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CYANOBACTERIA-RICH DIET REDUCES GROWTH RATES OF THE HYACINTH SILTSNAIL *FLORIDOBIA FLORIDANA* (GASTROPODA: HYDROBIIDAE)

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ABSTRACT

The freshwater gastropod genus *Floridobia* comprises 13 species in Florida, 11 of which are endemic to unique freshwater springs. Recent overgrowth of mat-forming filamentous algae and cyanobacteria in Florida spring runs could negatively impact growth, reproduction, and ultimately, the persistence of these native snail species. To determine the effect of nuisance cyanobacteria on siltsnail growth, we fed a cosmopolitan species, *Floridobia floridana*, diets composed of algae commonly found in Florida springs. Diets consisted of a) the cyanobacteria *Lyngbya* sp., b) a non-cyanbacteria control consisting of the eukaryotic yellow-green alga *Vaucheria* sp., and c) a mixture of both species. We predicted snails fed *Lyngbya* would have reduced growth due to low highly unsaturated fatty acid (HUFA) content. Snails fed *Vaucheria* were predicted to have an intermediate growth rate, and the highest growth was predicted for the mixed diet because multi-algal diets typically provide superior nutrition for grazers. Snails in all treatments were fed equal carbon content weekly for a period of 15 weeks. At the conclusion of the study, snails fed *Lyngbya* or *Vaucheria* had reduced relative growth rates compared to those fed the mixed diet (p = 0.0002). Reduced growth rates most likely resulted from poor nutritional content of *Lyngbya*, although cyanobacteria cell morphology may have also played a role. Our study suggests that though *Vaucheria* may provide adequate nutrition, continued increases in the standing crop of cyanobacteria in Florida springs could threaten the persistence of endemic siltsnails. Efforts to preserve the integrity of these springs should therefore focus on limiting cyanobacteria blooms.

KEY WORDS freshwater springs, gastropod, filamentous algae, *Lyngbya, Vaucheria*

INTRODUCTION

Extinction rates of plants and animals are increasing worldwide and may not peak for decades (Pimm & Raven, 2000). One of the most affected groups is the freshwater mollusks; of the 693 recorded animal extinctions since 1500, 42% were molluscan. The number of extinct gastropod species alone—260—outnumbers extinct tetrapods (Lydeard *et al*., 2004). Freshwater gastropods are particularly prone to becoming endangered or extinct because many are habitat specialists with relatively restricted ranges (Lydeard *et al*., 2004). Unfortunately, a lack of basic ecological knowledge of many species makes it difficult to determine their conservation status or identify potential threats to their persistence (Brown *et al*., 2008; Lysne *et al*., 2008; Strong *et al*., 2008). The gastropod family Hydrobiidae is experiencing multiple challenges (Brown *et al*., 2008; Mehlhop & Vaughn, 1994); over 74% of the species in this family have a conservation status of <G2 (Imperiled—at high risk of extinction due to very restricted range, very few populations, steep declines, etc.) or greater (Brown *et al*., 2008).

Hydrobiids are prosobranch gastropods, occur worldwide in fresh and brackish water (Mladenka & Minshall, 2001; Shelton, 2005; Thompson, 1968), and are known for their high degree of endemism (Brown *et al*., 2008; Shelton, 2005). An estimated 1,250 species of hydrobiid snails have been described, with many yet to be discovered (Strong *et al*., 2008). Twenty-six species of Hydrobiidae are recognized in peninsular Florida, many of which are highly endemic to freshwater springs (Strong *et al*., 2008; Thompson, 1968).

Florida's freshwater springs are threatened by aquifer depletion, water diversion, habitat destruction, and water quality issues (Mehlhop &Vaughn, 1994). Recently, a significant shift in the algal community composition has occurred in springs throughout central Florida. In particular, two species of filamentous algae are quickly becoming dominant: the cyanobacteria *Lyngbya* sp. and the yellow-green *Vaucheria* sp. (Stevenson *et al*., 2007). Increasing nitrate concentrations have been implicated in the shift in the algal community composition although there is some evidence that decreased grazer abundance resulting from low dissolved oxygen concentrations may also be responsible (Heffernan *et al*., 2010).

Cyanobacteria such as *Lyngbya* are generally considered to be nutritionally inadequate food sources for aquatic organisms (Basen et al., 2012; Brett *et al*., 1997; Schmidt & Jónasdóttir, 1997; Skoog, 1978). Several reasons for this have been cited, including cyanobacterial cell morphology, toxicity, and poor nutritional content. Many cyanobacteria have a thick gelatinous sheath that surrounds the filaments, which might inhibit the ability of grazers to ingest it (de Bernardi & Giussani, 1990; Van Donk *et al*., 2011; Komárek et al., 2003) or lead to active avoidance (Engström *et al*., 2001). The toxic effects of cyanobacteria have been demonstrated in pulmonate (Lance *et al*., 2007) and prosobranch (Lance *et al*., 2008) gastropods, as well as crustaceans (Engström *et al*., 2001), and include sublethal effects on growth and fecundity (Lance *et al*., 2007). Cyanobacteria tend to have low highly unsaturated fatty acid or 'HUFA' content and considerable evidence suggests that HUFAs and other lipids (Basen et al., 2012; Basen *et al*., 2011) are key components of the diets of grazers (Brett *et al*., 1997).

Vaucheria sp. belongs to the Xanthophyceae, a group of yellow-green algae that reproduce asexually and sexually (via zoospores). In Florida, *Vaucheria* sp. are a major nuisance algae that tend to be found in sites with high alkalinity and nitrogen: the percent coverage of this species reaches 100% in some Florida springs (Stevenson *et al*., 2007). The potential of these algae as a food source for native snails has not been evaluated. However, many species of Xanthophyta contain essential HUFAs that have been used for production of HUFAs for human consumption (Řezanka *et al*., 2010). Thus, *Vaucheria* may provide superior nutrition for grazers when compared to cyanobacteria.

If *Lyngbya* sp. consitutes poor quality food for grazers, continued increases in the standing crop of this filamentous cyanobacteria in spring runs could result in decreased growth and reproduction and threaten the persistence of endemic silt snails in Florida springs.

Our study focused on the hydrobiid siltsnail *Floridobia floridana* Frauenfeld, 1863 (Hyacinth Siltsnail). Although *F. floridana* is widespread, other congeners have a more restricted distribution; 11 of 13 species in the genus are known only from a single spring (Fig. 1; Thompson, 2004).

FIGURE 1

Floridobia sp. occurrences throughout central Florida. Numbers indicate location of spring endemics. Hatches and shading indicate range for *F. fraterna* and *F. floridana*, respectively. 1: Ichetucknee Springs - *Floridobia mica*; 2: Hunter Spring - *Floridobia helicogyra*; 3: Green Cove Spring - *Floridobia porterae*; 4: Silver Glen Springs - *Floridobia leptospira*; 5: Alexander Springs - *Floridobia alexander*; 6: Seminole Springs - *Floridobia vanhyningi*; 7: Rock Springs - *Floridobia petrifons*; 8: Wekiva Springs - *Floridobia wekiwae*; 9: Palm Spring- *Floridobia ponderosa*; 10: Blue Spring - *Floridobia parva*; 11: Benson's Mineral Spring - *Floridobia monroensis*.Cartographers: D. Bell, J. Duryea, K. Mueller, and S. Pesci.

Through this study, we hoped to provide insight into the potential impact of changing algal community composition on *F. floridana*, as well as on other less widely distributed members of the genus (*i.e*., spring endemics).

Much evidence suggests that uni-algal diets constitute an inadequate diet for most grazers (Brett *et al*., 1997; Foster *et al*., 1999; Gatenby *et al*., 1997; Wacker & von Elert, 2002). Thus, we predicted that *F. floridana* would have the fastest growth rate on a mixed diet of *Vaucheria* sp. and *Lyngbya* sp., intermediate growth rates on Vaucheria sp. which may contain essential HUFAs, and slowest growth on the cyanobacteria *Lyngbya* sp. We also predicted higher mortality in snails fed a uni-algal diet of *Lyngbya* sp. because snails may not be able to consume the filaments and thus would lack adequate energy for growth and survival.

MATERIALS & METHODS

Study Organism

In August 2010, 360 *F. floridana*, spring water, and limestone rocks were collected from the boil of Volusia Blue Spring in Volusia County, FL. Blue Spring is a first magnitude spring (Scott *et al*., 2002). The spring run is 25 m wide and 320 m long and flows out of the Floridan aquifer into the St. Johns River (Scott *et al*., 2002). Water temperature is on average 23°C year round which allows species such as the Florida manatee (*Trichechus manatus latirostris*) to use the spring run as a thermal refuge in winter (Gibbs *et al*., 2008).

Floridobia floridana populations in Blue Spring appear to be annual (Fig. 2). This very small siltsnail (2.8-3.5 mm adult size) is a relatively cosmopolitan species that occurs throughout the northern half of the Florida peninsula (Thompson, 1968).

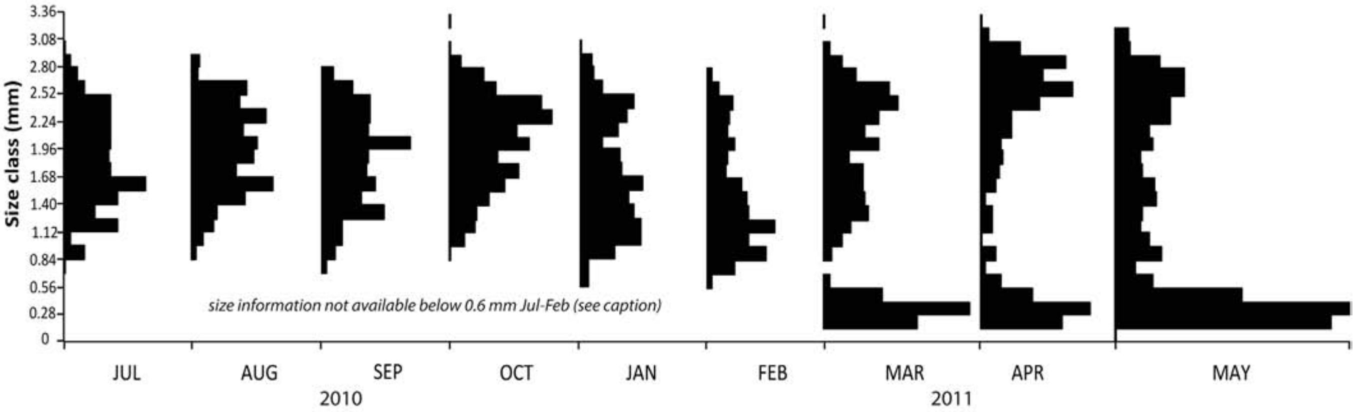

FIGURE 2

Histograms of *Floridobia floridana* growth rates from Volusia Blue Spring over a nine month period with 0.14 mm size classes. Size information for < 0.6 mm not available until Mar 2011 due to collecting restrictions.

Spring water and limestone rocks were sterilized to remove potential pathogens. One limestone rock, autoclaved spring water, and 10 snails were added to each clear plastic cylindrical container (12 cm diameter, 14 cm height). Containers were placed in an E8 Conviron controlled environmental chamber at 22°C with a 12:12h light:dark cycle. Air was bubbled slowly into each container to maintain dissolved oxygen concentrations.

The treatments (n=12 for each treatment) consisted of various diets of filamentous algae that occur naturally in Blue Spring 1) *Vaucheria*, which served as a non-cyanobacteria control, 2) *Lyngbya*, a cyanobacteria, or 3) a mixture of *Vaucheria* and *Lyngbya*. Containers were assigned to groups using a randomized block design. Before the study began, snails were fed *Lyngbya* sp. *ad libitum* for three weeks and then starved for 48 h. *Lyngbya* sp. was used for the initial stage of the experiment because it was easily grown in large amounts. Water was replaced every three weeks, and limestone rocks were autoclaved and containers replaced every six weeks to limit bacterial and algal growth in the containers. The study was terminated after four months due to snail reproduction; all data shown here are from measurements taken prior to first reproduction.

Vaucheria sp. was cultured in Alga-Gro Freshwater Media (Carolina Biological Supply) with artificial spring water (Gibbs, 2003) and *Lyngbya* sp. was cultured in Soil-Water Medium (Carolina Biological Supply). Cultures were re-started every two weeks in order to feed the snails during the logarithmic phase of algal growth (pers. comm. M. Patterson, Fisheries Biologist, US Fish & Wildlife Service, June 2010.) Cultures were uni-algal but not axenic, and cheesecloth was placed at the opening of the algae flasks to prevent contamination and allow aeration (Gatenby *et al*., 1997).

Throughout the study, snails in all treatments were fed equal carbon content, although the absolute amount of carbon varied from week to week due to variations in algal growth. Mean carbon content per cell was determined by average cell volume * predetermined picoggrams (10-12g) of carbon (pgC) per cell of similar algae species (from Rocha & Duncan, 1985). In our cultures, *Vaucheria* sp. primarily reproduced sexually, thus zoospore cell volume was used. *Lyngbya* sp. filaments were on average 210 µm in length and *Vaucheria* sp. zoospores were 5 µm long. Prior to counting, *Lyngbya* was vortexed to loosen filaments. When indicated, cultures were concentrated via centrifugation. Cell concentrations were determined with a hemocytometer. The carbon content (as pgC/mL) was calculated as mean carbon content per cell (pgC) * cells/mL. As necessary, the cultures with the highest pgC/mL were diluted until the carbon content was equal to the culture with the lowest pgC/mL. As a result, 5 mL of algae with equal carbon content was added weekly to each treatment.

Growth was determined by measuring shell length from the tip of the apex to the middle of the aperture under a dissecting microscope using digital calipers (Mitutoyo Model CD-6"CX). Measurements were taken approximately every 21days (d) for 103 d. The number of dead snails in each container was recorded. The minute size of these snails made it impossible to label (and thus track growth of) individual snails; thus, we calculated the mean length of all snails in each container and tracked changes in average length between measurements. Because this approach is sensitive to the loss of snails, containers were excluded from growth rate analyses, if mortality was observed.

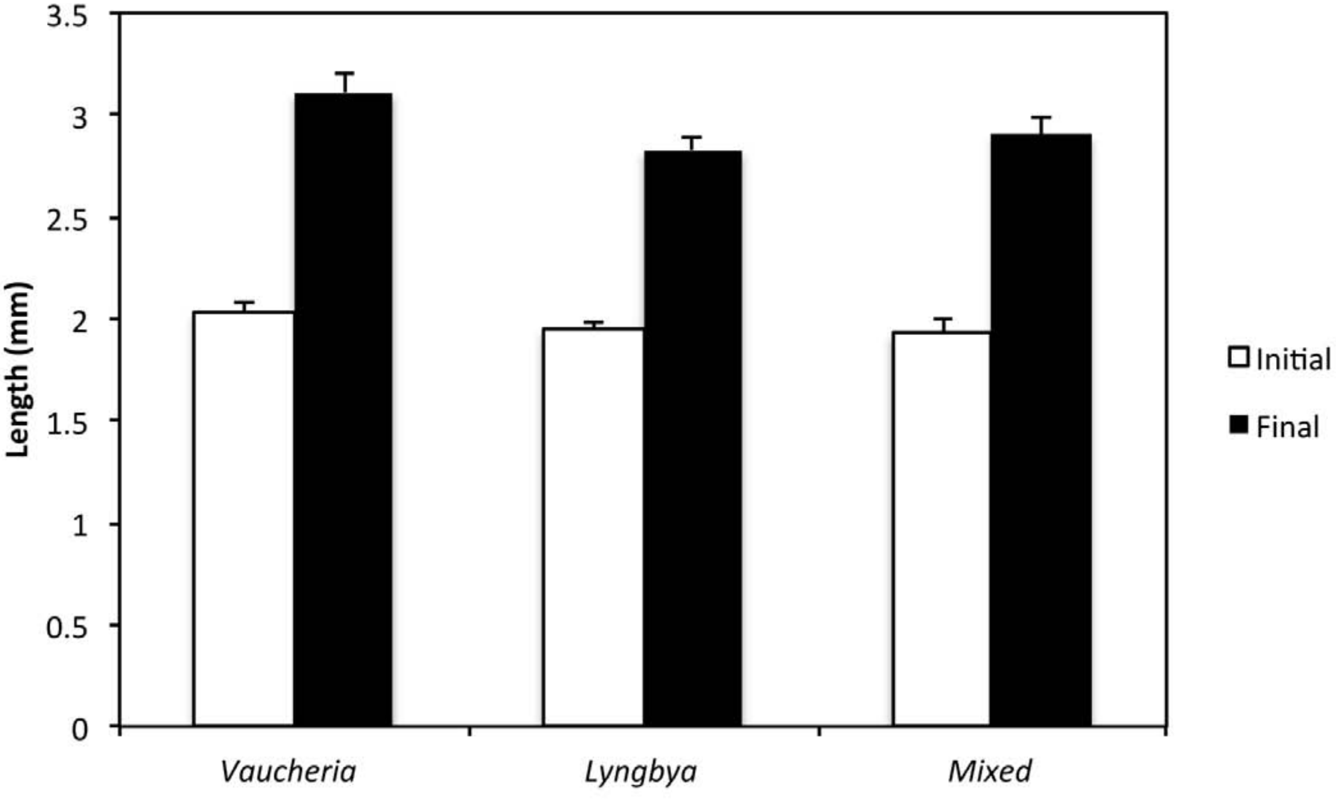

FIGURE 3

Mean length of *Floridobia floridana* before and after a 15-week diet of *Vaucheria* sp. (control), *Lyngbya* sp., or a mixture of the two algal species with ± 1 SEM. Containers with mortality not included.

Although there were no significant differences in initial sizes among treatments (Fig. 3), we used relative growth rate to account for differences in size. Relative growth was calculated as:

$$\text{relative growth rate} = \frac{\left(\ln\left(\text{final length}\right) - \ln\left(\text{initial length}\right)\right)}{t}$$

where t = duration of experiment in days

ANOVA as implemented in JMP ver 6.0.2 (SAS Institute, Cary, NC) was used to test for differences in the mean change in relative growth rate between measurement periods. Where indicated, t-tests with Bonferroni's correction for multiple comparisons were performed to determine which treatments were different.

Mortality data were analyzed using a nominal logistic model with survival (0,1) as the response variable and treatment as the main effect and a likelihood Chi-square, also with JMP ver 6.02.

RESULTS

Snails in all treatments increased in size over the course of the study (Fig. 3). There were no significant differences in initial (p = 0.23) or final absolute length (p=0.052) across treatments. However, snails in the *Lyngbya* treatment had the lowest overall absolute growth rate (K = 0.011, 0.008, and 0.01 mm/d for *Vaucheria, Lyngbya* and mixed diets respectively). Also, at the end of the study, the mean relative growth rate of snails fed *Lyngbya* or *Vaucheria* was lower than snails fed the mixed diet (p = 0.0002; Fig. 4). Mortality was very low overall and did not differ significantly across treatments (0.88%, 1.2%, 0.86%, for *Vaucheria, Lyngbya*, and mixed diet, respectively).

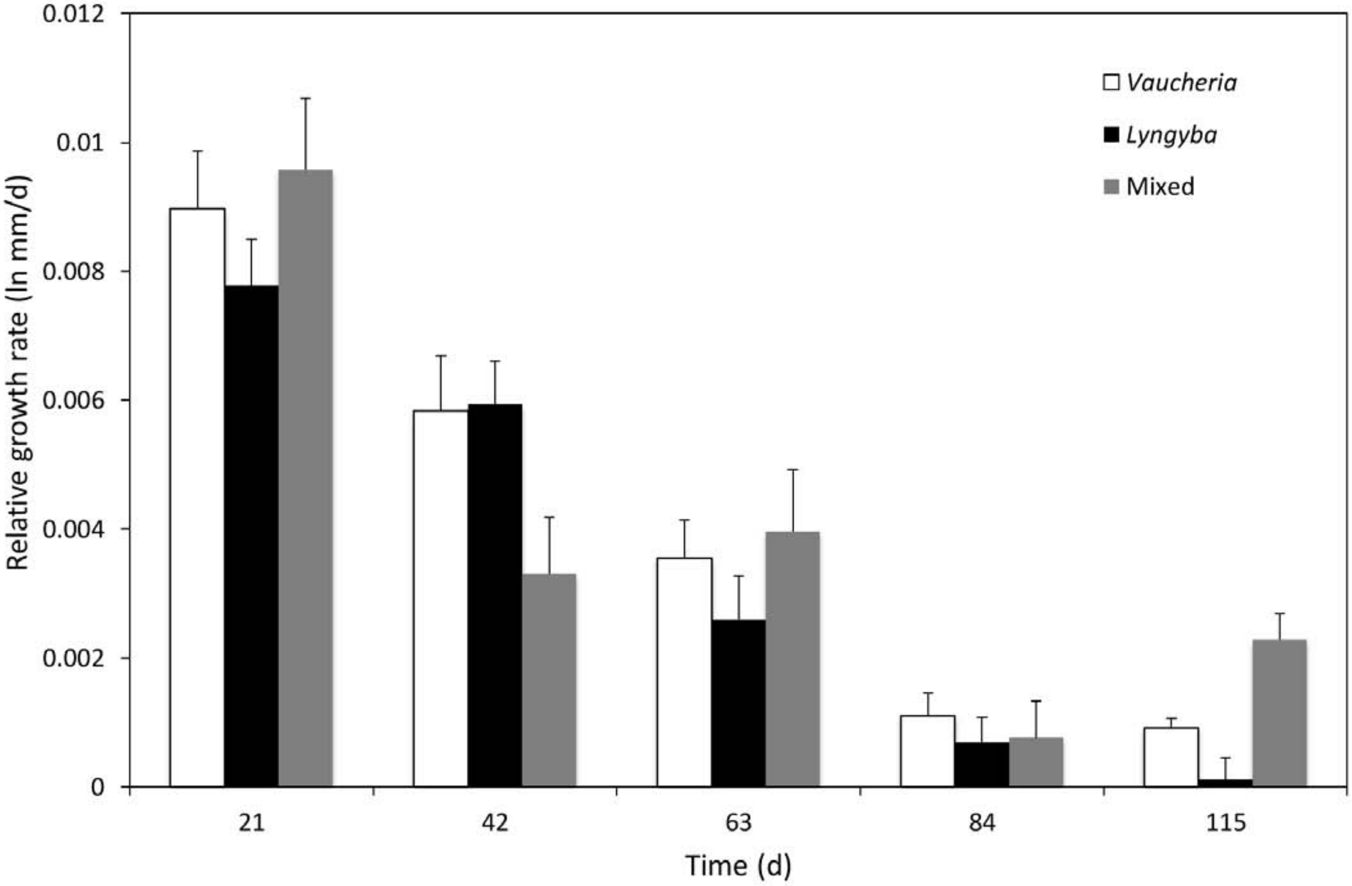

FIGURE 4

Relative growth rate of Floridobia floridana on diets of Vaucheria sp., Lyngbya sp., or a mixture of the two algal species with ± 1 SEM. Bars not connected by same letter are significantly different (p = 0.0002). Containers with mortality not included.

DISCUSSION

Consistent with our hypothesis, a uni-algal diet of the cyanobacteria *Lyngbya* sp. or the yellow green *Vaucheria* sp. negatively affected relative growth rate of *Floridobia floridana* compared to the mixed diet. This effect was only seen during the final study period, when snails fed a mixed diet continued to grow and those on the uni-algal diet either did not grow (*Lyngbya*) or grew at a reduced rate (*Vaucheria* sp.) The mixed diet may have provided additional nutrients absent in a diet of *Vaucheria* or *Lyngbya* alone. The negative effects of uni-algal diets are well-documented: the growth rate of *Villosa iris* was highest when fed two green algae and a diatom as compared to a uni-algal diet (Gatenby *et al*., 1997). Similarly, post-settlement growth of *Dreissena polymorpha* was higher on a mixed diet of four algal species compared to one species of cyanobacteria (Wacker & von Elert, 2002). Growth rates of the marine snail *Turbo sarmaticus* were also highest when fed a mixture of three marine algae (Foster *et al*., 1999). However, is it interesting that in our study a mixed diet that contained cyanobacteria would lead to higher relative growth rate, as cyanobacteria, particularly *Lyngbya* (see below), are expected to provide little additional nutrition (Brett *et al*., 1997; but see Schmidt & Jónasdóttir, 1997).

Our hypothesis that the relative growth rate of snails fed *Vaucheria* would be greater than those fed *Lyngbya* was not supported for any of the study periods. However, the overall absolute growth rates for the *Vaucheria* (0.011 mm/d) and mixed diets (0.01 mm/d) were higher than that of the *Lyngbya* diet (0.008 mm/d), and similar to those of natural populations of the closely related hydrobiid *Pyrgulopsis robusta* in good (low competition) conditions. In contrast, growth rates as low as that of the *Lyngyba* treatment occurred only in *P. robusta* in poor (high competition conditions) (Riley *et al*., 2008). Thus, although our relative growth rate data suggest only that the mixed diet yielded higher relative growth rate than a uni-algal diet, the absolute growth rate data indicate that *Vaucheria* may be a superior food source for *F. floridana*, perhaps owing to HUFA (Brett *et al*., 1997) or other lipid content (i.e., sterols; Basen *et al*., 2012). The smaller final size and slower growth rate of snails fed *Lyngbya* are not surprising, given that congeners have low fatty acid content (Rajeshwari & Rajashekar, 2011) and are known to be low quality food sources, even among cyanobacteria (Nagarkar *et al*., 2004).

Other nutrients (such as phosphorus or P) may also have played a role in the slower relative and overall absolute growth rate of snails fed *Lyngbya* sp. However, the effects of P limitation on grazer growth have been observed in nutrient poor systems (Stelzer & Lamberti, 2002); whereas natural concentrations of P in many Florida springs (Stevenson *et al*., 2007) and in our laboratory-maintained populations are above that which would constrain algal growth.

The size of the algal cells could also have impacted *F. floridana's* ability to feed. The ideal algal cell size for hydrobiids decreases with snail body size, with snails of sizes similar to those in our study unable to ingest cells larger than 125-150 μm (Fenchel & Kofoed, 1976). While *Vaucheria* zoospores (~5 μm) fall well within this range, *Lyngbya* sp. filaments at 210 μm may not be as easily consumed.

Although we did not measure whether *Lyngbya* in our study produced toxins, natural populations of *F. floridana* are unlikely to be exposed to high levels of *Lyngbya* toxins. Thorough sampling of *L. wollei* blooms in first magnitude springs across Florida have not detected saxitoxins, lyngbyatoxins, or debromoaplysiatoxins (PBS & J., 2006).

In contrast with our predictions, mortality was similar across all treatments. The occurrence of mortality may indicate that none of the diets we provided were of particularly high quality; studies of other freshwater gastropods exposed to cyanobacteria displayed no mortality during an 8-wk study period (Lance *et al*., 2007; 2008).

The late effects on relative growth rate in our study suggest that a longer period may be necessary to detect larger differences in growth rates. Lance *et al*. (2007) also found that long exposure times were necessary to see effects of cyanobacteria (esp. toxins) on growth rates. Thus, the smallest possible snails should be collected for future studies.

If the trend we saw in our laboratory study translates into slower growth rates in natural populations, increasing dominance of cyanobacteria in Florida springs and spring runs could have possible negative implications for persistence of *F. floridana* and other siltsnails. For example, individuals may take longer to reach maturity and do so at smaller body size. This could reduce fecundity and lead to decreases in population size that threaten the continued survival of these species.

This study of *F. floridana* is instrumental to learning more about the ecology of this species and other closely related endemics. Understanding how increases in filamentous algae in Florida springs will affect growth rate of endemic siltsnails can help direct conservation efforts and provide guidelines for maintaining healthy populations. Our results suggest that efforts to preserve the integrity of these springs should focus on limiting algal blooms. However, because the cause of blooms is not completely clear (Heffernan *et al*., 2010; but see Stevenson *et al*., 2007), future studies should also work to identify spring recharge areas, surrounding land use, and water quality entering the recharge areas to determine the causes of the increasing dominance of filamentous algae.

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Freshwater Mollusk Conservation Society

OUR PURPOSE

The Freshwater Mollusk Conservation Society (FMCS) is dedicated to the conservation of and advocacy of freshwater mollusks, North America's most imperiled animals. Membership in the society is open to anyone interested in freshwater mollusks who supports the stated purposes of the Society which are as follows:

1) Advocate conservation of freshwater molluscan resources;

2) Serve as a conduit for information about freshwater mollusks;

3) Promote science-based management of freshwater mollusks;

4) Promote and facilitate education and awareness about freshwater mollusks and their function in freshwater ecosystems;

5) Assist with the facilitation of the National Strategy for the Conservation of Native Freshwater Mussels (Journal of Shellfish Research, 1999, Volume 17, Number 5), and a similar strategy under development for freshwater gastropods.

OUR HISTORY

The FMCS traces it's origins to 1992 when a symposium sponsored by the Upper Mississippi River Conservation Committee, USFWS, Mussel Mitigation Trust, and Tennessee Shell Company brought concerned people to St. Louis, Missouri to discuss the status, conservation, and management of freshwater mussels. This meeting resulted in the formation of a working group to develop the National Strategy for the Conservation of Native Freshwater Mussels and set the ground work for another freshwater mussel symposium. In 1995, the next symposium was also held in St. Louis, and both the 1992 and 1995 symposia had published proceedings. Then in March 1996, the Mississippi Interstate Cooperative Research Association (MICRA) formed a mussel committee. It was this committee (National Native Mussel Conservation Committee) whose function it was to implement the National Strategy for the Conservation of Native Freshwater Mussels by organizing a group of state, federal, and academic biologists, along with individuals from the commercial mussel industry. In March 1998, the NNMCC and attendees of the Conservation, Captive Care and Propagation of Freshwater Mussels Symposium held in Columbus, OH, voted to form the Freshwater Mollusk Conservation Society. In November 1998, the executive board drafted a society constitution and voted to incorporate the FMCS as a not-for-profit society. In March 1999, the FMCS held it's first symposium "Musseling in on Biodiversity" in Chattanooga, Tennessee. The symposium attracted 280 attendees; proceedings from that meeting are available for purchase. The second symposium was held in March 2001 in Pittsburgh, Pennsylvania, the third in March 2003 in Raleigh, North Carolina, the fourth in St. Paul, Minnesota in May 2005, the fifth in Little Rock, Arkansas in March 2007, the sixth in Baltimore, Maryland in April 2009, the seventh in Louisville, Kentucky in 2011, and the eighth in Guntersville, Alabama in 2013. The society also holds workshops on alternating years, and produces a newsletter four times a year.

FMCS SOCIETY COMMITTEES

Participation in any of the standing committees is open to any FMCS member. Committees include:

Awards
Environmental Quality and Affairs
Gastropod Distribution and Status
Genetics
Guidelines and Techniques
Information Exchange - Walkerana and Ellipsaria
Mussel Distribution and Status
Outreach
Propagation and Restoration

TO JOIN FMCS OR SUBMIT A PAPER

Please visit our website for more information at http://www.molluskconservation.org

Or contact any of our board members or editors of WALKERANA to talk to someone of your needs. You'll find contact information on the back cover of this publication.