacinth treatment remained distinctly different sizes across all 12 weeks (see Figure 2; note lack of overlap or convergence in lines). Unlike snails fed water lettuce, all size classes fed water hyacinth attained significantly different maximum sizes from one another ($p < 0.01$ for contrast comparisons of all initial size classes).
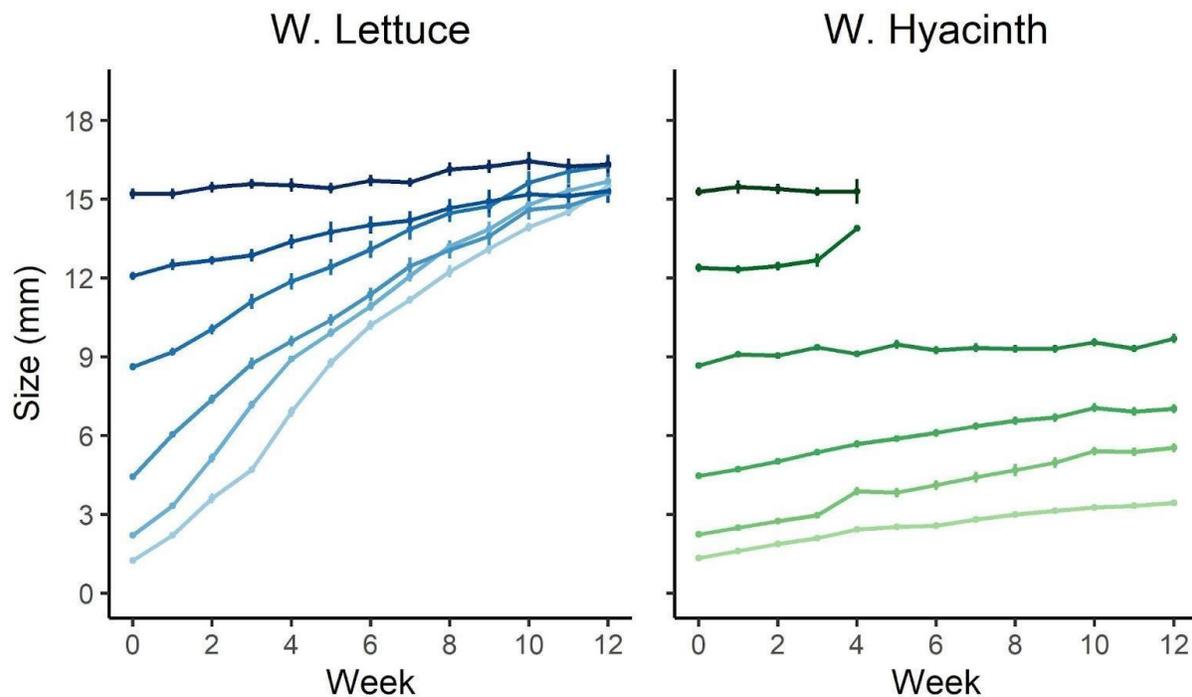

Figure 2. Effect of initial size class and detritus type on snail growth over 12 weeks. Data points represent the mean size of surviving snails of an initial size class and detritus treatment (n=10) over the 12-week experiment, and error bars represent +/- SE. Snails were provided

with either water lettuce or water hyacinth detritus *ad libitum* each week as their sole food source. Each line represents the weekly mean size for an initial snail size class (darker: larger size class, lighter: smaller size class) and detritus type (blue: water lettuce, green: water hyacinth). Overall, snails fed water lettuce converged on similar endpoint sizes, while snails fed water hyacinth did not. Lines that terminate prior to week 12 indicate the death of all snails of a given initial size class and detritus treatment.

A general linear model predicting maximum snail size based on initial size class and detritus type found that these variables strongly contributed to variation in maximum snail size (adjusted $R^2 = 0.959$), consistent with the visual trends seen in Figure 1 and Figure 2. Snails that consumed water hyacinth grew significantly less than snails that consumed water lettuce (GLM, food type, $p < 2.2e\text{-}16$). Additionally, the effects of food type on growth differed across initial size classes, with smaller snails experiencing greater negative effects of water hyacinth consumption on growth (GLM, food type * initial size class interactions, $p < 0.01$).

Snail reproduction:

The water hyacinth treatment reduced snail reproduction: snails fed water hyacinth produced significantly fewer cumulative eggs than snails fed water lettuce within all starting size classes (p<0.001 for all contrasts). In the water hyacinth treatment, only adult snails (8mm, 12mm, 15mm) reproduced (see Figure 3), and by week 5 of the experiment, reproduction halted for 12mm and 15mm snails due to 100% mortality. Out of all starting size classes in the water hyacinth treatment, only 8mm snails both reproduced and survived the full 12 weeks of the experiment.
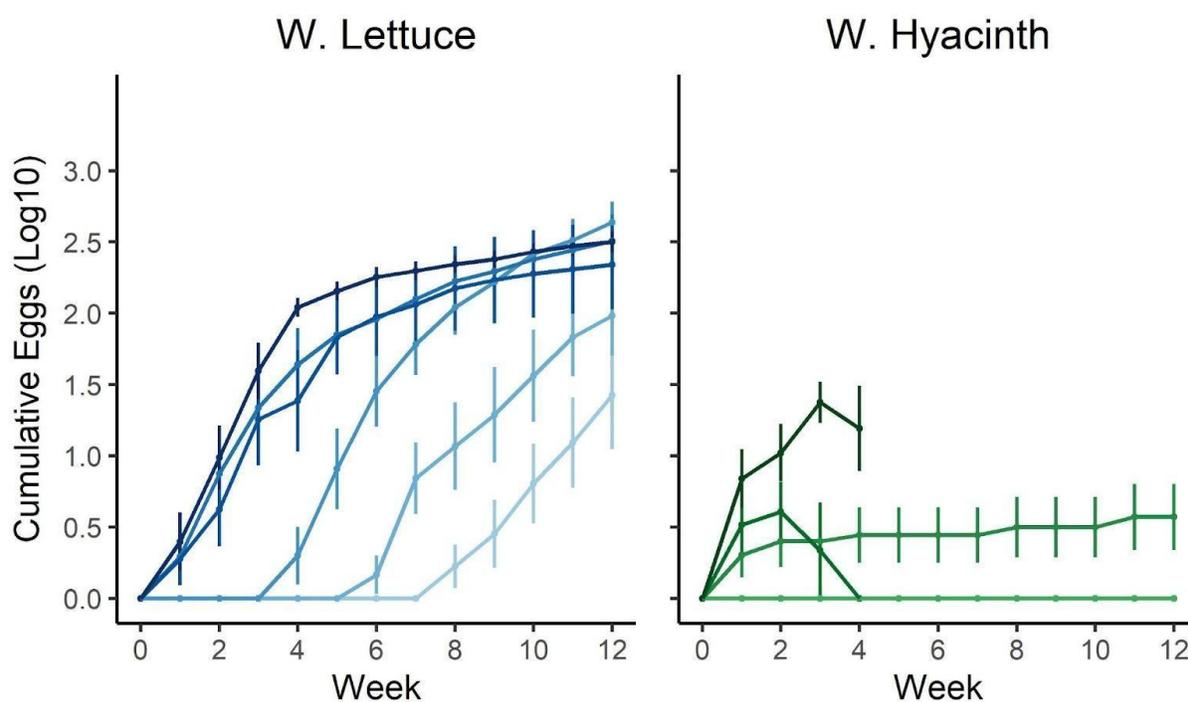
Figure 3. Effect of initial size class and detritus type on log-transformed snail cumulative egg production over 12 weeks. Data points represent the mean size of surviving snails of each initial size class and detritus treatment (n=10) each week, and error bars represent +/- SE. Snails were provided with either water lettuce or water hyacinth detritus *ad libitum* each week as their sole food source. Each line represents the weekly mean of log-transformed cumulative egg production for an initial snail size class (darker: larger size class, lighter: smaller size

class) and detritus type (blue: water lettuce, green: water hyacinth). Lines that terminate prior to week 12 indicate the death of all snails of a given initial size class and detritus treatment. Overall, snails fed water hyacinth produced fewer cumulative eggs than snails fed water lettuce.

Snail mortality:

The water hyacinth treatment increased mortality rates for large adult snails (12mm and 15mm). As seen in Figure 4, by the end of the experiment, all (100%) of the 12mm and 15mm adult snails fed water hyacinth were dead, while in water lettuce treatments, only 10% of 12mm snails and 20% of 15mm snails were dead. Smaller adult snails (8mm) experienced somewhat higher mortality when fed water hyacinth (30% mortality) than water lettuce (0% mortality). Juvenile snails (1mm, 2mm, 4mm) fed water hyacinth did not experience large differences in mortality when fed water hyacinth versus water lettuce (0-10% mortality in all treatment groups).
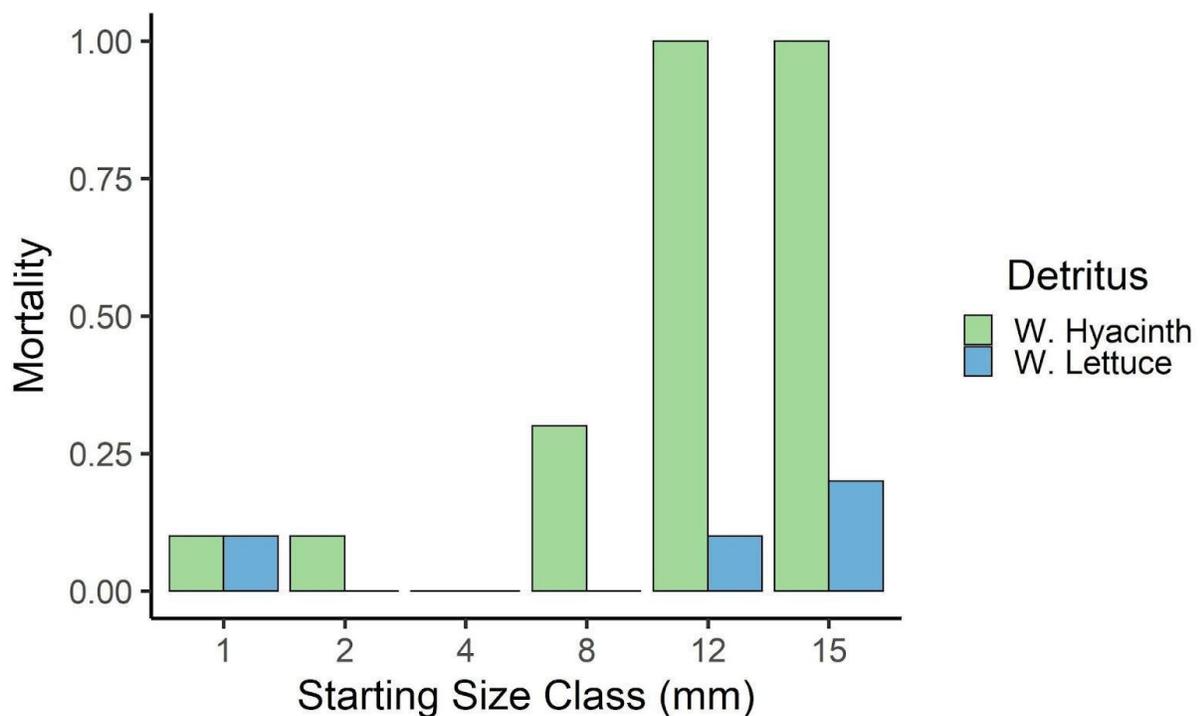
Figure 4. Snail mortality after 12 weeks by initial size class and detritus type. Bars represent the proportion of dead individuals of each initial size class and detritus treatment at the end of the 12-week experiment (n=10). Snails were provided with either water lettuce or

water hyacinth detritus *ad libitum* each week as their sole food source. Fill color indicates detritus type (blue: water lettuce, green: water hyacinth). Overall, snails of starting size class 12mm and 15mm fed water hyacinth experienced the highest mortality after 12 weeks (100% dead).

DISCUSSION:

In this study, I investigated the effects of water hyacinth detritus on the life history traits of *B. glabrata* individuals of different starting sizes. Based on the accumulation of adult biomass and lack of juvenile recruitment associated with the pulse input of water hyacinth detritus in Desautels et al. (in prep), I hypothesized that small, juvenile snails would experience negative effects on growth, reproduction, and mortality when consuming a water hyacinth diet rather than a high-quality water lettuce diet, and that large, adult snails would not experience significant differences in growth, reproduction, and mortality.

In accordance with prior work in other systems, diet quality (i.e., assignment to a water hyacinth or water lettuce detritus diet) had significant impacts on *Biomphalaria glabrata* life history traits. Specifically, this study found that: (1) water hyacinth detritus reduced *B. glabrata* growth and reproduction across all size classes; (2) water hyacinth and water lettuce detritus produced different growth trajectories for *B. glabrata;* (3) water hyacinth detritus resulted in heightened mortality for large, adult *B. glabrata* individuals. Consistent with studies by Zandonà et al. (2011) and Richman et al. (2015), a poor-quality diet (water hyacinth) was associated with reduced growth, reduced fecundity, and presumed delays in juvenile maturation. Notably, the patterns that we observed across life stages in *B. glabrata* were similar to those found in beetles in a prior study: juveniles fed low quality diets experienced delayed maturation while adults experienced reduced survival (Katsuki et al. 2012). However, based on these results, the effects of water hyacinth detritus consumption on adult and juvenile *B. glabrata* were not sufficient to explain the juvenile bottleneck population dynamics observed in the Desautels et al. mesocosm, suggesting that other environmental or biological factors may have been responsible. Future

work seeking to explain these dynamics should consider the effects of water hyacinth as a supplement to a high-quality diet and the effects of detritus on egg hatching.

First, this study showed that a water hyacinth diet reduced growth and reproduction for all snails regardless of initial size class. Over the course of the experiment, within each starting size class, snails fed water hyacinth remained at smaller sizes than snails fed water lettuce. While these effects were not exclusive to juveniles, as predicted, they nonetheless demonstrated that a water hyacinth diet did not support as much snail growth as a water lettuce diet. Water hyacinth had similar negative effects on cumulative reproduction; within all starting size classes, consumption of water hyacinth detritus rather than water lettuce detritus resulted in lower cumulative egg counts. Juvenile snails that consumed water hyacinth never reproduced, potentially because the quality of water hyacinth detritus as a food source was not sufficient for juvenile snails to reach sexual maturity. Juvenile snails fed water hyacinth never attained sizes of 7mm or greater, which has previously been described as the minimum shell diameter at first reproduction (Pimentel 1957). All starting size classes of snails fed water lettuce, on the other hand, ultimately grew larger than 7mm and laid eggs, suggesting that water lettuce served as a sufficiently high-quality diet for juveniles to sexually mature and for snails of all starting size classes to eventually reproduce.

In addition to reductions in growth and reproduction, the water hyacinth diet altered patterns of snail growth across all six size classes. In the water lettuce treatment, regardless of initial size class, all snails converged on similar maximum sizes, with more rapid initial growth that leveled out as the experiment continued (see Figure 2). The sizes reached by snails fed water lettuce in this study fall within previously proposed maximum size ranges for *Biomphalaria glabrata* (Benson 2019). That is, the water lettuce diet appears to have provided sufficient

resources for most size classes to grow to their shared maximum size under these conditions. In contrast to the similar sizes attained by all snails fed water lettuce, snails fed water hyacinth remained distinctly different sizes based on their initial size class for the length of the experiment. Additionally, neither juvenile nor small adult snails in the water hyacinth treatment reached the proposed maximum size of *B. glabrata* in typical conditions (Benson 2019). That is, the water hyacinth treatment did not appear to provide sufficient resources for snails to grow to a shared maximum size. Lastly, in the water hyacinth treatment, 8mm snails and larger had an approximately flat growth trajectory, suggesting that they lacked resources to grow beyond this size, while a similar flat growth trajectory occurred only at the largest size (15mm) in the water lettuce treatment (see Figure 2). If larger snails in the water hyacinth treatment were unable to grow or maintain their bodies due to poor resource quality, these snails may have been losing soft tissue biomass within their shells, but we could not observe such shrinking in our experiment because snail shells themselves do not shrink. Future work could use destructive methods to document shrinking in tissue biomass for snails in similar poor resource conditions. Overall, further energetic studies of snail growth using water hyacinth and water lettuce resources would help inform our understanding of these trajectories.

Although the aforementioned effects of growth and reproduction occurred across all starting size classes, only adult snails experienced substantial differences in mortality when fed water hyacinth detritus. The two largest adult size classes in the water hyacinth treatment (12mm and 15mm snails, in or near the proposed maximum range of Benson 2019) experienced 100% mortality by week 5 of the experiment. In addition to their large size, these 12mm and 15mm snails reproduced at high levels prior to their death. While 12mm and 15mm snails in the water lettuce treatment also reproduced substantially, they did not suffer from increased mortality. Out

of all snails fed water hyacinth, only smaller adult snails (8mm) both reproduced and survived the course of the 12-week experiment, and even these snails experienced a moderate increase in mortality compared to 8mm snails fed water lettuce; thus, it seems likely that a combination of the cost of reproducing and the higher absolute cost of maintaining a larger body size explains heightened mortality rates for adult snails in this treatment. The water hyacinth diet may not have provided sufficient resources for both reproduction and body growth at larger sizes, resulting in increased mortality. Given these potential trade-offs in allocation to growth, reproduction, and mortality, future work on the impacts of water hyacinth on *B. glabrata* life history traits through the lens of dynamic energy budget theory (Kooijman 2010) may be valuable. Prior work in *B. glabrata* has also used dynamic energy budget models to link life history trait variation to broader population dynamics (Malishev and Civitello, 2019), which would be particularly useful when considering further connections between the effects of water hyacinth observed in this study and the prior mesocosm experiment of Desautels et al.

The results of this study also provide further context for the water hyacinth shredding mesocosm experiment described in Desautels et al. (in prep). As stated previously, in Desautels et al., the addition of water hyacinth detritus to snail populations was associated with an increase in snail growth and reproduction, but a failure in juvenile recruitment. However, the effects of water hyacinth detritus on snail life history traits in this study were not sufficient to fully explain this phenomenon, and size-based effects on mortality actually ran counter to predictions based on observed mesocosm trends. Based on the growth and reproduction of large snails and lack of juvenile recruitment in the mesocosm experiment, I initially predicted that juvenile *B. glabrata* given a water hyacinth diet in this study would face negative effects on life history traits (reduced growth, heightened mortality, and reduced cumulative reproduction), while adult snails

given a water hyacinth diet would not face these negative effects. However, in this study's life

table experiment, a water hyacinth diet negatively impacted the growth and reproduction of *B.*

glabrata snails regardless of initial size class, suggesting that all snails in this detritus treatment

experienced decreased overall health. Given these results, along with low levels of juvenile

mortality and high levels of adult mortality in the water hyacinth treatment, it seems unlikely that

consumption of water hyacinth as a sole food source -- and subsequent size-based effects on

snail life history traits -- was responsible for the juvenile bottleneck and increase in adult growth

and reproduction observed by Desautels et al. Alternatively, in the mesocosm experiment, the

utilization of water hyacinth as a diet subsidy along with a higher-quality periphyton diet may

have had different effects on life history traits than the uniform water hyacinth diet provided in

this life table experiment. Water hyacinth may also have impacted egg hatching rates in the

mesocosm by interfering with egg mass oxygenation, contributing to the observed juvenile

recruitment failure. Future research considering the effects of water hyacinth as a subsidy rather

than a complete diet and testing for potential effects of water hyacinth on *B. glabrata* egg

hatching may help further explain the mechanism underlying the water hyacinth-associated *B.*

glabrata juvenile recruitment bottleneck.

It is also worth noting that snails in this study were fed *ad libitum*, and at several points

during the experiment, snails in all 6 size classes fed water lettuce consumed all available

detritus and were thus fed increasing amounts (see Table 4 for weekly detritus amounts). On the

other hand, snails fed water hyacinth did not consume all detritus offered regardless of size class,

and were thus offered consistent amounts each week based on their initial size class. This

provides further evidence that water hyacinth is not a highly desirable or digestible food source

for snails. No known toxins have been isolated from water hyacinth, and both water hyacinth and

water lettuce have been shown to contain high levels of the essential nutrients phosphorus and nitrogen (Reddy & De Busk 1985). However, it is possible that the waxy cuticle of water hyacinth, which prevents living leaves from decaying, interferes with snail digestion. Further research is required to determine whether this cuticle or other characteristics make water hyacinth a particularly low-quality sole food source for *B. glabrata*. In future research, it may also be valuable to compare the effects of a water hyacinth detritus diet to the effects of starvation to determine whether water hyacinth consumption has an active detrimental effect on snails, such as toxicity, or whether it simply has such low digestibility or nutritional value that it is unable to provide snails with adequate resources for growth, reproduction, and survival.

Overall, water hyacinth detritus negatively impacted all life history traits studied in *Biomphalaria glabrata*. However, counter to my predictions, these effects were not exclusive to juvenile snails. Instead, water hyacinth detritus reduced growth and reproduction for *B. glabrata* individuals regardless of initial size class, altered the growth trajectories of snails across size classes, and greatly increased mortality for larger snails. While the effects of water hyacinth on *B. glabrata* life history traits in this study did not align with the juvenile bottleneck dynamics observed in Desautels et al., they nonetheless provide insight into the ecological effects of diet on individuals and the potential role of size in mediating *B. glabrata* resource allocation and mortality in low-quality food environments. Given the controlled nature of this experiment, these results are may not be directly generalizable to the field, but they do provide evidence that increased *B. glabrata* growth and reproduction in prior population-level work (e.g., Desautels et al., in prep), and the positive association observed between water hyacinth presence and *Biomphalaria* abundance in the field (e.g., Mutuku et al. 2019, Plummer 2005) are not due to consumption of water hyacinth detritus alone. In fact, based only on the results of this study,

water hyacinth shredding would be expected to reduce the abundance and total biomass of *B. glabrata* populations in the field if the resulting detritus were consumed as a sole food source. Clearly, associations between snail hosts and water hyacinth are more complex than previously assumed, and further study is needed to fully understand the ways that *B. glabrata* may interact with and be affected by this plant across varied environments. These findings contribute to a detailed, growing understanding of how a critical schistosomiasis host is impacted by invasive water hyacinth and raise questions that merit further study about the effects of size, food quality, and energy acquisition and allocation on *Biomphalaria glabrata*.

REFERENCES:

Albright, T. P., Moorhouse, T. G., & Mcnabb, T. J. (2004). The Rise and Fall of Water Hyacinth in Lake Victoria and the Kagera River Basin, 1989-2001. In *73 J. Aquat. Plant Manage* (Vol. 42).

Ayanda, O. I., Ajayi, T., & Asuwaju, F. P. (2020). Eichhornia crassipes (Mart.) Solms: Uses, Challenges, Threats, and Prospects. In *Scientific World Journal* (Vol. 2020). Hindawi Limited. https://doi.org/10.1155/2020/3452172

Benson, A. J. (2019, August 9). *Biomphalaria glabrata (Say, 1818)*. Nonindigenous Aquatic Species. Retrieved March 14, 2022, from https://nas.er.usgs.gov/queries/factsheet.aspx?SpeciesID=1029

Bicudo, D. D. C., Fonseca, B. M., Bini, L. M., Crossetti, L. O., Bicudo, C. E. D. M., & Araújo-Jesus, T. (2007). Undesirable side-effects of water hyacinth control in a shallow tropical reservoir. *Freshwater Biology*, *52*(6), 1120–1133. https://doi.org/10.1111/j.1365-2427.2007.01738.x

Cedeno-Leon, A., & Thomas, J. D. (1982). Competition Between Biomphalaria glabrata (Say) and Marisa cornuarietis (L.): Feeding Niches 707-721. In *Journal of Applied Ecology* (Vol. 19, Issue 3).

Centers for Disease Control and Prevention. (2019, August 14). *CDC - Schistosomiasis - Biology*. Centers for Disease Control and Prevention. Retrieved November 1, 2021, from https://www.cdc.gov/parasites/schistosomiasis/biology.html.

Civitello, D. J., Angelo, T., Nguyen, K. H., Hartman, R. B., Starkloff, N. C., Mahalila, M. P., Charles, J., Manrique, A., Delius, B. K., Bradley, L. M., Nisbet, R. M., Kinung'hi, S., & Rohr, J. R. (2022). Transmission potential of human schistosomes can be driven by resource competition among Snail Intermediate hosts. *Proceedings of the National Academy of Sciences*, *119*(6). https://doi.org/10.1073/pnas.2116512119

Civitello, D. J., Fatima, H., Johnson, L. R., Nisbet, R. M., & Rohr, J. R. (2018). Bioenergetic theory predicts infection dynamics of human schistosomes in intermediate host snails across ecological gradients. In *Ecology Letters* (Vol. 21, Issue 5, pp. 692–701). Blackwell Publishing Ltd. https://doi.org/10.1111/ele.12937

Coles, G. C. (1973). The effect of diet and crowding on the shedding of schistosoma mansoni cercariae by biomphalaria glabrata. *Annals of Tropical Medicine and Parasitology*, *67*(4), 419–423. https://doi.org/10.1080/00034983.1973.11686909

Colley, D. G., Bustinduy, A. L., Secor, W. E., & King, C. H. (2014). Human schistosomiasis. *The Lancet*, *383*(9936), 2253–2264. https://doi.org/10.1016/S0140-6736(13)61949-2

De Roos, A. M., Persson, L., & McCauley, E. (2003). The influence of size-dependent life-history traits on the structure and dynamics of populations and Communities. *Ecology Letters*, *6*(5), 473–487. https://doi.org/10.1046/j.1461-0248.2003.00458.x

Desautels, D. J., Wang, Y., Ripp, A., Beaman, A., Andea, S., Hartman, R. B., & Civitello, D. J. (2022). Nutritional effects of invasive macrophyte detritus on *Schistosoma mansoni* infections in snail intermediate hosts. [Manuscript in review]. Biology Department, Emory University.

Desautels, D. J., Hartman, R. B., Shaw, K. E., Maduraiveeran, S., & Civitello, D. J. (2022). Divergent effects of invasive macrophytes on population dynamics of a snail intermediate host of Schistosoma Mansoni. *Acta Tropica*, *225*. https://doi.org/10.1016/j.actatropica.2021.106226

Desautels, D. J., Hartman, R. B., Weber, M. E., & Civitello, D. J. (2022). Plant invasion and control alter population dynamics of snail intermediate hosts and transmission potential of human schistosomes. [Unpublished manuscript]. Biology Department, Emory University.

Gérard, C., & Théron, A. (1997). Age/size- and time-specific effects of schistosoma mansoni on energy allocation patterns of its snail host Biomphalaria glabrata. *Oecologia*, *112*(4), 447–452. https://doi.org/10.1007/s004420050331

Hussner, A., Stiers, I., Verhofstad, M. J. J. M., Bakker, E. S., Grutters, B. M. C., Haury, J., van Valkenburg, J. L. C. H., Brundu, G., Newman, J., Clayton, J. S., Anderson, L. W. J., & Hofstra, D. (2017). Management and control methods of invasive alien freshwater aquatic plants: A review. In *Aquatic Botany* (Vol. 136, pp. 112–137). Elsevier B.V. https://doi.org/10.1016/j.aquabot.2016.08.002

Pimentel, D. (1957). Life History of Australorbis Glabratus, The Intermediate Snail Host of Schistosoma. In *Source: Ecology* (Vol. 38, Issue 4).

Katsuki, M., Okada, Y., & Okada, K. (2012). Impacts of diet quality on life-history and reproductive traits in male and female armed beetle, Gnatocerus Cornutus. *Ecological Entomology*, *37*(6), 463–470. https://doi.org/10.1111/j.1365-2311.2012.01390.x

Kilham, S. S., Kreeger, D. A., Lynn, S. G., Goulden, C. E., & Herrera, L. (1998). COMBO: a defined freshwater culture medium for algae and zooplankton. In *Hydrobiologia* (Vol. 377).

Kooijman, S. A. L. M. (2010). *Dynamic Energy Budget Theory for metabolic organisation*. Cambridge University Press.

Malishev, M., & Civitello, D. J. (2019). Linking Bioenergetics and Parasite Transmission Models Suggests Mismatch between Snail Host Density and Production of Human Schistosomes. *Integrative and Comparative Biology*, *59*(5), 1243–1252. https://doi.org/10.1093/icb/icz058

McManus, D. P., Dunne, D. W., Sacko, M., Utzinger, J., Vennervald, B. J., & Zhou, X.-N. (2018). Schistosomiasis. *Nature Reviews Disease Primers*, *4*(1). https://doi.org/10.1038/s41572-018-0013-8

Mkumbo, O. C., & Marshall, B. E. (2015). The Nile perch fishery of Lake Victoria: Current status and management challenges. *Fisheries Management and Ecology*, *22*(1), 56–63. https://doi.org/10.1111/fme.12084

Mutuku, M. W., Laidemitt, M. R., Beechler, B. R., Mwangi, I. N., Otiato, F. O., Agola, E. L., Ochanda, H., Kamel, B., Mkoji, G. M., Steinauer, M. L., & Loker, E. S. (2019). A search for snail-related answers to explain differences in response of schistosoma mansoni to praziquantel treatment among responding and persistent hotspot villages along the Kenyan Shore of Lake Victoria. *American Journal of Tropical Medicine and Hygiene*, *101*(1), 65–77. https://doi.org/10.4269/ajtmh.19-0089

Nakazawa, T. (2011). The ontogenetic stoichiometric bottleneck stabilizes herbivore-autotroph dynamics. *Ecological Research*, *26*(1), 209–216. https://doi.org/10.1007/s11284-010-0752-9

Olsen, A., Kinung'hi, S., & Magnussen, P. (2015). Schistosoma mansoni infection along the coast of Lake Victoria in Mwanza region, Tanzania. *American Journal of Tropical Medicine and Hygiene*, *92*(6), 1240–1244. https://doi.org/10.4269/ajtmh.14-0676

Plummer, M. L. (2005). Impact of invasive water hyacinth (Eichhornia crassipes) on snail hosts of schistosomiasis in Lake Victoria, East Africa. *EcoHealth*, *2*(1), 81–86. https://doi.org/10.1007/s10393-004-0104-8

Reddy, K. R., & De Busk, W. F. (1985). Nutrient removal potential of selected aquatic macrophytes. *Journal of Environmental Quality*, *14*(4), 459–462. https://doi.org/10.2134/jeq1985.00472425001400040001x

Richman, S. E., Leafloor, J. O., Karasov, W. H., & McWilliams, S. R. (2014). Ecological implications of reduced forage quality on growth and survival of sympatric geese. *Journal of Animal Ecology*, *84*(1), 284–298. https://doi.org/10.1111/1365-2656.12270

Thomas, J. D., Nwanko, D. I., & Sterry, P. R. (1985). The feeding strategies of juvenile and adult Biomphalaria glabrata (say) under simulated natural conditions and their relevance to ecological theory and Snail Control. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, *226*(1243), 177–209. https://doi.org/10.1098/rspb.1985.0090

Villamagna, A. M., & Murphy, B. R. (2010). Ecological and socio-economic impacts of invasive water hyacinth (Eichhornia crassipes): A review. In *Freshwater Biology* (Vol. 55, Issue 2, pp. 282–298). https://doi.org/10.1111/j.1365-2427.2009.02294.x

Villar-Argaiz, M., & Sterner, R. W. (2002). Life history bottlenecks in diaptomus clavipes induced by phosphorus-limited algae. *Limnology and Oceanography*, *47*(4), 1229–1233. https://doi.org/10.4319/lo.2002.47.4.1229

Zandonà, E., Auer, S. K., Kilham, S. S., Howard, J. L., López-Sepulcre, A., O'Connor, M. P., Bassar, R. D., Osorio, A., Pringle, C. M., & Reznick, D. N. (2011). Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. *Functional Ecology*, *25*(5), 964–973. https://doi.org/10.1111/j.1365-2435.2011.01865.x

APPENDIX A:

Tables of p-values for pairwise contrasts of estimated marginal means using the Tukey method (R package: emmeans) based on general linear models fitted to experimental data (R package: stats).

Table 1. Size contrasts by detritus type within each starting size class.

Starting Size Class	Detritus Type Contrast	p-value	Significance
1	W. hyacinth - W. lettuce	1.8 e-52	***
2	W. hyacinth - W. lettuce	7.9 e-46	***
4	W. hyacinth - W. lettuce	3.6 e-37	***
8	W. hyacinth - W. lettuce	1.8 e-29	***
12	W. hyacinth - W. lettuce	8.7 e-8	***
15	W. hyacinth - W. lettuce	3.8 e-2	*

Table 2. Size contrasts by starting size class within water lettuce detritus treatment.

Starting Size Class Contrast	p-value	Significance
1 - 2	8.9 e-1	
1 - 4	10.0 e-1	
1 - 8	1.3 e-1	
1 - 12	10.0 e-1	
1 - 15	3.2 e-2	*
2 - 4	8.4 e-1	
2 - 8	7.1 e-1	
2 - 12	7.6 e-1	
2 - 15	3.6 e-1	

4 - 8	9.9 e-2	
4 - 12	10.0 e-1	
4 - 15	2.3 e-2	*
8 - 12	6.9 e-2	
8 - 15	9.9 e-1	
12 - 15	1.5 e-2	*

Table 3. Log-transformed cumulative egg count contrasts by detritus type within each starting size class.

Starting Size Class	Detritus Type Contrast	p-value	Significance
1	W. hyacinth - W. lettuce	8.1 e-5	***
2	W. hyacinth - W. lettuce	5.6 e-9	***
4	W. hyacinth - W. lettuce	1.7 e-13	***
8	W. hyacinth - W. lettuce	8.2 e-10	***
12	W. hyacinth - W. lettuce	5.4 e-6	***
15	W. hyacinth - W. lettuce	2.2 e-4	***

APPENDIX B:

Table of weekly detritus amounts given in life table experiment by starting size class and detritus type. Detritus amount listed is the amount that snails were offered the week *prior to* data collection. First week of data collection (just prior to addition of snails to life table cups and provision of detritus) is Week 0.

Table 4. Weekly detritus amounts by starting size class and detritus type.

Week	Starting Size Class (mm)	Detritus Type	Detritus Amount (mg)
1	1	W. Hyacinth	15
1	2	W. Hyacinth	25
1	4	W. Hyacinth	50
1	8	W. Hyacinth	100
1	12	W. Hyacinth	200
1	15	W. Hyacinth	350
1	1	W. Lettuce	15
1	2	W. Lettuce	25
1	4	W. Lettuce	50
1	8	W. Lettuce	100
1	12	W. Lettuce	200
1	15	W. Lettuce	350
2	1	W. Hyacinth	15
2	2	W. Hyacinth	25
2	4	W. Hyacinth	50
2	8	W. Hyacinth	100
2	12	W. Hyacinth	200
2	15	W. Hyacinth	350
2	1	W. Lettuce	15
2	2	W. Lettuce	25
2	4	W. Lettuce	50
2	8	W. Lettuce	100
2	12	W. Lettuce	200
2	15	W. Lettuce	350
3	1	W. Hyacinth	15
3	2	W. Hyacinth	25
3	4	W. Hyacinth	50
3	8	W. Hyacinth	100
3	12	W. Hyacinth	200

3	15	W. Hyacinth	350
3	1	W. Lettuce	25
3	2	W. Lettuce	50
3	4	W. Lettuce	100
3	8	W. Lettuce	200
3	12	W. Lettuce	350
3	15	W. Lettuce	350
4	1	W. Hyacinth	15
4	2	W. Hyacinth	25
4	4	W. Hyacinth	50
4	8	W. Hyacinth	100
4	12	W. Hyacinth	250
4	15	W. Hyacinth	350
4	1	W. Lettuce	50
4	2	W. Lettuce	100
4	4	W. Lettuce	100
4	8	W. Lettuce	200
4	12	W. Lettuce	350
4	15	W. Lettuce	350
5	1	W. Hyacinth	15
5	2	W. Hyacinth	25
5	4	W. Hyacinth	50
5	8	W. Hyacinth	100
5	12	W. Hyacinth	200
5	15	W. Hyacinth	350
5	1	W. Lettuce	75
5	2	W. Lettuce	100
5	4	W. Lettuce	150
5	8	W. Lettuce	200
5	12	W. Lettuce	350
5	15	W. Lettuce	350
6	1	W. Hyacinth	15
6	2	W. Hyacinth	25
6	4	W. Hyacinth	50
6	8	W. Hyacinth	100
6	12	W. Hyacinth	NA
6	15	W. Hyacinth	NA
6	1	W. Lettuce	100
6	2	W. Lettuce	150
6	4	W. Lettuce	200
6	8	W. Lettuce	270
6	12	W. Lettuce	350

6	15	W. Lettuce	350
7	1	W. Hyacinth	15
7	2	W. Hyacinth	25
7	4	W. Hyacinth	50
7	8	W. Hyacinth	100
7	12	W. Hyacinth	NA
7	15	W. Hyacinth	NA
7	1	W. Lettuce	150
7	2	W. Lettuce	200
7	4	W. Lettuce	200
7	8	W. Lettuce	270
7	12	W. Lettuce	350
7	15	W. Lettuce	350
8	1	W. Hyacinth	15
8	2	W. Hyacinth	25
8	4	W. Hyacinth	50
8	8	W. Hyacinth	100
8	12	W. Hyacinth	NA
8	15	W. Hyacinth	NA
8	1	W. Lettuce	200
8	2	W. Lettuce	200
8	4	W. Lettuce	200
8	8	W. Lettuce	270
8	12	W. Lettuce	350
8	15	W. Lettuce	350
9	1	W. Hyacinth	15
9	2	W. Hyacinth	25
9	4	W. Hyacinth	50
9	8	W. Hyacinth	100
9	12	W. Hyacinth	NA
9	15	W. Hyacinth	NA
9	1	W. Lettuce	200
9	2	W. Lettuce	200
9	4	W. Lettuce	270
9	8	W. Lettuce	270
9	12	W. Lettuce	350
9	15	W. Lettuce	350
10	1	W. Hyacinth	15
10	2	W. Hyacinth	25
10	4	W. Hyacinth	50
10	8	W. Hyacinth	100
10	12	W. Hyacinth	NA

10	15	W. Hyacinth	NA
10	1	W. Lettuce	200
10	2	W. Lettuce	200
10	4	W. Lettuce	270
10	8	W. Lettuce	270
10	12	W. Lettuce	350
10	15	W. Lettuce	350
11	1	W. Hyacinth	15
11	2	W. Hyacinth	25
11	4	W. Hyacinth	50
11	8	W. Hyacinth	100
11	12	W. Hyacinth	NA
11	15	W. Hyacinth	NA
11	1	W. Lettuce	200
11	2	W. Lettuce	220
11	4	W. Lettuce	270
11	8	W. Lettuce	270
11	12	W. Lettuce	350
11	15	W. Lettuce	350
12	1	W. Hyacinth	15
12	2	W. Hyacinth	25
12	4	W. Hyacinth	50
12	8	W. Hyacinth	100
12	12	W. Hyacinth	NA
12	15	W. Hyacinth	NA
12	1	W. Lettuce	200
12	2	W. Lettuce	220
12	4	W. Lettuce	270
12	8	W. Lettuce	270
12	12	W. Lettuce	350
12	15	W. Lettuce	350