

ENVIRONMENTALLY MEDIATED CONSUMER CONTROL OF ALGAE  
PROLIFERATION IN FLORIDA SPRINGS

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL  
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT  
OF THE REQUIREMENTS FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2013

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To Harold and Judy Liebowitz

## ACKNOWLEDGMENTS

I would like to express my deepest gratitude to my advisor, Matt Cohen, for his endless encouragement, scientific curiosity, brilliance, kindness, and good humor. He has been an inspiration to me throughout this process, and I cannot thank him enough. I also sincerely thank my wonderful committee, Tom Frazer, Jim Heffernan, Ed Philips, and Mark Brenner, for their insights, patience, and support.

I could not have accomplished all the gator-defying field work without the help the mild-mannered man of steel, Larry Korhnak. I am also extremely grateful to many people for field and lab assistance, including Crystal Hartman, Ashley Aarensen, Jenn Dragunchuk, Josh Bellamy, Loren Matthews, Chad Foster, David Kaplan, Jonathan Field, and Jennifer Hoyer.

Many sources of financial support allowed me to focus on this work. The NSF IGERT program on Adaptive Management: Wetlands, Water, and Watersheds, under Mark Brown's leadership, provided financial support and a stimulating intellectual community of colleagues. Assistantships through the School of Natural Resources and the Environment, teaching experience with the biology department, and a grant from the Three Rivers Fnpc, Inc., provided generous additional financial support and training experiences.

Last, but far from least, I send warm thanks to my amazing family and friends for all their love, humor, creativity, and general fabulousness. My parents, Harold and Judy Liebowitz, my sisters, Debbie Kantor, Naomi Maron, and Esther Glahn, all my beloved nieces and nephews, and my aunt Sharon Green and uncle Jay Friedman were all bastions of support and encouragement throughout this process. And I'd like to send warm thanks to my friends who were particularly involved in motivating me through the

endgame: Margaret Tolbert, Gaby stocks, Jennifer Hoyer, Kelly Biedenweg, Bill Bryson, Daphna Davidson, Jesse Rodin, Jonathan Field, Sarah Norman, Sonora and Kevin Thomas, Kathleen McKee, and Danielle and Adam Watts. Thank you all!

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Abstract of Dissertation Presented to the Graduate School  
of the University of Florida in Partial Fulfillment of the  
Requirements for the Degree of Doctor of Philosophy

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May 2013

Chair: Matthew J. Cohen  
Major: Interdisciplinary Ecology

Herbivores control autotroph biomass across many aquatic ecosystems, therefore the loss of grazing pressure can induce dramatic changes in autotroph abundance and composition. Symptoms of consumer loss in aquatic systems (e.g., nuisance algae accumulation) may be similar to those of the alleviation of nutrient limitation, necessitating discrimination between these mechanisms. We tested the hypothesis that the loss of top-down control by aquatic gastropods explains benthic filamentous algae proliferation in Florida's iconic springs and spring-fed rivers. We further hypothesized that the dominant grazer control is dissolved oxygen (DO) concentration, which varies substantially among, and longitudinally within, springs. Finally, we hypothesized that algae-domination may persist if algae biomass exceeds a critical herbivore escape level, beyond which grazers can no longer constrain algae accumulation. We used three scales of investigation to test these hypotheses: state-wide observational surveys, *in situ* grazer experiments in Ichetucknee Springs, and artificial stream mesocosms with controlled dissolved oxygen levels. We observed a strong and temporally

consistent negative association between algae and gastropod biomass in the surveys (gastropods predicting up to 40% of the algal variation), and gastropods were by far the most important explanatory variable in multivariate prediction models as well. DO concentrations were significantly, but only modestly, positively associated with gastropod density in field surveys, but hypoxic conditions strongly reduced gastropod survival and grazing rates in controlled experiments. The *in situ* grazing experiments showed that high, but ecologically relevant, densities of grazers ( $>100 \text{ g m}^{-2}$  wet weight *Elimia spp.*) could inhibit algal bloom formation, but even  $> 330 \text{ g m}^{-2}$  of *Elimia spp.* could not reduce high algal biomass consistently, suggesting that algal biomass may escape herbivore control and entrain spring systems in algal-dominated states. Recognition that the fauna are not merely passive recipients of the system effects, but direct drivers themselves, may suggest new approaches to restoration and help focus future research.

## CHAPTER 1 INTRODUCTION

### **Top-Down Versus Bottom-Up Control of Algae Proliferation**

Aquatic ecosystems are changing on a global scale (Diaz 2001, Steffen and Tyson 2001, Lotze et al. 2006); one of the symptoms of these changes is the proliferation of nuisance algal blooms, which can have impacts ranging from aesthetic changes to disruption of ecological function and large-scale degradation of aquatic resources (Estes et al. 2011, Barnosky et al. 2012). A long-running debate has questioned the causes of these changes in primary producer biomass and composition, with early ecological enquiry asking whether “top-down” (herbivorous consumers) versus “bottom-up” (nutrient enrichment) factors control primary producers (Hairston et al. 1960, Power 1992, Hillebrand 2002). Models aimed at understanding the controls on primary producer diversity and productivity became progressively more sophisticated in the attempt to explain highly variable experimental outcomes (Leibold et al. 1997), incorporating the concurrent and interactive effects of both top-down and bottom-up factors, with a multitude of potential mediating factors such as plant edibility and species compositional change (Leibold et al. 1997), ecosystem type and producer evenness (Hillebrand et al. 2007), and positive feedbacks leading to hysteretic interactions and alternative stable states (Dent et al. 2002, Scheffer et al. 2008).

Despite the complexity and context-dependence of the controls on the ecosystems, aquatic ecosystem management efforts have generally focused on nutrient control as the most tractable driver amenable to intervention and remediation. However, emerging evidence suggests that system declines often attributed to nutrient enrichment may instead primarily be responses to loss of consumer control and trophic cascades

(Heck and Valentine 2007, Gruner et al. 2008, Baum and Worm 2009, Estes et al. 2011, Poore et al. 2012). Once consumer control is lost and algal blooms proliferate, these ecosystem changes can be maintained as alternative stable states as a consequence of a variety of mechanisms, such as “herbivore escape” mechanisms, in which algal biomass amasses to an extent that it escapes herbivore control through loss of palatability (Scheffer et al. 2008). Therefore, the evaluation of the role of grazers in ecosystems of interest, as well as the factors controlling those grazer populations, may be an important step in successfully managing and restoring ecosystems.

### **Controls of Aquatic Grazer Populations**

There are a large number of anthropogenic changes that can lead to the decline of aquatic fauna, including habitat destruction through dams, dredging, or severe flow reductions. Water-quality changes are equally as important, particularly factors such as increasing temperature, heavy siltation, nutrient enrichment, pesticides, pharmaceuticals, and a host of emerging contaminants. In relatively unmodified ecosystems, such as those located on protected lands, low dissolved oxygen (DO) concentration can be one of the strong controls on aquatic faunal health. The highly visible effects of marine and estuarine hypoxic (low DO) or anoxic (no DO) waters, so-called “dead zones” (Rabalais et al. 2002), have prompted significant investigation, leading to an early definition of hypoxia at  $2.9 \text{ mg O}_2 \text{ L}^{-1}$  (Diaz and Rosenberg 1995), later refined to hypoxia at  $< 2.0 \text{ mg O}_2 \text{ L}^{-1}$  and severe hypoxia at  $< 0.5 \text{ mg O}_2 \text{ L}^{-1}$  (Diaz 2001). However, those levels may underestimate the DO concentrations necessary for healthy ecosystems, due to subtle sublethal effects (Vaquer-Sunyer and Duarte 2008). Fresh water systems encompass similar ranges (Malard and Hervant 1999), but have received less systematic attention so far.

## Exploring the Role of Consumers in Florida's Springs

Northern Florida has one of the highest densities of large freshwater springs in the world, with more than 700 named springs that provide both aesthetic and economic benefits to the State. Seminal work in aquatic ecology was made possible by the short-term chemostatic and hydrostatic properties of these systems (Odum 1957b), but recently there have been dramatic increases in nitrate levels in many springs. Nuisance algal blooms have developed and submerged aquatic vegetation (SAV) has declined in many of these springs as well, leading to detrimental impacts on aquatic habitats, human health, and ecosystem aesthetics. Nutrient increases have been implicated as the cause of nuisance algal blooms, and management attention has overwhelmingly focused on nutrient tracking and control (Stevenson et al. 2004, 2007, Brown et al. 2008b). Several lines of evidence, however, suggest that nitrate enrichment may not be the primary driver of algal proliferation in these systems, and that alternative mechanisms such as consumer effects should be considered (Heffernan et al. 2010b).

The following chapters use three scales of inquiry to test three main hypotheses: 1) top-down control is a strong driver in Florida's springs, and therefore the loss of this consumer control helps explain the observed benthic filamentous algal proliferation; 2) hypoxia controls the presence and grazing rate of dominant invertebrate grazer in the springs, *Elimia* spp.; and 3) the algal-dominated state can become persistent if grazer populations remain low or the algae reach an herbivore escape density (a large biomass of algae that grazers can no longer diminish). We conducted observational surveys across the state to detect an ecosystem-level signal of the grazer effect on algae, the potential for alternative states (the persistence of high or low algae states), and environmental controls on grazers. We then used *in situ* experiments to refine our

understanding of grazer impacts, by demonstrating causal effects of gastropods on algae, and enumerating grazer thresholds and herbivore escape densities leading to hysteretic processes. Lastly, we used artificial stream mesocosms to control DO levels and test the effects of varying degrees of hypoxia on grazer survival and grazing rates at multiple grazer and algal biomass levels.

## CHAPTER 2 ECOSYSTEM-LEVEL PATTERNS OF CONSUMER CONTROL OF ALGAL PROLIFERATION IN FLORIDA'S SPRINGS

### **Introduction**

Broad-scale shifts in the composition and abundance of aquatic plant assemblages, in particular the proliferation of nuisance algae, have focused attention on causal mechanisms of aquatic ecosystem degradation. A large and well-studied suite of potential drivers of algal blooms has been invoked, including increased nutrient delivery and changes in stoichiometry (Dodds 2006, Hillebrand et al. 2007, Elser et al. 2007), reductions in consumer biomass (Gruner et al. 2008, Hillebrand 2009, Poore et al. 2012), changes in light availability (Odum 1956, Hillebrand 2005), and changes in water velocity and hydrology (Hoyer et al., 2004, Riseng et al., 2004), along with myriad interactions. Nutrients are often considered the most influential drivers of algal blooms and thus are the focus of management interventions, and there are a plethora of well documented instances of nutrient enrichment leading to algal dominance, particularly in coastal and estuarine regions, lakes, and large rivers (Carpenter et al. 1995, Jørgensen and Richardson 1996, Worm and Lotze 2006, Conley et al. 2009). However, evidence suggests that herbivore control, specifically consumer removal of primary producer biomass through consumption or mechanical disturbance, can be an equally strong force in structuring ecosystems (Hillebrand 2002, Heck and Valentine 2007, Gruner et al. 2008, Baum and Worm 2009, Estes et al. 2011), removing an average of 59% of algal standing stock across aquatic ecosystems (Hillebrand 2009).

A decline in grazer populations can lead to algal-dominated systems, which can persist if grazer populations remain low or if algae reach a critical herbivore escape density. Scheffer et al. (2008) modeled the equilibria of primary producer-dominated

versus herbivore-dominated states, finding that both press and pulse declines in the herbivore population can induce a switch to a stable algal-dominated state, if high algal biomass or older growth forms are sufficiently resistant to grazing. Grazers have a particularly strong influence on early algal successional stages and reduce colonization potential for many filamentous forms, with much weaker effects on adult algal forms (Alstyne et al. 1999; Korpinen et al. 2008). Once algae accumulate beyond an herbivore escape density, these positive feedback mechanisms allow the persistence of unpalatable algal growth forms or species; systems can thus stabilize into an algal-dominated state, insensitive to even intense grazing pressure (Gliwicz 1990, van de koppel et al. 1996, Gragnani et al. 1999, Lotze and Worm 2000). Alternatively, if algal growth rate is higher than the grazer consumption rate, but algae do *not* reach an herbivore escape density, algal biomass could be reduced by additional influx of grazers or increases in overall consumption rates (Lamberti et al. 1987, Suding and Hobbs 2009).

Causes of gastropod declines and extinctions worldwide include hydrological modification, habitat loss, declining water quality and quantity, invasive species, and their many potential interactions (Brown et al. 2008a, Lysne et al. 2008). Low dissolved oxygen (DO), in particular, can directly cause mortality through suffocation, or indirectly through slowing of feeding rates and resulting starvation, reduced fecundity, and behavioral changes that increase predation vulnerability (Diaz 2001, Wu 2002, Cheung et al. 2008).

### **Grazers, Oxygen, and Algae in Florida's Springs**

Northern Florida has the highest density of large freshwater springs in the world, with more than 700 named springs that provide both aesthetic and economic value

(Bonn and Bell 2003, Scott et al. 2004). Nuisance algal blooms have developed and submerged vascular plants (or submerged aquatic vegetation, SAV) have declined in many of these springs over the last few decades, leading to detrimental impacts on habitat, human health and aesthetics (Fig. 2-1). Increases in nitrate ( $\text{NO}_3^-$ ) concentration have been implicated as the cause of nuisance algal blooms, and management attention has overwhelmingly focused on nutrient reduction and related remediation (Stevenson et al. 2004, Brown et al. 2008). However, several lines of evidence suggest that nitrate enrichment may not be the primary driver of algal proliferation in these systems, and that alternative mechanisms, including loss of top-down control, should be considered (Heffernan et al. 2010).

Given the well-established effects of grazers on primary producers in streams in the southeastern US (Mulholland et al. 1991, Hill 1992, Rosemond et al. 2000), explicit consideration of the potential for top-down controls of algal proliferation in Florida's springs is critically needed. Studies in east coast temperate streams found gastropods from the family Pleuroceridae (mostly of the genera *Juga* and *Elimia*) to be particularly important consumers, with natural populations reaching 1,000 individuals  $\text{m}^{-2}$  and accounting for up to 95% of invertebrate biomass (Newbold et al. 1983, Rosemond et al. 2000, Stewart and Garcia 2002), and eating a wide array of algae, from diatoms to green algae and phaeophytes (Hill, Boston & Steinman 1992). Although population densities of gastropods are poorly characterized in Florida's springs, pleurocerids can reach "extraordinary levels" (Walsh 2001), documented up to 579  $\text{g m}^{-2}$  (Dutoit 1979), suggesting a potentially strong role of these herbivores in controlling algal biomass. The potential for consumer regulation has only recently been explored in Florida's springs

(Dormsjo 2008), and there have been no broad-scale investigations of top-down controls.

Despite evidence of DO declines and increasing incidence of hypoxia ( $\text{DO} < 2 \text{ mg L}^{-1}$ ; Diaz 2001) in springs (Heffernan et al. 2010), the effects of DO on freshwater gastropods in Florida's springs are poorly understood. Previous laboratory studies found slight associations between oxygen stress and metabolic patterns in the lab, and weak relationships between DO and gastropod distributions in the field (Berg and Ockelmann 1959, Hanley and Ultsch 1999). More recently, Dormsjo (2008) and Liebowitz (Chapter 3) found that *Elimia* spp. suffered high mortality and potential sub-lethal effects under hypoxic conditions in Ichetucknee springs, as well as in laboratory streams (Liebowitz, Chapter 4). Pleurocerids are heavy-shelled, particularly slow snails with low dispersal rates (Brown et al. 2008a), making them slow to repopulate, which suggests potentially persistent effects of even pulse hypoxic events that cause mortality.

## **Hypotheses**

We sought to test the hypothesis that gastropod grazers control algal accumulation in Florida's springs, and that the loss of this consumer control can lead to algal blooms. We further hypothesized that dissolved oxygen influences grazer abundance, and that feedbacks between algal accumulation and decreased grazing ability create the potential for bifurcation into high or low algal states. These hypotheses led to three linked predictions: (1) gastropods and algae biomass will exhibit a negative association in the field surveys; (2) DO concentrations will be positively associated with gastropod densities; and (3) that below a certain gastropod biomass threshold, residuals of algal biomass will be uniformly high, while above that gastropod threshold,

the algal residuals will be bimodally distributed (either high or low), consistent with positive feedbacks that create alternative states.

## **Methods**

### **Study Site Selection**

We selected eight spring runs from the more than 700 named springs in Florida to represent gradients in environmental variables of interest, and to satisfy the following several criteria. First and foremost, we used existing data on biological, chemical and physical attributes (Scott et al. 2004, Stevenson et al. 2004) to group springs based on three binary classifications: (1) high vs. low nitrate concentration (breakpoint at  $0.35 \text{ mg L}^{-1}$ , which corresponds to the recently adopted numeric nutrient criterion; Obreza et al. 2011); (2) high vs. low vent DO concentration (using the widely cited threshold of hypoxia at  $2 \text{ mg L}^{-1}$ ; Diaz 2001); and (3) high vs. low algal abundance (breakpoint at 50% cover using Stevenson et al. (2004) for most springs, and qualitative high vs. low using Scott et al. (2004) if quantitative data were lacking). This created eight groupings, from which one spring per group was selected (Table 2-1) based on discharge and spring length criteria. Mean discharge of each spring was between  $0.3$  and  $2.8 \text{ m}^3 \text{ s}^{-1}$  ( $2^{\text{nd}}$  magnitude springs; Meinzer 1928). Additionally, springs required a spring run (the lotic ecosystem downstream of the vent) at least 200 m long before confluence with another water body, and with no tidal or salinity influence. This criterion was applied to permit investigation of within-system longitudinal DO variation, while minimizing the number of confounding factors. In addition to the eight springs selected, we also obtained measurements in three additional springs from the Ichetucknee Springs complex to further populate the sample of low-DO systems. We note that this study design necessarily precluded consideration of larger, iconic  $1^{\text{st}}$  magnitude springs (e.g.

Silver Springs, Rainbow Springs). Additionally, we treat the results pertaining to nitrate and DO associations with algae with caution, as the sampling design specifically crossed those variables with algal abundance, therefore bivariate associations are not random.

### **Field Sampling Design**

A hierarchical sampling design was employed to document variation among springs as well as within springs along a longitudinal gradient (e.g., downstream changes in DO or nitrate). Observational surveys were conducted three times during 2009 in each of the eleven springs to examine temporal effects. At each spring, we selected three sites spanning from the spring vent (Site 1) to locations 200 m or more downstream. Downstream sites in high-DO springs were 100 m from the vent (Site 2) and 200 m from the vent (Site 3). In low-DO springs, we varied the location of Site 3 to ensure that DO levels at all Site 3 locations were above  $2 \text{ mg L}^{-1}$  at the trough of diel DO variation, measured using a YSI 6920 multi-parameter sonde (Yellow Springs Instruments, Yellow Springs, OH) deployed for hourly measurements over an extended period (4-6 days) prior to sampling.

### **Field Sampling Protocols**

At each site, we installed two line transects from bank to bank (10 m apart from each other along the bank and perpendicular to spring flow), with three sampling locations evenly spaced along each transect. At each of these sample locations, we estimated benthic cover of filamentous algae, diatoms, and submerged aquatic vegetation (SAV) using a 5-step Braun-Blanquette scale (Braun-Blanquet 1932) (binned at 1=0-5%, 2=6-25%, 3=26-50%, 4=51-75%, 5=76-100%). At each sampling location, we also collected biomass, and estimated canopy cover using a densitometer, surface

flow velocity using the float method, and DO, water temperature, specific conductance, and pH using a YSI 6920 multi-parameter sonde. At each site we also collected a 500-mL water sample into an acid-washed polyethylene bottle that was acidified to pH 2.0 using hydrochloric acid, and stored below 4°C until analysis. Water samples were filtered using a 0.45-µm glass fiber filter and analyzed within 28 days for nitrate (cadmium reduction method; EPA 353.2) and soluble reactive phosphorus (ascorbic acid method; EPA 365.1).

We sampled vegetation and large invertebrates using a Hess-type invertebrate sampler with a circular footprint of 0.086 m<sup>2</sup>, modified with a mesh cap to keep samples from escaping in deeper water, and with a larger mesh size (ca. 1 mm) that facilitated sampling in flocculent and diverse substrates. Because previous studies reported, and site reconnaissance confirmed, that gastropods are often dominant grazers, we used a method well suited to gastropod assessment across a variety of substrates, but less accurate for smaller invertebrates. The sampler was placed on the substrate and pushed down 1-2 cm to create a bottom seal. At deep-water sites where the sampler had to be completely submerged (> 0.75 m), a mesh cover with a single access slit was attached to the top of the sampling device to ensure none of the sample was lost. Vegetation was clipped at the roots and guided into the mesh bag attached to the downstream side of the sampler. The sediment was then agitated to collect remaining invertebrates and algae. Sampling was considered complete when three consecutive sweeps with a hand net yielded no additional biota. Samples were transferred into labeled containers, and placed on ice for transport to the lab.

## **Biomass Sample Processing**

Samples were separated into four biomass categories and rinsed with DI water to remove sediment and foreign materials. Plant species were categorized into coarse groupings of: 1) submerged aquatic vegetation (SAV), primarily *Sagittaria kurziana* and *Vallisneria americana*; 2) filamentous algae; 3) aquatic plants of other species (e.g., *Ceratophyllum sp.*, *Hydrocotyle sp.*, *Chara sp.*); and 4) invasive species (*Hydrilla verticillata* and *Elodea densa*). Each biomass component was blotted dry, weighed for wet mass, oven dried at 70 °C for 24 hours, and reweighed for dry mass. Aquatic vegetation other than *Sagittaria* and *Vallisneria* was not included in subsequent statistical analyses, as it accounted for a trivial fraction of total biomass. Invertebrates were sorted from the samples by hand, taking 1-6 hour processing time per sample depending on sample biomass and complexity. Invertebrates were separated by major taxa (family level for gastropods, order level for all others), counted, blotted dry, weighed, dried at 70 °C for 24 hours and then reweighed for dry mass. Because of sampling and sorting constraints, insect and decapod abundances and biomass measurements were considered informative, but incomplete representations of prevailing conditions, particularly for smaller taxa (e.g., chironomids).

## **Data Analysis**

Wet and dry weight were strongly correlated for all biomass categories (SAV:  $y = 0.12 + 0.08*x$ ,  $r = 0.97$ ,  $p < 0.001$ ; Algae:  $y = -2.90 + 0.33*x$ ,  $r = 0.95$ ,  $p < 0.001$ ; Gastropods:  $y = -0.11 + 0.64*x$ ,  $r = 0.99$ ,  $p < 0.001$ ). Because each metric of algal presence has biases, the correlation between filamentous algal biomass and estimated % cover was evaluated ( $y = -3.074 + 1.869*x$ ;  $r = 0.844$ ,  $p < 0.0001$ ). The five samples (out of 480) that showed a large discrepancy between % cover and biomass were

omitted from further analysis. Additionally, cover and biomass showed discrepancies at Site 1 in Mill Pond as a result of the dominant algal growth form, whose structural disaggregation during sampling rendered mass recovery extremely low. Algal data from this site were excluded from all statistical analyses unless otherwise noted.

We examined the data in a hierarchical manner. At the highest level, data were averaged for each spring, and segregated by sampling period; analysis of these data provided the lowest statistical power and therefore the most conservative assessment of the inferred relationships. Data were analyzed subsequently at the site level (i.e., three sites per spring), which is likely more appropriate, because the distance between sites is sufficient to preclude gastropod traversal (Huryn et al. 1997). Additionally, because key environmental variables (e.g.,  $\text{NO}_3^-$ , SRP, light, water velocity) showed non-systematic but significant differences among sites within springs, we considered the site level independent for these analyses. Bivariate analyses were examined at multiple levels of hierarchy, but multivariate analyses were run on the intermediate “site” level data aggregation. Finally, we assessed bivariate relationships between algae, gastropods, and DO at the individual sample level, but did not analyze these data using multivariate approaches.

Biomass of algae and gastropods, as well as environmental variables, exhibited skew and were natural-log transformed prior to statistical analysis. All regressions were initially run for each sampling period separately, yielding nearly identical results. Data were also checked for temporal differences using the Kruskal-Wallis test, with no significant effect of time of sampling period on algae biomass ( $\chi^2 = 0.39$ ,  $p = 0.82$ ) or gastropod biomass ( $\chi^2 = 0.64$ ,  $p = 0.73$ ), so data were pooled across sampling periods.

We used bivariate and multivariate general regression models to test relationships between environmental metrics, gastropod biomass, and algal biomass; all analyses were performed in STATISTICA v10 (Statsoft, Inc.). To test prediction 1 (a negative relationship between algae and grazers) we first modeled the pairwise association between algal and gastropod biomass at each level of data aggregation. In a second model, we used multiple regression to assess the conditional effects of grazers and other drivers (i.e., nutrients, water velocity and light); a third model included the entire suite of environmental variables. The corrected Akaike Information Criteria ( $AICc = 2k + n(\ln(RSS/n)) + (2k(k+1))/(n-k-1)$ , where  $k$  is the number of parameters and  $n$  is the number of samples) was used to compare models. We also explored the role of grazer composition on algal abundance by analyzing the effect of fractional biomass of the four primary snail families (Pleuroceridae [*Elimia spp.*], Hydrobiidae, Planorbidae, and Viviparidae) on algal biomass.

To test prediction 2 (a positive relationship between DO and grazers) we used the same basic approach as for prediction 1. We first evaluated the bivariate association between DO and grazers, and then considered the suite of environmental controls by building multivariate models at two levels of complexity, the first using the most likely controls on grazers, and the second including all measured environmental variables. To further explore whether high-DO springs ( $> 2.0 \text{ mg L}^{-1}$  DO at the vent) exhibited different longitudinal behavior than low-DO springs ( $< 2.0 \text{ mg L}^{-1}$  DO at the vent), we compared patterns of algal and gastropod biomass with distance downstream for springs grouped by DO concentration.

To test prediction 3 (bi-modality of algal biomass above a grazer density threshold, suggesting positive feedbacks that create alternative ecosystem states) we examined the residuals of the relationship between gastropod and algal biomass. Because bimodality was expected to be manifest at or above a critical gastropod density, we evaluated bimodality above and below five gastropod biomass thresholds: 12, 16, 20, 26, and 33 g m<sup>-2</sup> gastropod dry weight; this analysis was performed using natural-log-transformed data, resulting in residual values that are likewise transformed. All bi-modality analyses were performed in R v.2.15.0 (R Foundation for Statistical Computing). Hartigan's diptest (diptest package in R) was employed to test the null hypothesis that residual distributions above and below each threshold are unimodal, with the alternative hypothesis of at least bimodality (Hartigan and Hartigan 1985). Using the mclust package in R, we compared the fit of the residuals to both a normal distribution ( $N$  is a normal distribution with mean  $\mu$  and standard deviation  $\sigma$ ), and a bimodal distribution given by  $P=q N(x, \mu_1, \sigma_1) + (1-q) N(x, \mu_2, \sigma_2)$ , where  $q$  is a mixing coefficient with values from 0 to 1 that describes the relative contribution to the density function of mode 1, and  $x$  is the residual algal biomass not explained by gastropods. We selected between unimodal and bimodal distributions using both the Bayesian Information Criteria (BIC) and the diptest to present the most consistent threshold.

## Results

### Biomass Composition and Predictors of Distribution Patterns

Twenty faunal taxa, including 9 mollusks, 7 insects and 4 decapods, were identified across the samples from the springs. The dominant taxa in terms of biomass were the gastropods, accounting for 60-100% of the biomass in each spring. Algal biomass averaged 153 g m<sup>-2</sup> (SD = 426, max = 3,419); SAV biomass averaged 49 g m<sup>-2</sup>

(SD = 86, max = 580), gastropod biomass averaged 67 g m<sup>-2</sup> (SD = 107, max = 589), decapod biomass averaged 0.6 g m<sup>-2</sup> (SD = 1.8, max = 18.3), and insect biomass averaged 0.04 g m<sup>-2</sup> (SD = 0.13, max = 1.4). The large mesh size needed to sample flocculent substrates led to low capture efficiency of insect and small decapod biomass, therefore they are not included in subsequent analyses as a consequence of likely underrepresentation in samples. We note, however, that for decapods, we found no significant relationship with algal biomass ( $p = 0.12$ ), and a significant, but weak positive relationship with DO ( $p = 0.001$ ,  $r^2 = 0.13$ ); similarly, insect biomass was uncorrelated with algal biomass ( $p = 0.54$ ), and was weakly, positively associated with DO ( $p = 0.013$ ,  $r^2 = 0.07$ ).

### **Algae Distributions**

Gastropod biomass was a strong predictor of algal biomass in the bivariate correlations at all three data aggregation levels (Fig. 2-2 A-C). In the most conservative analysis (“spring” level data averaging, separated by sampling period), the gastropod and algae association was significant and consistent across all sampling periods (period 1,  $n=10$ ,  $p = 0.01$ ,  $r = -0.79$ , slope = -2.3; period 2,  $n = 11$ ,  $p = 0.003$ ,  $r = -0.83$ , slope = -2.9; period 3,  $n = 11$ ,  $p = 0.01$ ,  $r = -0.72$ , slope = -2.4). This relationship remained strong and consistent when the data were pooled across seasons ( $n = 32$ ,  $p < 0.0001$ ,  $r = -0.76$ ). At the most ecologically relevant, intermediate “site” level of aggregation, the same held true: the negative algae and grazer relationship was consistent across sample periods, as well as for pooling all samples (combined data:  $n = 88$ ,  $p < 0.0001$ ,  $r = -0.61$ , slope = -1.29). The sign and magnitude of the gastropod effect indicated that the gastropods were the strongest and most consistent predictor of algal biomass, but other bivariate relationships were also significant (Table 2-2). At the individual sample

level, we still observed strongly significant relationships, but lower explanatory power for individual and pooled sampling periods (combined data:  $n = 451$ ,  $p < 0.0001$ ,  $r = -0.35$ , slope =  $-0.56$ ).

Among the multivariate algal biomass prediction models of varying complexity (Table 2-3), model 2 includes only those factors most often associated with algal blooms (nutrients, canopy, water velocity, and consumers), and explains ~45% of the variation in algal biomass. However, only gastropods (negative relationship) and light (positive relationship) are statistically significant predictors. Inclusion of the full suite of measured environmental factors improved overall explanatory power (adj.  $R^2 = 0.53$ ), but not  $AIC_c$  suggesting that model 3 may be overfit, with additional variables not adding sufficient additional predictive power. Despite the improved overall fit and explanatory power, only one variable (gastropod biomass) in model 3 was a statistically significant predictor.

The fraction of biomass as *Elimia spp.* was negatively correlated with algal biomass ( $r = -0.458$ ,  $p < 0.0001$ ), whereas % hydrobiids ( $r = 0.348$ ,  $p = 0.001$ ) and % planorbids ( $r = 0.278$ ,  $p = 0.009$ ) were positively correlated with algal biomass. However, % *Elimia spp.* was also positively correlated with overall gastropod biomass ( $r = 0.401$ ,  $p = 0.0001$ ), while hydrobiids and planorbids were both negatively correlated. Gastropod populations dominated by *Elimia spp.* had much higher biomass overall (up to  $575 \text{ g m}^{-2}$ ) than those dominated by other species (planorbids up to  $113 \text{ g m}^{-2}$ , hydrobiids up to  $106 \text{ g m}^{-2}$ ).

### **Gastropod Distributions**

Dissolved oxygen concentration was a significant predictor of gastropod biomass across two of the three data aggregation levels. At the highest, “spring” level of

aggregation, where sample size limits power and any longitudinal changes in DO are lost to averaging, the DO-gastropod relationship was marginally significant and weakly positive, ( $p = 0.08$ ,  $r = 0.31$ ; Fig. 2-3A). At the “site” level of aggregation, the relationship was highly significant ( $p < 0.001$ ,  $r = 0.42$ ), but heavily influenced by three points from one spring site (i.e. MP Site 1), the exclusion of which reduced the strength of the DO-grazer association (Fig. 2-3B). At the fully disaggregated “sample” level, the DO-gastropod regression was significant ( $p = 0.0003$ ,  $r = 0.32$ ; Fig. 2-3C). Only two springs, both low-DO sites, had significant within-spring relationships between DO and gastropod biomass (MP,  $p = 0.01$ ;  $r = 0.91$ ; RS,  $p = 0.0006$ ;  $r = 0.91$ ). Notably, Williford springs had DO levels of  $0.8 \text{ mg L}^{-1}$  at the vent, yet as much as  $27 \text{ g m}^{-2}$  of gastropods.

Inclusion of additional predictive factors in Model 2 (Table 2-4) indicated that all but temperature exerted a significant univariate effect, and together explained 50% of gastropod variation. Model 3 included the full suite of environmental variables, with all but temperature and SRP exerting a significant effect. DO, water velocity, and light were all positive predictors, whereas  $\text{NO}_3^-$ , conductivity, pH, and SAV were negative predictors, together explaining over 60% of gastropod variation. However, Model 2 showed a slightly more favorable AICc score due to its parsimony, suggesting that Model 3 may be overfit.

### **Longitudinal Patterns**

Longitudinal gastropod biomass changes were different across springs. Over 70% of low-DO springs (5 of 7) showed significant ( $p < 0.05$ ) positive associations between gastropod biomass and distance downstream; one more was marginally significant ( $p = 0.09$ ) and the last had no significant relationship. Gastropods increased

significantly downstream in only one of the four high-DO springs; gastropods also significantly decreased in one and showed no relationships in the other two.

Longitudinal gradients of gastropod and algal biomass differed between high- and low-DO springs (Fig. 2-5). In low-DO springs, gastropod biomass increased from upstream to downstream; gastropod densities decreased downstream in high-DO springs, though they had a higher overall population than low-DO springs throughout ( $F(1,85) = 9.1, p = 0.003$ ). Longitudinal patterns of algal biomass were slightly different, decreasing with downstream distance in both high and low-DO springs. This trend was weaker in the low-DO springs than in high-DO springs, and low-DO springs had higher overall algae biomass ( $F(1,82) = 7.3, p = 0.008$ ).

### **Bimodality**

Hartigan's diptest indicated that gastropod densities were unimodal below all potential threshold levels, but that above these thresholds, unimodality could be rejected for all except at a threshold of  $26 \text{ g m}^{-2}$  (Table 2-5). BIC model comparisons (i.e., bi-modal vs. unimodal model fits) were generally in agreement with this output. However, as the gastropod threshold increased beyond  $26 \text{ g m}^{-2}$ , distributions become more ambiguous. Above a threshold of  $26 \text{ g m}^{-2}$ , goodness-of-fit became more equivocal (i.e., less than three units separate models). With a threshold of  $33 \text{ g m}^{-2}$ , residuals both above and below the threshold were better described by bi-modal distributions. Because ambiguity in the data distributions first appeared at  $26 \text{ g m}^{-2}$ , we selected  $20 \text{ g m}^{-2}$  as the most defensible threshold for which model residuals are presented (Fig. 2-6). The probability of gastropod biomass exceeding this  $20 \text{ g m}^{-2}$  threshold varies dramatically with DO conditions (Fig. 2-4); 10% of observations with

DO below 1 mg L<sup>-1</sup> exceeded critical snail densities, whereas more than 70% of observations exceeded this snail biomass threshold at sites with DO > 5 mg L<sup>-1</sup>.

## Discussion

### Gastropod Control of Filamentous Algae Blooms

This study lends strong support for the claim that consumers play an important role in controlling algal proliferation in Florida's springs. We found that gastropod biomass exhibited a strong negative relationship with algal biomass across all levels of data aggregation in this study, including at the entire spring-level, for which our sample size was small. This is consistent with recent meta-analyses of grazer experiments illustrating the paramount role of consumers in controlling primary producers across most aquatic ecosystems (Hillebrand 2002, 2009, Hughes et al. 2004, Gruner et al. 2008, Poore et al. 2012). Observational surveys such as this study and others (Riseng *et al.* 2004) complement and contextualize the experimental manipulations that dominate the assembled evidence for top-down controls. While experimental manipulations provide stronger inference about causality and constitute the next step in exploring this mechanism in Florida's springs, this large-scale observational survey demonstrates the plausibility of consumer, and specifically gastropod, control on algal biomass in the context of complex ecosystems.

Negative correlations between *Elimia* spp. biomass and algal abundance suggest that this group plays a particularly important role in top-down control in Florida Springs. Gastropod populations dominated by other major gastropod families (Hydrobiidae, Planorbidae, and Viviparidae) were, in contrast, weakly but positively associated with algal biomass. *Elimia* spp. are generalist grazers with a demonstrated ability to shift algal composition and selectively remove filamentous cyanobacteria, keeping these

species from accumulating (Tuchman and Stevenson 1991, Feminella and Hawkins 1995). They have been documented at exceedingly high biomass levels dominating southeastern streams (Hill 1992) and have been shown to control algal accumulation in natural stream systems (Brown *et al.* 2008a). However, this association may also reflect covariation between species composition and density (rather than greater grazing efficacy of *Elimia* at the individual level), as *Elimia*-dominated populations reach five-fold higher biomass levels than any other species in these systems.

Although gastropods consistently exhibited the strongest bivariate relationship with algae, a number of additional variables (phosphate (SRP), conductivity, temperature, and submerged aquatic vegetation (SAV), all positive) had significant bivariate correlations. Moreover, multivariate models including the full suite of environmental factors explained 15% more algal variability than gastropods alone. However, in those more complex multivariate models, all variables except for gastropods were no longer significant, suggesting complex interactions among variables that cannot be discerned with the low power of this particular study design. Notably,  $\text{NO}_3^-$ , the variable most widely cited as the cause of algal proliferation, showed no association with algal biomass in this study. We caution, however, that the expectation of a relationship may be confounded by the study design which intentionally selected springs with high and low algal cover at high and low nitrate levels.

The debate over “top-down versus bottom-up” control of autotrophs has shifted to acknowledge the complex and nuanced interactions and contingencies that mediate the strengths of multiple drivers. Although studies suggest that nutrients generally increase primary producer biomass in the absence of sufficient herbivory (Gruner *et al.* 2008), a

host of physical, chemical, physiological, and ecological factors can mediate this pattern (Borer et al. 2005, Gruner et al. 2008, Poore et al. 2012). This study addressed only some of these potential mediating factors, yet the fact that grazer biomass was consistently the best predictor of algal biomass, while all other variable showed weaker associations, strongly supports the conclusion that grazers exert a dominant effect on algal proliferation in these systems.

### **Dissolved Oxygen and Additional Controls on Gastropods**

Our results support the hypothesis that dissolved oxygen influences gastropod biomass, but also that other stressors need to be considered to understand grazer declines. At most levels of data aggregation there was a significant positive relationship between DO and gastropod biomass, but DO only explained 18% of gastropod variation. At the most conservative ("spring") level of analysis, the association was in the expected positive direction, but was not statistically significant. Whereas hypoxic sites generally had low gastropod density, there were exceptions to this pattern. The Williford Spring site, for example, had DO levels of  $0.8 \text{ mg L}^{-1}$  at the vent, but also had relatively high gastropod biomass ( $27 \text{ g m}^{-2}$ ) at that location.

Discerning critical levels of dissolved oxygen for aquatic organism health has proven to be a vexing problem. Both chronic hypoxia and pulses of severe hypoxia or anoxia affect gastropod populations, potentially leading to apparent low correspondence between concurrent DO and gastropod levels—for instance, a brief low-DO pulse could decimate a community, but not be in evidence if DO measurements are taken days later. Hypoxia can cause a range of responses, including depressed feeding and reproduction rates as a consequence of shifting metabolic pathways (Kapper and Stickle 1987, Hanley and Ultsch 1999), behavioral modifications that can lead to

predator vulnerability, and direct mortality (Sagasti et al. 2001, Wu 2002, Cheung et al. 2008). Gastropods may be able to behaviorally adjust to conditions of hypoxia by taking advantage of the DO concentration gradient at the air-water interface in shallow waters (Wu 2002). However, multivariate cross-system analyses of water depth, DO, and velocity indicated no interactive effects in this study. We observed that at DO concentrations below  $1 \text{ mg L}^{-1}$ , gastropod density was uniformly low, while at higher DO concentrations there was a wide range of gastropod densities. However, as DO levels can fluctuate through time, as well as vary spatially in microhabitats, our observations may have been insufficient to capture the dynamics of DO effects in space and time (Garvey 2007). Experimental manipulation of dissolved oxygen to discern effects on grazing rates and gastropod mortality is clearly needed.

Longitudinal patterns of DO within springs further illustrate the nuanced effect of DO on gastropods, and their commensurate effects on algal density. Low-DO springs uniformly showed significant gastropod increases downstream, whereas the longitudinal patterns in high-DO springs were inconsistent. Additionally, low-DO springs consistently had greater algal abundance and fewer gastropods at all sites despite gastropod increases downstream. Overall, the low gastropod abundances at the vent of low-DO springs suggests that gastropods benefit from reoxygenation downstream. That they continue to exhibit lower biomass in low-DO springs, may suggest both reduced survivorship and lower fecundity from low-level physiological stress. One explanation for higher algal biomass at downstream locations in low-DO springs may be high rates of longitudinal drift of sloughed algal tissues which subsequently settle downstream; there is strong

evidence of this effect in Alexander Springs (Cohen et al. 2012), one of our low-DO sites.

Whereas our evidence supports the conclusion that DO influences grazer populations in Florida springs, the influence of other factors is clearly evident in the data. In the multivariate regressions, most of the environmental variables added significant predictive power, collectively explaining 60% of gastropod variability, while DO alone explained only 20%. Nitrate concentrations, conductivity, pH, and SAV were all negatively correlated with gastropod abundances, whereas light, water velocity, and DO were positively correlated. These factors generally align with the expected direction of effect. The effect of nitrate is of particular interest. Several studies have shown lethal impacts of nitrate on aquatic fauna. However, gastropods generally appear to be less susceptible to these effects, particularly at the relatively low levels seen in the springs (Camargo et al. 2005, Mattson et al. 2007, Brown et al. 2008b). Conductivity, on the other hand, is generally negatively associated with freshwater gastropods (Lodge *et al.* 1987), with potential implications for coastal springs susceptible to salt water intrusion, and those where connate sea water influences spring water chemistry (e.g., Alexander Springs; Davis *et al.* 2001). The negative relationship with SAV observed in multivariate analyses is not present in the bivariate relationship, and is surprising, as gastropods frequently graze on strapleaf vegetation; however, they do aggregate more densely on complex vegetative forms such as the macro-algae *Chara* spp. (Liebowitz, pers. obs.), and the highest biomass aggregations were on bare rocky or sandy substrates. Gastropods have been found to be positively associated with light availability (possibly due to positive phototaxis), and flow relationships can vary greatly depending on habitat

and life-stage (Johnson and Brown 1997). Together, these factors suggest that the persistence of low nutrients and conductivity, cooler water, and high flow and DO are characteristics of optimal gastropod habitat. This suite of variables helps set our expectations of grazer abundances; those variables that are amenable to management may be candidates for restorative action.

Our results suggest that gastropod populations respond predictably to environmental drivers, however a large fraction of the variance in snail biomass remains unexplained. This is particularly salient in light of evidence of gastropod declines in this data versus earlier surveys (DuToit 1979), and observations made by local biologists who noted declines in Manatee Springs (R. Mattson, pers. comm.), and in Ichetucknee Springs (J. Stevenson, pers. comm.; C. Parenteau, pers. comm.). Although gastropod densities are relatively undocumented in Florida, extreme declines are seen throughout the southeastern US (Neves et al. 1997, Brown et al. 2008a, Lysne et al. 2008); for example, 65% of Alabama's gill-breathing snails are either "extinct, endangered, threatened, or of special concern," a listing of  $\geq$  G2 by the Nature Conservancy (Lydeard and Mayden 1995). It seems likely that the faunal responses to anthropogenic changes in Florida's springs exhibit similar patterns.

A wide range of emerging contaminants may be complicit in broadly observed grazer declines, and are potentially important to understanding residual biomass variation in the models. Pesticides and herbicides entering the water, either unintentionally from terrestrial applications or wastewater, or intentionally as part of aggressive aquatic weed management, could negatively impact invertebrate health, and remain one of the understudied realms of conservation science (Lawler *et al.* 2006). In a

review of the effects of the management of invasive aquatic flora, Brown et al. (2008b) outlined a number of herbicides used currently or historically in Florida's springs, including copper and diquat, both of which are toxic to invertebrates. A recent survey of four springs in the St. Johns River Water Management District found low levels of a variety of pesticides (such as DEET), herbicides, and wastewater compounds—all were below levels of concern for DEP; however, recent studies have found that pesticides can cause ecological degradation at levels within legal bounds. For example, the most commonly used fungicide in the US, Chlorothalonil (US EPA 2004), has been shown to have strong negative effects on invertebrates (including gastropods) and lead to algal blooms at ecologically relevant levels of contamination (McMahon et al. 2012). Additionally, few studies have looked at the effects of cocktails of various compounds, which could potentially act as multiple sources of stress and therefore have large negative impacts in combination (Phelps et al. 2006).

### **Gastropods, Algae, and Hysteretic Ecosystem Change**

Feedbacks between organisms and environmental factors can create multiple persistent ecosystem configurations under the same environmental drivers. The suite of examples in which such alternative stable states are observed is growing (e.g., van de Koppel et al. 2001; Beisner et al. 2003; Schroder et al. 2005; Heffernan 2008), as is recognition of the importance of these behaviors for environmental management (Suding and Hobbs 2009). Our analysis of model residuals suggests the presence of a critical gastropod density, below which algal biomass is uniformly high and above which two ecosystem configurations might persist. In theoretical models of algal escape from herbivore control (Scheffer et al. 2008), this condition arises in part in response to the historical trajectory of the system, depending on whether the system has experienced a

stressor that allowed algae to grow beyond herbivore control. If *Elimia spp.* populations declined because of a lethal pulse of low DO or some other stressors, their slow recolonization rates could release herbivore control long enough to allow algal blooms, even if contemporary gastropod densities are, in other systems, sufficient to keep algal biomass at low levels. This escape density may persist long after bloom conditions initiate and gastropod populations recover, due to less palatable growth forms or altered species compositions after succession.

Bi-modality is only one line of evidence in support of the presence of alternative stable states within spring ecosystems, and this study was only able to consider variation across sites, rather than detecting change within a given system over time. However, these results offer a plausible scenario to guide future research. The gastropod threshold level yielding the clearest division between unimodal and bimodal distributions was  $20 \text{ g m}^{-2}$  dry weight of gastropods (equivalent to  $\sim 30 \text{ g m}^{-2}$  wet weight of gastropods), suggesting that systems with lower gastropod biomass will generally have algal biomass at nuisance levels, but systems above that threshold may or may not, depending on the historical trajectory of the spring's ecological community.

### **Management Implications**

Our results indicate that loss of top-down control is a plausible factor leading to algal proliferation in Florida's springs, and that dissolved oxygen, along with several other environmental variables, helps explain patterns of gastropod abundance and distribution. Photosynthetic oxygen production and atmospheric reaeration are negligible in aquifers, suggesting that DO levels in groundwater are primarily a function of water age and microbial respiration. In short, older water and DOC-enriched water (which allows respiratory consumption of DO) generally have the lowest DO levels in

the Floridan Aquifer (Malard and Hervant 1999, Martin and Dean 2001). Therefore, on a practical level, DO may be changed by (1) droughts or anthropogenic use of younger (shallower) aquifer water leading to lower flows and relative dominance of older water, and (2) groundwater organic matter enrichment leading to higher microbial respiration and DO depletion. Given that DO plays a role in grazer abundance, and that both pulse and press hypoxia could harm populations, ensuring adequate flows and reducing organic pollutants merit attention in management plans. Further work to isolate and experimentally verify the validity of DO as a restoration objective are needed, but our results support both ongoing investigation and management attention.

Restoration plans for springs with nuisance filamentous algae should consider potential alternative states and herbivore escape densities. Our conclusion from these surveys that high-algae or low-algae states exist, and can persist, is supported by the *in situ* experiments (Chapter 3) which found that low algae biomass can be maintained by  $> 100 \text{ g m}^{-2}$  of gastropod wet weight, but that even  $> 330 \text{ g m}^{-2}$  of gastropod wet weight may not be enough to decrease an algal bloom. Additionally, preliminary evidence suggests that *Elimia* can inhibit further growth of *Lyngbya*, but not decrease the existing standing crop (Liebowitz, unpublished data). These findings suggest that once a system has reached a high algal state, it will likely remain in that state unless "reset" in some manner. While further study is necessary to explore the critical transitions between states, it is likely that mature algal growth forms would have to be cleared to allow grazers to access early algal successional stages that they are capable of controlling. Alternative stable states and simple time lags are difficult to distinguish without

systematic long term monitoring of spring biology, which is a critical gap in the State of Florida's exemplary monitoring program.

Where gastropod populations fall below the 20 g m<sup>-2</sup> dry weight (~ 30 g m<sup>-2</sup> wet weight) threshold, reintroducing native gastropods may be a tool for restoration, though the cause of grazer declines would first have to be ameliorated. Sixty percent of the springs in this study currently have gastropod populations above the threshold, indicating that these biomass levels are a viable target for most springs. Low-DO conditions may require large-scale changes in water consumption to mitigate further declines, and DO is also impacted by climate-induced variations in discharge that are beyond local management control. Other factors, such as organic matter inflows (e.g., from septic tanks, wastewater sprayfields), are slightly more tractable to management oversight. In cases where low populations are a result of an historic pulse event from which populations were slow to recolonize, breeding and restocking native grazers to their historic ranges and densities could be an effective intervention (Lysne et al. 2008, Whelan et al. 2012).

Table 2-1 Categorization of springs study sites. The 32 springs with sufficient prior data to be considered for the study were categorized by NO<sub>3</sub><sup>-</sup>, DO and Algae % cover as described in the methods. The column “# Springs” displays the number of these 32 springs that fit in each category. The cell to the right of each displays the spring chosen for the survey and its basic chemical composition. Three additional low DO springs were added: Mission (MS), Mill Pond (MP), and Devil’s Eye (DE).

		High Algae (> 50% cover)		Low Algae (< 50% cover)	
		# Springs	Sampled spring	# Springs	Sampled spring
High N (>0.35 mg L <sup>-1</sup> )	High DO (>2mg L <sup>-1</sup> )	6	Blue Hole (BH) NO <sub>3</sub> <sup>-</sup> = 0.66 mg L <sup>-1</sup> DO = 2.1 mg L <sup>-1</sup> F-03 Algae = 87%	6	Gilchrist Blue (GB) NO <sub>3</sub> <sup>-</sup> = 1.7 mg L <sup>-1</sup> DO = 4.0 mg L <sup>-1</sup> Qualitative low
	Low DO (<2 mg L <sup>-1</sup> )	5	Manatee (MN) NO <sub>3</sub> <sup>-</sup> = 1.8 mg L <sup>-1</sup> DO = 1.6 mg L <sup>-1</sup> F-03 Algae = 94%	5	Rock Springs (RS) NO <sub>3</sub> <sup>-</sup> = 1.3 mg L <sup>-1</sup> DO = 1.0 mg L <sup>-1</sup> Qualitative low
Low N (<0.35 mg L <sup>-1</sup> )	High DO (>2 mg L <sup>-1</sup> )	3	Fern Hammock (FH) NO <sub>3</sub> <sup>-</sup> = 0.12 mg L <sup>-1</sup> DO = 6.0 mg L <sup>-1</sup> F-03 Algae = 54%	5	Cypress (CP) NO <sub>3</sub> <sup>-</sup> = 0.3 mg L <sup>-1</sup> DO = 5.2 mg L <sup>-1</sup> F-03 Algae = 0%
	Low DO (<2 mg L <sup>-1</sup> )	1	Alexander (AX) NO <sub>3</sub> <sup>-</sup> = 0.07 mg L <sup>-1</sup> DO = 1.6 mg L <sup>-1</sup> F-03 Algae=74%	1	Williford (WL) NO <sub>3</sub> <sup>-</sup> = 0.07mg L <sup>-1</sup> DO = 1.4 mg L <sup>-1</sup> F-03 Algae = 13%
Additional Springs			Mill Pond (MP) NO <sub>3</sub> <sup>-</sup> = 0.23 mg L <sup>-1</sup> DO = 0.39 mg L <sup>-1</sup> F-03 Algae=52%		
			Mission (MS) NO <sub>3</sub> <sup>-</sup> = 0.51 mg L <sup>-1</sup> DO = 0.83 mg L <sup>-1</sup> F-03 Algae=86.4%		
			Devil’s Eye (DE) NO <sub>3</sub> <sup>-</sup> = 0.39 mg L <sup>-1</sup> DO = 0.42 mg L <sup>-1</sup> F-03 Algae = 67%		

Table 2-2. Pearson r correlations for primary variables of interest included in the regression models, averaged at the intermediate “site” level of data aggregation. Numbers marked with asterisks are significant at  $p < 0.05$ .

	SRP	NO <sub>3</sub> <sup>-</sup>	DO	Cond.	Temp.	pH	Vel.	Light	Algae	SAV
SRP	-									
NO <sub>3</sub> <sup>-</sup>	0.16*	-								
DO	-0.12	-0.11								
Conductivity	0.36*	-0.07	0.01	-						
Temperature	0.41*	-0.03	-0.02	0.56*	-					
pH	0.04	-0.47*	0.27*	-0.02	-0.22	-				
Velocity	0.34*	0.16	-0.16	-0.07	0.19	-0.14	-			
Light	0.17	0.24*	0.28*	0.42*	0.17	-0.15	-0.05	-		
Algae	0.24*	0.12	-0.12	0.56*	0.42*	0.00	0.06	0.17	-	
SAV	0.63*	0.31*	-0.07	0.22*	0.26*	-0.17	0.47*	0.15	0.20	-
Gastropods	-0.13	-0.06	0.42*	-0.33*	-0.13	-0.14	0.12	0.32*	-0.61*	-0.16

Table 2-3. Three general linear regression models for algae biomass, with a suite of potential explanatory variables, N = 74. Model 1 uses the hypothesized predictor alone, model 2 includes the suite of common drivers of algal abundance, and model 3 includes all sampled environmental variables (data aggregation at site level; n=74 for all model).

	Variable	SS	Df	MS	F	P	Beta ( $\beta$ )	Adjusted R <sup>2</sup>	Whole Model F	Whole model p	AICc
Model 1	Intercept	600.7	1	600.7	95.95	<0.0001		0.37	44.54	<0.0001	135.76
	Gastropods	278.8	1	278.8	44.54	<0.0001	-0.62				
	Error	450.7	72	6.26							
Model 2	Intercept	9.8	1	9.8	1.89	0.202		0.45	14.47	<0.0001	126.58
	Gastropods	302.6	1	302.6	58.23	<0.0001	-0.67				
	SRP	2.3	1	2.3	0.44	0.511	0.06				
	NO <sub>3</sub> <sup>-</sup>	0.03	1	0.03	0.01	0.938	-0.01				
	Velocity	17.5	1	17.5	3.37	0.071	0.18				
	Canopy	58.2	1	58.2	11.19	0.001	0.31				
	Error	353.4	68	5.4							
Model 3	Intercept	10.4	1	10.4	2.22	0.141		0.53	9.25	<0.0001	126.31
	Gastropods	101.1	1	101.2	21.55	<0.0001	-0.54				
	SRP	3.9	1	3.9	0.83	0.366	-0.11				
	NO <sub>3</sub> <sup>-</sup>	6.2	1	6.2	1.31	0.256	0.12				
	DO	3	1	3	0.65	0.425	0.08				
	Conductivity	14.6	1	14.6	3.12	0.082	0.24				
	Temperature	10.6	1	10.6	2.26	0.138	0.18				
	pH	2.3	1	2.3	0.49	0.488	0.08				
	Velocity	9.4	1	9.4	2.00	0.162	0.15				
	Canopy	6.8	1	6.8	1.46	0.232	0.14				
	SAV	2.7	1	2.7	0.57	0.454	0.09				
	Error	295.6	63	4.7							

Table 2-4. Three general linear regression models for gastropod biomass with a suite of potential explanatory variables, N = 77. Model 1 uses the hypothesized predictor alone, model 2 includes the suite of common predictors of gastropod abundance, and model 3 includes all sampled environmental variables.

	Variable	SS	df	MS	F	p	Beta (β)	Adjusted R <sup>2</sup>	Whole Model F	Whole model p	AICc
Model 1	Intercept	113.2	1	113.2	46.9	<0.0001		0.228	23.402	<0.0001	67.92
	DO	56.5	1	56.5	23.4	<0.0001	0.49				
	Error	181.1	75	2.4							
Model 2	Intercept	0.05	1	0.1	0.0	0.846		0.555	16.793	<0.0001	24.56
	DO	22.1	1	22.1	15.9	<0.0001	0.39				
	Temperature	1.8	1	1.8	1.3	0.263	0.11				
	Velocity	10.7	1	10.7	7.7	0.007	0.25				
	Canopy	41.2	1	41.2	29.6	<0.0001	0.50				
	SAV	8.9	1	8.9	6.4	0.014	-0.23				
	Conductivity	35.4	1	35.4	25.4	<0.0001	-0.54				
	Error	97.4	70	1.4							
Model 3	Intercept	7.5	1	7.5	6.1	0.016		0.609	14.129	<0.0001	25.53
	SRP	2.9	1	2.9	2.4	0.128	0.17				
	NO <sub>3</sub> <sup>-</sup>	7.2	1	7.2	5.9	0.018	-0.22				
	DO	29.8	1	29.8	24.3	<0.0001	0.43				
	Conductivity	28.7	1	28.7	23.4	<0.0001	-0.51				
	Temperature	0.2	1	0.2	0.2	0.688	-0.04				
	pH	14.2	1	14.2	11.6	0.001	-0.33				
	Velocity	12.2	1	12.1	9.9	0.002	0.27				
	Canopy	29.3	1	29.3	24.0	<0.0001	0.50				
	SAV	8.9	1	8.9	7.2	0.009	-0.28				
	Error	82	67	1.2							

Table 2-5. BIC model comparisons and Hartigan's diptest for unimodality at varying grazer biomass thresholds. Each gastropod threshold was tested for fit for curves with 1 vs. 2 modes. Shown is the best of the two models for each threshold, and the BIC difference between it and the less fit model. Hartigan's statistic tests the null hypothesis that the distribution is unimodal, with significant p-values indicating multimodality. Where BIC and the diptest disagree on the number of modes, "1 or 2" is listed.

Gastropod threshold (g m <sup>-2</sup> )	# Modes	BIC diff.	Mean 1	Mean 2	Variance	Hartigan's Diptest: D	p-value
<12.1	1	5.072	-0.263	-	2.525	0.072	0.459
>12.1	2	8.663	-2.827	2.006	1.659	0.072	0.009
<15.6	1	3.859	-0.085	-	2.75	0.051	0.795
>15.6	2	7.77	-2.793	2.208	1.77	0.074	0.015
<20.1	1	3.436	-0.201	-	3.182	0.047	0.825
>20.1	2	8.01	-2.769	2.284	1.729	0.077	0.014
<25.7	1	3.252	0.117	-	3.358	0.044	0.928
>25.7	1 or 2	8.76	-2.73	2.508	1.709	0.071	0.07
<33.1	1 or 2	1.216	-3.543	0.612	1.818	0.029	0.993
>33.1	2	16.723	-2.74	2.599	0.488	0.085	0.018



A



B

Figure 2-1. Photographs of the same location at the Ichetucknee Headspring (A) in 1976 (photo: C. DuToit) and (B) 2010 (photo: D. Liebowitz), exhibiting a system shift from primary producer dominance by a diverse community of submerged aquatic macrophytes to dominance by the filamentous cyanobacterium *Lyngbya sp.*

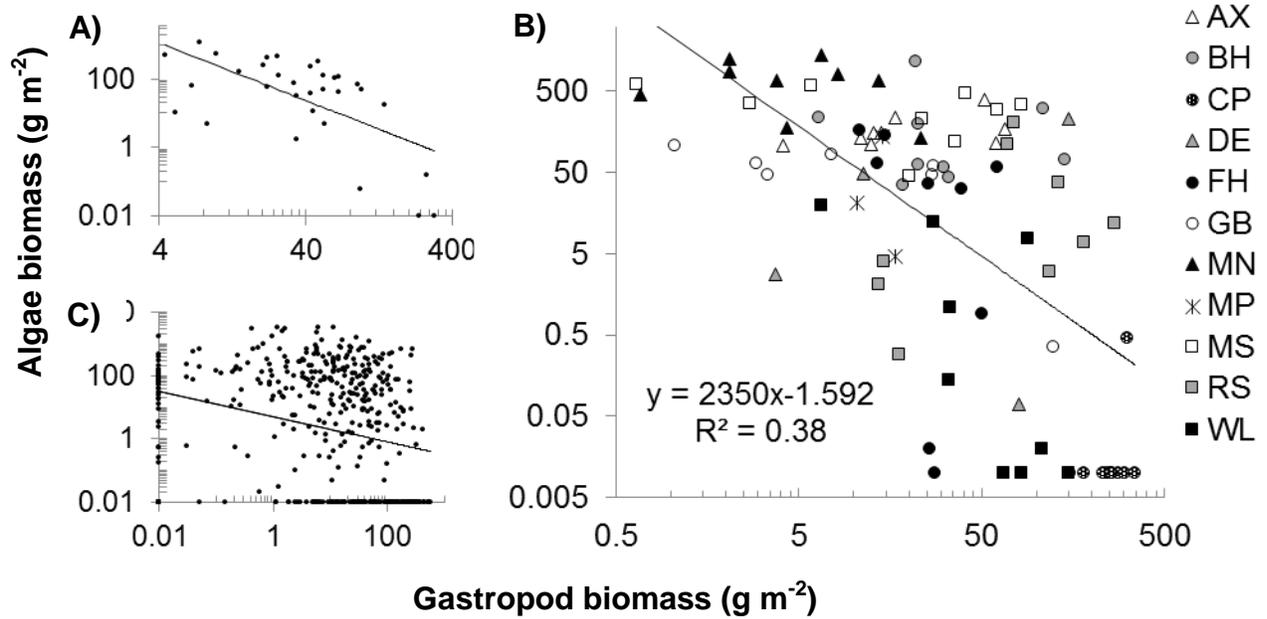


Figure 2-2. Bivariate regressions of gastropods and algae at three scales of data aggregation. (A) Spring level data averaging ( $11,609.4x^{-1.68}$ ,  $R^2 = 0.37$ ,  $p < 0.001$ ); (B) site level data averaging coded by spring (fit on figure); and (C) non-averaged, sample level data ( $y = 5.0x^{-0.40}$ ,  $R^2 = 0.08$ ,  $p < 0.001$ ). Note the different axis-scales on each graph.

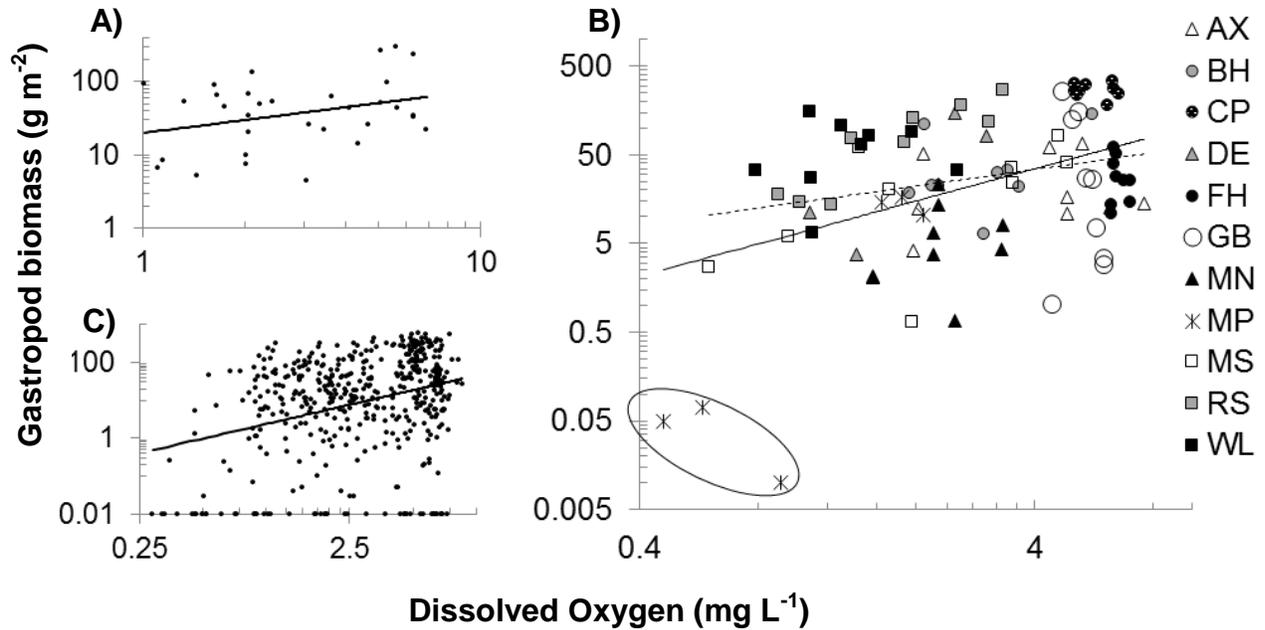


Figure 2-3. Bivariate regression of gastropods and DO at three scales of data aggregation. (A) Spring level data averaging ( $y = 19.9x^{0.59}$ ,  $R^2 = 0.10$ ,  $p = 0.08$ ); (B) site level coded by spring with symbols—sites represented by circles are high DO springs, all others are low DO springs; solid line is the fit including the circled MP outliers ( $y = 6.5x^{1.21}$ ,  $R^2 = 0.19$ ,  $p < 0.001$ ), dashed line is the fit without MP ( $y = 14.3x^{0.62}$ ,  $R^2 = 0.07$ ,  $p = 0.01$ ); (C) sample level, non-aggregated data ( $y = 2.4x^{1.28}$ ,  $R^2 = 0.10$ ,  $P < 0.001$ ).

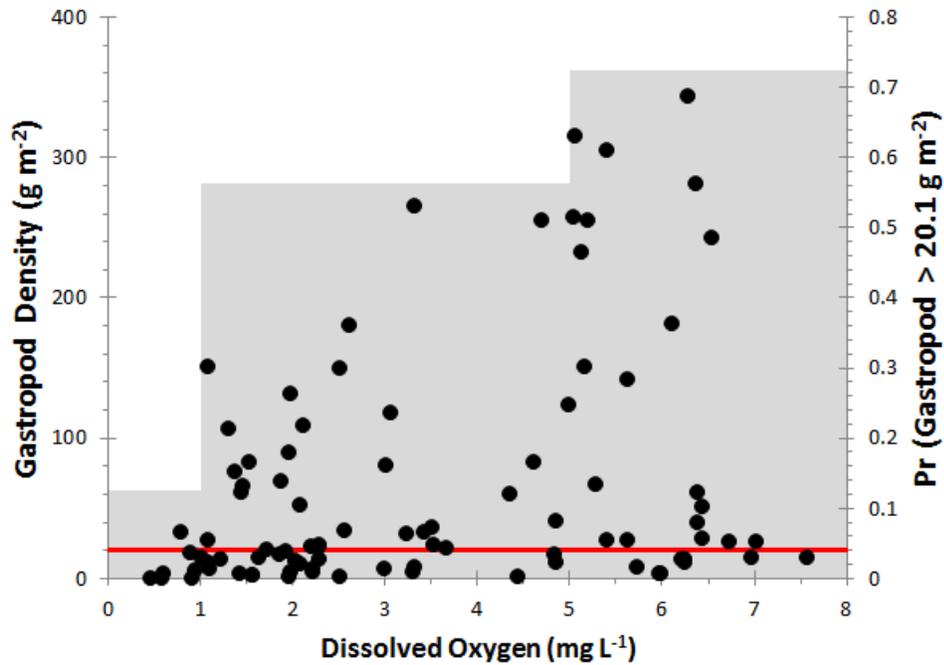


Figure 2-4. Probability of gastropod biomass being above the proposed threshold of  $20.1 \text{ g m}^{-2}$  (above red line), as a function of DO, presented for breaks at  $\text{DO} = 0\text{-}1 \text{ mg L}^{-1}$  (severe hypoxia),  $1\text{-}5 \text{ mg L}^{-1}$  (mild hypoxia to normoxia), and above  $5 \text{ mg L}^{-1}$  (above DEP threshold, high oxygen).

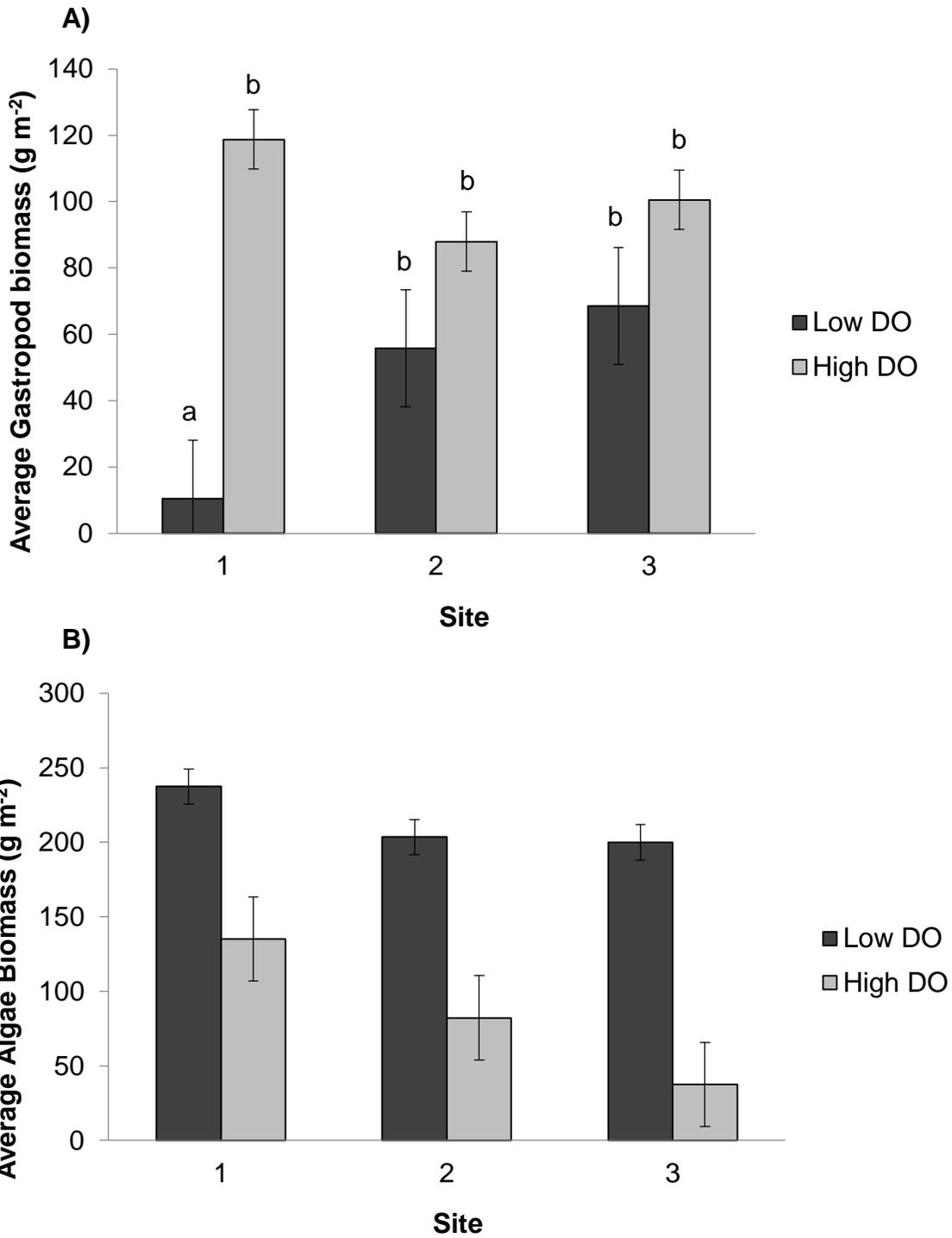


Figure 2-5. Longitudinal behavior of gastropod biomass (A), and algal biomass (B) by longitudinal site for high vs. low DO springs. Bars represent means  $\pm$  standard error. ANOVA F and p values for the log transformed data for: A) Gastropods vs. DO level (9.11, 0.002), Site (4.39, 0.02), and DO\*Site (8.69, < 0.001); and B) Algae vs. DO level (7.33, 0.01), Site (0.06, 0.94), and DO\*Site (0.33, 0.72). Significantly different values (Tukey's HSD,  $p < 0.05$ ) are marked in lower-case letters.

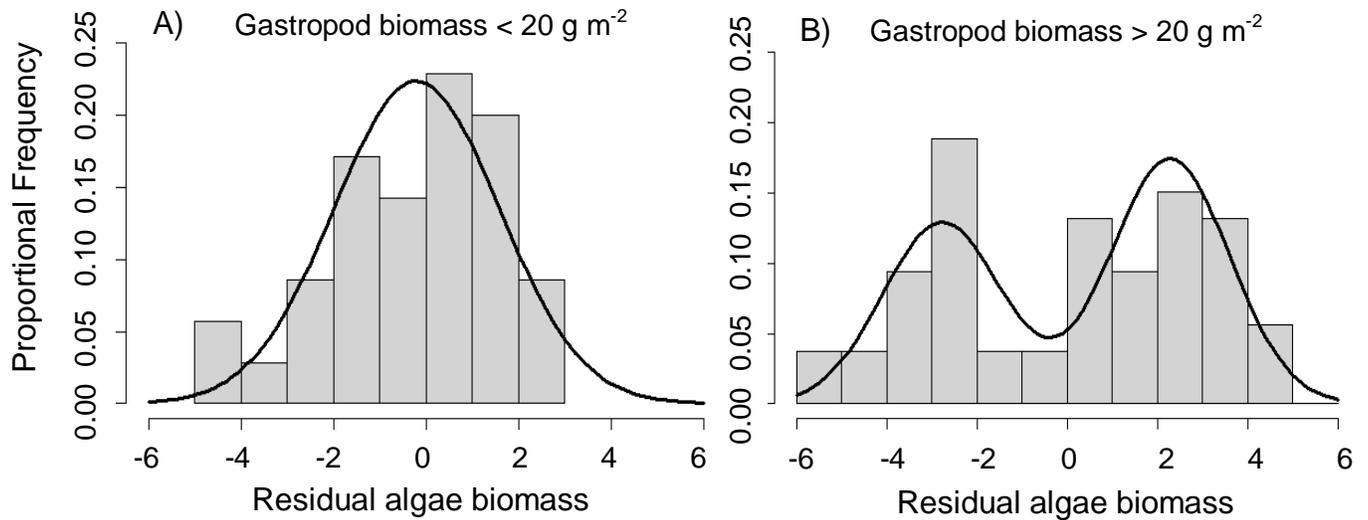


Figure 2-6. Histograms of residuals of the regression of algal biomass with gastropod biomass, fit for unimodal vs. bimodal distributions. Graphs contain the values and fit curves for gastropod biomass < 20.1 g m<sup>-2</sup> (A), and >20.1 g m<sup>-2</sup> (B). BIC fitting data and Hartigan's diptest are presented in table 2-5.

## CHAPTER 3 EXPERIMENTAL EVIDENCE OF GRAZER CONTROL OF FILAMENTOUS ALGAE IN A SPRING-FED RIVER

### **Introduction**

Consumer control of primary producers is increasingly viewed as a key driver of ecosystems states, with evidence of significant top-down control in terrestrial ecosystems (Estes et al. 2011), and even stronger effects in aquatic systems (Cyr and Pace 1993, Borer et al. 2005, Heck and Valentine 2007, Gruner et al. 2008, Estes et al. 2011). Proliferation of nuisance algae has motivated a focus on aquatic ecosystems where bottom-up nutrient and top-down grazer impacts are potentially confounded due to the similarity of the symptoms.

Experimental studies have provided clear evidence of top-down control. A meta-analysis of 25 consumer-control experiments (Cyr and Pace 1993) found grazers remove a median of 79% of biomass, and another meta-analysis of 89 stream experiments found ambient grazer densities reduced periphyton biomass in 70% of studies (Feminella and Hawkins 1995). A much larger meta-analysis of 865 experimental studies across multiple ecosystem types concluded that grazers remove on average 59% of periphyton biomass, with no significant differences between aquatic biomes (Hillebrand 2009).

Despite this convergence of evidence, considerable uncertainties and mediating factors remain regarding the presence and strength of consumer control across systems, therefore top-down controls should be demonstrated on a case-by-case basis. Positive relationships between consumers and algae are observed in 10% of the studies (Hillebrand 2009), and many additional factors directly and indirectly affect primary producer biomass, such as hydrology (Poff et al. 1990, Riseng et al. 2004), light (Odum

1956, Phlips et al. 2000), nutrients (Biggs 2000, Dodds 2007), and a host of indirect effects (Holomuzki et al. 2010). The strengths of consumer “top-down” effects are conditional, and depend on a wide variety of factors, such as consumer composition (Lamberti et al. 1987, Steinman 1996, Shurin et al. 2002), water velocity (Opsahl et al. 2003, Poff et al. 2003), and light (Mallory and Richardson 2005). Dissolved oxygen (DO) influences grazer survival as well as grazing rates (Diaz 2001, Wu 2002), and contaminants may also reduce grazer populations (McMahon et al. 2012). Population density is an important factor for grazing, as higher density populations will generally have higher cumulative grazing rates until they overcrowd to the point of competition (Feminella and Hawkins 1995).

Loss of consumer control can lead to algal blooms (Lamberti et al. 1989, Worm and Lotze 2006), and positive feedback mechanisms can make bloom conditions stable (Scheffer et al. 2008), and thus resistant to restoration. In the simplest conceptualization of the autotroph/grazer relationship (assuming only energetic caps on grazer biomass, and type I functional response), higher autotroph productivity is linearly related to higher cumulative grazing rates, leading to increasing herbivore biomass and consistent fractional standing crop consumption (Rosenzweig 1971, Feminella and Hawkins 1995, Borer et al. 2005). Under such circumstances, healthy grazer populations can potentially keep algal biomass consistently low. In many examples, however, non-linear threshold effects have been observed with varying grazer densities. In these cases, when grazer populations fall below threshold levels (for any exogenous reason), algal biomass proliferates. If grazer populations can rebound and resume consumption at a higher rate than algal growth, and the algae is still palatable, the system will ultimately

return to low-biomass equilibrium (Steinman et al. 1987, Suding and Hobbs 2009). However, if algae become less palatable or inaccessible as biomass accumulates (Gliwicz 1990, Alstynne et al. 1999, Gragnani et al. 1999, Lotze and Worm 2000, Korpinen et al. 2008), an algal-dominated state can persist or even become exacerbated by further diminishing the grazer population (Scheffer et al. 2008).

### **Case Study: Florida's Springs**

In north Florida, the karstic Floridan Aquifer feeds the highest density of large artesian springs in the world, with > 30 first magnitude springs ( $> 2.8 \text{ m}^3 \text{ s}^{-1}$ ) and > 700 named springs (Scott et al. 2004). These springs exhibit remarkable stability in flow, temperature and water chemistry, and the chemostatic properties have made them foci for ecological research (Odum 1956, 1957a, 1957b, Heffernan et al. 2010a). Alarming ecosystem changes have occurred in many springs in the past 20 years, as the autotrophs switched from dominance by submerged aquatic macrophytes to proliferations of dense mats of filamentous algae (Stevenson et al. 2004). The causes of these transitions, however, remain unclear (Heffernan et al. 2010b). Nutrient enrichment has been a leading explanation for these algal blooms (Stevenson et al. 2007), but a review of the available data suggests that the role of nutrients is equivocal, while the role of consumers and the factors that control them merit greater attention (Heffernan et al. 2010b).

Research in similar systems in the southeast US has shown that grazers, and gastropods in particular, exert strong control algal accumulation (Hill et al. 1992, Rosemond et al. 1993, Brown et al. 2008a), and recent studies suggest that this could be an important driver in Florida's springs as well. Heffernan et al. (2010b), for example, found that DO concentrations and qualitative measures of grazer presence were

significant predictors of algal biomass in a study spanning 28 springs. Additionally, Chapter 2 demonstrated that algal biomass and gastropod biomass were negatively correlated across multiple sites in 11 springs, while other potential predictors of algal biomass (nutrients, flow) were not significant predictors of algal biomass. In addition, these investigations found that in low-gastropod environments ( $< 30 \text{ g m}^{-2}$  wet weight), algal biomass was always high, whereas in the presence of higher gastropod biomass ( $> 30 \text{ g m}^{-2}$ ), algae biomass could be either high or low. Dormsjo (2008) provided the first experimental evidence of consumer control of algae in Florida's springs.

Conducting experiments with 10-cm diameter petri dish enclosures, she found that grazers could impact algal accumulation under high oxygen conditions, but not in severely hypoxic sites. Studies that combine experiments and field observations, although rare, are required to ensure that patterns observed in nature are consistent with hypothesized mechanisms (Feminella and Hawkins 1995). This study uses an experimental approach to test mechanisms of algal control by grazers suggested by ecosystem-level surveys (Chapter 2).

## **Hypotheses**

We sought to test two hypotheses regarding grazer controls of algal abundance in springs. The first is that grazer density controls algal accumulation. This leads to the prediction that experimental manipulations of grazer density will influence algal standing crop, with high densities leading to reduced algal biomass. The second hypothesis is that grazers are able to control algal accumulation when initial algae conditions are low, but are unable to do so when the initial algae biomass is high (i.e., algae have a critical escape density from gastropod consumers). The predictions that follow from this hypothesis are that a) at low initial algal biomass, high snail biomass will maintain low

algal levels, but that b) algal biomass will not decline with any level of grazer biomass when initial algal abundance is high.

## Methods

### Study Site and Biota

The Ichetucknee River, located in the Ichetucknee Springs State Park in north-central Florida (Fig. 3-1), originates at the Ichetucknee Head spring and flows for 8 km before discharging into the Santa Fe River. It is fed along its length by nine named springs with distinct chemistry. Median Ichetucknee Head spring discharge is  $1.3 \text{ m}^3 \text{ s}^{-1}$ . Additional spring flows along the length of the river result in median river discharge of  $9\text{--}12 \text{ m}^3 \text{ s}^{-1}$  (Heffernan et al. 2010a). As a state park, the Ichetucknee is relatively well protected, but is subject to heavy recreational pressure during the summer. A carrying capacity study (DuToit 1979) led to seasonal visitation restrictions, which have enabled submerged aquatic vegetation recovery (Kurz et al. 2003, 2004). Nuisance algal levels are ill-defined, but EPA Rapid Bioassessment Protocols suggest biomass thresholds at  $> 50 \text{ g m}^{-2}$  AFDM, or  $> 100 \mu\text{g chl a m}^{-2}$  (Barbour et al. 1999), which have been recorded near many of the spring vents (Frydenborg 2006, Liebowitz et al, in prep.).

The aquatic fauna of the Ichetucknee River includes a wide variety of invertebrate species, with biomass dominated by the Pleurocerid snail *Elimia floridensis*. The genus *Elimia* can account for up to 95% of biomass in some southeastern US streams (Newbold et al. 1983, Hill et al. 2001). *Elimia* spp. are known to be generalist grazers, capable of preventing accumulation of filamentous algae, particularly cyanobacteria (Tuchman and Stevenson 1991). Early surveys of three locations in the Ichetucknee River report a mean *Elimia* density of  $315 \text{ g m}^{-2}$  wet weight (range 138-579  $\text{g m}^{-2}$ ; DuToit 1979). Recent sampling found that *Elimia* accounted for 86% of

invertebrate abundance in the river, but only 10% in the feeder springs where algal proliferation is most severe (Dormsjo 2008). While high *Elimia* density is still occasionally observed in the river (surveys in 2010 recorded one site with 517 g m<sup>-2</sup>; D. Liebowitz, unpublished data), most densities were much lower in these surveys, averaging 37 g m<sup>-2</sup> at nine spring and river locations.

### **Gastropod Surveys**

On 6/13/11, we conducted a survey of the biota in 10 cross-sectional transects, each approximately evenly spaced along the length of the Ichetucknee River, from the Headspring (HS) to the South Take-out (ST). Three samples were collected per transect, evenly spaced across the width of the river. A modified Hess-type sampler (area = 0.084 m<sup>2</sup>) was used to collect all biomass within the sampling device and each sample was individually bagged, labeled, and kept on ice until processing within 72 hours. Each sample was washed and sorted to family level taxa, and all gastropod shells were checked for the presence of bodies, to ensure empty shells were not included. Animals were tamped with paper towel to remove excess water, then weighed. Wet weights were converted to dry weights using an empirically derived relationship from surveys in Chapter 2 ( $y = 0.64x - 0.11$ ,  $R^2 = 0.98$ ), and the three samples per transect were averaged to assess the range of variation in consumer density within the Ichetucknee River.

### **Experimental Design and Field Sampling**

Experiments were conducted at four sites (Fig. 3-1C) with slightly different water chemistry (DO; nitrate, NO<sub>3</sub><sup>-</sup>; orthophosphate, PO<sub>4</sub><sup>3-</sup>), channel morphology, canopy cover, and DO regimes (Fig. 3-2). Diel DO profiles taken for a week at each site between 21 February and 30 March 2011 (Fig. 3-2) show the magnitude of variation in

this key environmental variable. Two sites were near spring vents (Ichetucknee Headspring, HS, high DO, 0.8 mg NO<sub>3</sub>-N L<sup>-1</sup>, 20 µg PO<sub>4</sub>-P L<sup>-1</sup>; Mill Pond, MP, low DO, 0.2 mg NO<sub>3</sub>-N L<sup>-1</sup>, 55 µg PO<sub>4</sub>-P L<sup>-1</sup>), and two were in the main stem of the river (Grassy Flats, GF, medium fluctuating DO, 0.38 mg NO<sub>3</sub>-N L<sup>-1</sup>, 41 µg PO<sub>4</sub>-P L<sup>-1</sup>; South Takeout, ST, high fluctuating DO, 0.31 mg NO<sub>3</sub>-N L<sup>-1</sup>, 40 µg PO<sub>4</sub>-P L<sup>-1</sup>).

*In situ* enclosures (flumes) were created using 15-cm diameter polyvinyl chloride (PVC) tubes, cut to 77-cm length and split laterally, creating a footprint area of 0.12 m<sup>2</sup>. Each flume was enclosed with nylon mesh (ca. 1-mm aperture) at the top and both sides, with one side affixed with hook and loop seals to allow repeated access. Strips of plastic lattice raised tiles 1-cm off the base of the flume to minimize sediment accumulation. Flumes were suspended with PVC stakes affixed on each corner to maintain flumes 10-cm below the water surface, and oriented parallel to the flow (Fig. 3-1D).

This study consisted of two experiments, the first initiated with low algae biomass, the second initiated with high algae biomass. Experiment 1 was conducted at all four previously described sites. Each site contained nine flumes, allowing three replicates of three treatments: (1) 0 snail enclosures (0 g m<sup>-2</sup>), (2) ambient snail densities where flumes were open on the ends (mesh was present, but unsealed at the upstream end to maintain consistent flow effects with the two sealed treatments, and absent in the downstream end), and (3) high snail enclosures (70 *E. floridensis* individuals per enclosure, equivalent to ~306 g m<sup>-2</sup> or ~ 600 adult individuals m<sup>-2</sup>). This high snail enclosure level of 306 g m<sup>-2</sup> wet weight is in the top 10% of pleurocerid biomass values from the 480 samples from Chapter 2 (which found a maximum

pleurocerid density of 845 g m<sup>-2</sup> wet weight in Cypress Spring); however, it is approximately the mean of the pleurocerid biomass found in Ichetucknee Springs in 1979 (Dutoit 1979) and approximates extant levels at GF, therefore an ecologically relevant value. Algae was grown on unglazed ceramic tiles (5 cm<sup>2</sup>), which were placed in each flume and allowed to incubate for five days prior to initiation of Experiment 1 on 4 February 2011. Tiles were collected at the start of the experiment, then at days 4, 12, and 28 of the experimental duration. Flumes were inspected every 2-3 days during which the top mesh was brushed clean, excess sediment was removed by gently agitating the water column to re-suspend and flush out the sediment, and dead snails were replaced, if necessary.

Experiment 2 was conducted only at HS, GF, and ST; MP was not used because of extremely high grazer mortality during Experiment 1. In this experiment, we used high initial algae biomass and four grazer density treatments. Each site contained 12 flumes, with three replicates of each treatments: (1) snail enclosures (0 g m<sup>-2</sup>), (2) low density enclosure (25 snails, 110 g m<sup>-2</sup>), (3) medium density enclosure (50 snails, 220 g m<sup>-2</sup>), and (4) high density enclosure (75-85 snails, 330 g m<sup>-2</sup>). Unglazed ceramic tiles were cultured for approximately two months prior to initiation of the experiment. However, during this culturing period, a wave of chironomid larvae infiltrated the flumes, necessitating tile clearing and additional algal accumulation time. This delayed the start and shortened the duration of the experiment, the end of which was constrained by the start of high park visitation. During Experiment 1 we observed very little algae accumulation at ST even in ungrazed conditions, either on tiles or in the river. Therefore tiles incubated at HS were transported to ST at the initiation of Experiment 2, to explore

whether algae did not naturally recruit there, or alternatively could not grow once established. Due to the shortened timeline of Experiment 2, tiles were collected at initiation (13 May 2011), and on days 7 and 13. Because GF was not affected by visitor pressure, the experiment was continued for an additional 10 days at that site.

Environmental variables were recorded for the duration of both experiments. We measured canopy cover using a densiometer, surface flow velocity using the float method, and DO, water temperature, specific conductance, and pH using a YSI 6920 multi-parameter sonde (Yellow Springs Instruments, Yellow Springs, OH). At each site we also collected a 500-mL water sample into an acid-washed polyethylene bottle that was acidified to pH 2.0 using hydrochloric acid, and stored below 4°C until colorimetric analysis for  $\text{NO}_3^-$  and SRP. For both experiments, three tiles were collected from the same location in each flume and placed in individual, labeled, sealable plastic bags. They were put on ice in a dark cooler for transport to the laboratory, and kept on ice until processed, within 48 hours of sample collection.

### **Laboratory Processing**

Each experimental tile was scraped with a clean razor blade, rinsed into a beaker using deionized water to a volume of 200 mL, and split into two subsamples of equal volume. Subsamples were individually filtered through a pre-combusted and pre-weighed Whatman GF/F glass fiber filter. One filter was frozen for Chlorophyll *a* analysis (Standard Methods 10200 H (APHA 2005), with the modification of pigment extraction in solution (Sartory and Grobbelaar 1984)). The other filter was processed for ash-free dry mass (AFDM) by drying the filters at 60° C for 24 hours, weighing them, combusting them with a muffle furnace at 500° C for 1 hour, and then reweighing them to calculate AFDM. Water samples were filtered using a 0.45- $\mu\text{m}$  glass fiber filter and

analyzed within 28 days for  $\text{NO}_3^-$  (as N, using EPA 353.2) and  $\text{PO}_4^{3-}$  (as P, using EPA 365.1).

## Data Analyses

Four response variables were examined individually and together to explore algal responses to grazing: (1) Chlorophyll *a*  $\text{g m}^{-2}$ , (2) AFDM  $\text{g m}^{-2}$ , (3) percent removal (%R), and (4) removal rate (RR). The first and second response variables are direct measurements. The third response variable is the percent of algae AFDM removed (Worm and Lotze 2006), comparing grazed biomass ( $A_G$ ) to ungrazed biomass ( $A_U$ ) treatments on the final collection date:

$$\%R = 100 \cdot (A_U - A_G) / A_U \quad (3-1)$$

The fourth response variable is the daily removal rate of AFDM per treatment:

$$RR = (\Delta A_U - \Delta A_G) / \Delta t \quad (3-2)$$

where  $A_U$  and  $A_G$  are the same as they are for %R, compared between sampling times, and  $t$  is the number of days of grazing. We note that RR and %R use the term “removal” rather than “consumption” to acknowledge that measured changes in biomass are a function of both direct consumption and bioturbation or other forms of mechanical disturbance and export (Cattaneo and Mousseau 1995).

All response variables were tested for normality using the Kolmogorov-Smirnov test. When variables deviated significantly from normality, we conducted the Box-Cox analyses to find the most appropriate transformation, with resulting lambdas consistently near zero, suggesting logarithmic transformations (Osborne 2010). Therefore, all response values were natural log transformed for statistical analyses, though averages are presented for untransformed values.

To test the significance of both site and treatment on each response variable, we used factorial ANOVA on natural log transformed data to detect significant differences among sample means at  $p < 0.05$ . Specifically, we examined site, treatment, and site\*treatment interactions. Tukey's HSD post-hoc tests were used to detect significant results ( $p < 0.05$ ) for factors without interaction effects (treatment, site). All analyses were performed using Statistica (v10 STATsoft, Inc.). The relationship of ungrazed samples with environmental variables was tested with ordinary least squares (OLS) regression using site means ( $n=4$ ), providing very low statistical power, but preliminary information for future study. To account for varying grazing pressure in ambient treatments between sites, we used linear regression on log-transformed data to assess bivariate relationships between grazer density and algal biomass.

## Results

### Spatial Patterns of Gastropod Grazers

Gastropods from six native families and one invasive species (family Thiaridae) were distributed along the river in a highly variable manner (Fig. 3-3), with the family Pleuroceridae (*Elimia spp.*) comprising the dominant biomass presence. Pleuroceridae were 57% of the biomass, and the family Ampullariidae (apple snails, a genus that primarily consumes macrophytes rather than algae (Dillon 2000)) was the second most abundant, accounted for 14% of the sampled gastropod biomass. The highest snail density was found at GF, with total gastropod biomass approaching levels seen by Dutoit (1979). Additional *Elimia* density metrics derived from the presence of gastropods in the ambient flumes on 3 March 2011 showed a wide range of densities, with 55-155 g  $m^{-2}$  at HS, 107-363 g  $m^{-2}$  at GF, 0 g  $m^{-2}$  at MP, and 4-128 g  $m^{-2}$  at ST.

## Experiment 1: Low Initial Algae Conditions

We observed strong and significant effects of *Elimia* grazing on all measures of algal accumulation, with visually apparent (Fig. 3-4) and statistically significant variability between sites (Fig. 3-5). Chlorophyll *a* and AFDM were significantly different for all treatments ( $p < 0.001$  for both response variables). Post-hoc comparisons ranked 0 grazers > ambient grazers > enclosed high grazers (Table 3-1). Algal AFDM and grazer biomass exhibited a strong negative relationship (Fig. 3-6), but with some variation between sites. However, combining all sites together showed the presence of a potential threshold at  $100 \text{ g m}^{-2}$  of gastropods: when gastropod biomass was below that level, algae began to accumulate beyond  $10 \text{ g m}^{-2}$ , a level equated with low-algae biomass across grazer studies (Feminella and Hawkins 1995). Above  $100 \text{ g m}^{-2}$  of gastropods, the algal AFDM ( $M = 2.6$ ,  $SD = 1.7 \text{ g m}^{-2}$ ) was not significantly different from the initial conditions ( $M = 2.1$ ,  $SD = 0.95 \text{ g m}^{-2}$ ;  $t(38) = 1.1$ ,  $P = 0.25$ ), whereas below  $100 \text{ g m}^{-2}$  of gastropods, algal AFDM ( $M = 16.4$ ,  $SD = 12.6 \text{ g m}^{-2}$ ) was significantly higher than the same initial conditions ( $t(42) = 5.5$ ,  $p < 0.001$ ). Mean percent removal (%R) differences between ambient (48%) and high grazer enclosure (78%) treatments was nearly significant ( $p = 0.057$ ) across all sites. However, there was strong evidence of a site-level effect for this response variable; HS and GF both averaged more than 90% algal removal in high grazer treatments, while snails at MP and ST were much less effective (14% to 64%; Fig. 3-5). There was no effect of treatment on removal rate (RR), suggesting that grazers consumed constant amounts per unit biomass. However, we did observe one significant site effect; HS and GF had average removal rates of 1.6 and  $1.0 \text{ g AFDM m}^{-2} \text{ d}^{-1}$ , respectively, which were both significantly higher than MP and ST, with 0.2 and  $0.1 \text{ g AFDM m}^{-2} \text{ d}^{-1}$ , respectively.

None of the environmental relationships with algal AFDM were statistically significant, which was expected because of low power ( $n = 4$ ) for detecting those effects. However, we note the few marginally significant factors: water velocity exhibited a negative relationship in enclosures ( $r = -0.94$ ,  $p = 0.06$ ), and DO concentration had a positive relationship in the enclosures ( $r = -0.92$ ,  $p = 0.08$ ).

## **Experiment 2: High Initial Algae Conditions**

We observed significant grazer effects on both Chlorophyll *a* and AFDM, but only for the highest grazing treatment ( $330 \text{ g m}^{-2}$  snail wet weight); intermediate densities ( $110 \text{ g m}^{-2}$  and  $220 \text{ g m}^{-2}$ ) were not significantly different from snail-free enclosures (Fig. 3-5). Grazing efficiency (%R) and removal rate (RR) were not significantly different across the three grazer treatments (Table 3-1). The highest snail density treatment ( $330 \text{ g m}^{-2}$ ) averaged 54% removal (strongly influenced by extreme removal of 82% at HS), while the intermediate ( $220 \text{ g m}^{-2}$ ) and low ( $110 \text{ g m}^{-2}$ ) treatments averaged only 30% and 23% removal, respectively. Removal rates varied significantly among sites and treatments, with a high of  $2.5 \text{ g m}^{-2} \text{ day}^{-1}$  in GF (at  $220 \text{ g m}^{-2}$ ) and lows of  $-0.09 \text{ g m}^{-2} \text{ day}^{-1}$  at ST ( $220 \text{ g m}^{-2}$ ) (Fig. 3-5).

Algae AFDM increased in all treatments at GF during the extra week of experimental duration (Fig. 3-7). At the end of the experiment, AFDM had reached  $49 \text{ g m}^{-2}$  in the snail-free enclosures, and the snail enclosure treatments were not significantly different ( $F = 0.4$ ,  $p = 0.8$ , Table 3-1). Although there was negligible snail mortality for the first three weeks (until 26 May) at GF, there was unexplained snail mortality between 26 May and 5 June. Final snail biomass averaged  $258 \text{ g m}^{-2}$  in the “330” treatments,  $187 \text{ g m}^{-2}$  in the “220” treatments, and  $100 \text{ g m}^{-2}$  in the “110” treatments.

Negligible biomass accumulation on tiles at ST during the 2-month culturing period necessitated transplanted algae for experiment 2 at that site. As such, the experiment tests whether algae simply did not recruit at that site, or if algal biomass was actively diminished. All grazed treatments at ST lost biomass whereas the 0 snail treatment gained a small amount of biomass, however none of the treatments were significant ( $F(3, 8) = 1.27, p = 0.35$ ).

## **Discussion**

### **Grazer Impacts in Low Initial Algae Conditions**

This study provides strong evidence that grazers can inhibit formation of algal blooms, given high snail density and low initial algae conditions. In the high grazer enclosures, the final AFDM was even lower than the negligible levels present at initiation of the experiment for HS, GF, and ST, while grazer enclosures exhibited positive but varying levels of algal accumulation. Ambient grazer levels were intermediate between grazer enclosure and enclosure treatments; all three treatments together exhibited a highly significant negative association between grazer and algal biomass, consistent with our central prediction for hypothesis 1, and with behaviors observed broadly across aquatic ecosystems (Hillebrand 2009) and specifically in streams (Feminella and Hawkins 1995).

That snails inhibited algal accumulation except at the hypoxic MP site, despite site variation in ungrazed algal productivity (Fig. 3-5), suggests that given sufficient snail biomass, algal productivity is transferred to secondary production or sloughed off, rather than accumulating at the primary producer level. This is consistent with the “paradox of enrichment” (Rosenzweig 1971), wherein increases in primary production are realized at higher trophic levels. It also reinforces the findings of Feminella and Hawkins (1995),

whose meta-analysis of stream grazing experiments found that grazers generally maintained low algae biomass ( $< 10 \text{ g m}^{-2}$ ), despite dramatically different production (0 to  $80 \text{ g AFDM m}^{-2}$ ) in grazer-free areas. This effect of higher grazing with higher productivity is also seen in the relatively consistent percentage of algae biomass consumed across productivity gradients (Cyr and Pace 1993, Worm and Lotze 2006).

The absence of algal accumulation at the South Take-out (ST) could have been a consequence of the high water velocities or low  $\text{NO}_3^-$  at this site. However, we suspect that a large factor was the “cage effect,” as the water at that point in the river had a relatively large suspended sediment load, which it deposited when the velocity decreased upon entering the flume through the mesh partial barrier. Therefore, the algal declines at ST may have been a result of sedimentation.

The ability of grazers at ambient densities to maintain a low algal biomass illustrates the management relevance of grazing effects. At HS and GF, ambient gastropod levels ( $94$  and  $217 \text{ g m}^{-2}$ , respectively) were able to maintain the same low algal biomass as enclosures ( $330 \text{ g m}^{-2}$ ) for about two weeks. By the end of the month, algal biomass in the ambient treatments at HS had accumulated significantly, whereas GF had increased only slightly (Fig. 3-7), likely because ambient snail levels at GF were closer to enclosure levels. The regression analysis of gastropod biomass versus algal biomass shows that gastropod density above approximately  $100 \text{ g m}^{-2}$  maintained algae AFDM at the same level as the initial conditions, with most points below  $4 \text{ g m}^{-2}$  algae AFDM and MP having two points at  $6 \text{ g m}^{-2}$  algae AFDM, perhaps slightly higher due to lower efficiency grazing from hypoxic stress. While grazed tiles remained at low biomass, ungrazed tiles reached  $51.2 \text{ g m}^{-2}$ , exceeding the US EPA nuisance algae

threshold of  $50 \text{ g m}^{-2}$  (Barbour et al. 1999) in a short period of time. Historical *Elimia* densities (mean =  $315 \text{ g m}^{-2}$ , minimum =  $138 \text{ g m}^{-2}$ ; DuToit 1979) far exceed this  $100 \text{ g m}^{-2}$  wet weight gastropod biomass threshold, suggesting that snails would have been capable of maintaining low algal biomass under historic conditions. In contrast, current average grazer levels are mostly below these values, except at GF.

### **Environmental Mediation of Grazer Impact**

It seems likely that DO impacts algal growth in grazed treatments by suppressing grazing rates. DO was not significantly related to AFDM in grazer enclosures as a direct driver of algae, but marginally significant in the enclosures ( $r = -0.92$ ,  $p = 0.08$ ) indicating impacts on grazing potential. The hypoxic MP site was the only one where grazer enclosures were ineffective and allowed algal biomass to increase, likely because of high snail mortality that did not occur in any of the other sites. Snails in the enclosures were sourced from the immediate vicinity of the experimental unit, and therefore presumed to be acclimated to the local conditions. We speculate that these snails may survive in this extremely low DO environment through behavioral modifications (Hanley and Ultsch 1999, Wu 2002, Cheung et al. 2008), specifically positioning themselves close to the air-water interface, particularly at night. The flumes prohibited access to the water surface, and thus precluded the behavioral coping mechanism. Although snails that died were replaced regularly, enclosure tiles were less completely grazed than at other sites, suggesting that DO stress may lower grazing rates, even where biomass may be high. This is consistent with results from chapter four, which found lower per capita grazing rates in hypoxic treatments. We caution that there was only one site with severe hypoxia, and the mortality could also have been caused by some other unmeasured factor. However, extreme low DO is stressful for most

aquatic organisms (Diaz 2001), prior research at this location also suggested higher mortality and torpor (Dormsjo 2008), and controlled DO experiments (Chapter 4) found a strong effect of hypoxia on the survival and grazing rate of pleurocerids.

Algal accumulation may also be mediated by environmental factors in the absence of grazing, with water velocity potentially influencing growth and proliferation (Poff et al. 1990). In the grazer treatments, only DO showed even marginal associations with AFDM, while in the grazer-free treatments, water velocity was marginally significant ( $r = -0.96$ ,  $p = 0.06$ ). Additionally, turbidity increases downriver (Wetland Solutions Inc. 2011), and as noted previously, settling of sediments at ST may smother algae and inhibit biomass accumulations. We discuss the environmental data despite the non-significant p-values, in order to avoid Type II errors (given the low power of  $N = 4$  and high correlations), with the caveat that further research is needed. These data suggest that it is worth testing the hypothesis that with high grazer density, environmental factors are secondary, but in the absence of strong grazer control, the environmental factors may affect primary producers more directly.

### **Grazer Impacts in Algae Bloom Conditions**

Grazing effects at high initial algal biomass (Experiment 2) were more variable by site and snail density, and suggested potential herbivore escape dynamics. Algal biomass decreased at the highest level of grazing ( $330 \text{ g m}^{-2}$ ) at all three sites for the first 2 weeks, indicating that grazing rates can exceed algal growth rates; if the system continued on that trajectory, it would eventually return to low algal standing crop. One important caveat is that at the one site with the extended experiment duration (GF), there was a reversal in the algal biomass control trajectory, leading to algal accumulation (Fig. 3-7). This could be due to the longer duration of the experiment, and

the rest of the sites may have eventually lost control of algal biomass as well (after the initial impacts of bioturbation leading to temporary declines in algal biomass), given a longer time-frame. Alternatively, this may have been a consequence of high gastropod mortality at GF during the extra week, wherein the “330 g m<sup>-2</sup>” treatment ended with approximately 258 g m<sup>-2</sup>. However, that is still well above the 100 g m<sup>-2</sup> gastropod biomass which prevented algal accumulation in low initial algae sites, illustrating the presence of hysteretic behavior. Different thresholds could also be a function of different algal assemblages in the blooms, as some may be more palatable than others. We were unable to run full palatability experiments, but preliminary trials suggest that *Elimia* can limit new growth in *Lyngbya* mats, but cannot decrease pre-existing biomass (Liebowitz, unpublished data).

Control of algae was less effective at intermediate gastropod densities. At HS, we observed net accumulation of algae at and below the 220 g m<sup>-2</sup> treatment, while at GF the intermediate gastropod density treatments did decrease AFDM by week 2, but AFDM growth was positive by week 3. This again could be due to snail mortality during that last week, but showed that gastropods could not reduce AFDM at the new average level of biomass treatments (330 = 258 g m<sup>-2</sup>, 220 = 187 g m<sup>-2</sup>, and 110 = 100 g m<sup>-2</sup>). Together, these findings suggest that grazer densities of ca. 330 g m<sup>-2</sup> are required to reverse algal accumulation.

As a restoration target, this grazer density is extremely high. Densities in excess of 330 g m<sup>-2</sup> are present, but rare in Florida’s springs. Recent surveys (450 samples across multiple sites and dates in 11 springs between 2008 and 2010) found only ~ 10% of samples were above this threshold (chapter 2). Only 25% of samples were above

110 g m<sup>-2</sup>, a density at which no evidence of biomass reduction was found in the current study. In short, all snail treatments in this experiment were high in relation to existing (natural) densities, suggesting that most locations have gastropod populations that are too low to reverse the trajectory of algal growth. We note, however, that this finding and the threshold at which reversal of nuisance algal biomass is possible, may depend on algal productivity at each site.

One mechanism for creating an herbivore escape density is the loss of palatability as algae reach mature growth forms, which inhibits herbivore population growth and allows autotrophic biomass accumulation that is unaffected by herbivory (Scheffer et al. 2008). This mechanism does not appear to hold in this study, where grazers continued to remove algae at similar rates (per unit snail; RR) in both experiments. Alternatively, simple declines in grazing efficiency at high prey density (i.e., type II functional response; Holling) or consumer satiation could lead to the cap in grazing as well.

The different thresholds of gastropod density needed to maintain low algal biomass from low initial algae conditions (100 g m<sup>-2</sup> snails) vs. the density needed to reduce algae from high algae initial states (> 330 g m<sup>-2</sup> snails) suggests a hysteretic system. This could arise from three potential scenarios for the longer term dynamics of algae-grazer interactions, which hinge on algal palatability and grazer population recovery. The first posits that if the algae removal in Experiment 2 was due to consumption and exogenous stressors are absent, the grazer population could recover and cumulative grazing rates would increase. This would allow the grazers to eventually reduce a bloom and return to an herbivore dominated system. In the second scenario, if

the algae are palatable but the exogenous stressor(s) remains, the grazer population density may remain low or further decline. Thus cumulative grazing rates would remain depressed, effectively maintaining an algal dominated state. In the third scenario, if the algal removal in experiment 2 was primarily due to mechanical interference, and the bloom formation is unpalatable or otherwise impenetrable, then the algal dominance would be entrenched, whether or not grazer populations ever recovered. As this study was temporally restricted and was not run long enough to capture long-term population dynamics, it remains unknown whether this system could eventually recover through a rebound of gastropod populations alone, or whether it would require a system “reset” to clear unpalatable algal species.

This study highlights the vital role of grazers in controlling aquatic autotroph biomass, and delineates threshold levels of gastropods needed to maintain herbivore dominated states in Ichetucknee Springs. Management attention is generally focused on the abiotic drivers of ecosystem change, but this study provides unequivocal evidence that healthy gastropod communities can forestall nuisance algal blooms, and even reduce them at sufficiently high grazer densities. Managing nuisance algae in Florida’s springs would likely benefit from research and management efforts that result in maintaining viable grazer populations.

A variety of stressors could affect gastropod populations, with DO only one of many potential factors, suggesting the importance of assessing multiple hypotheses to fully understand the drivers of ecosystem change (Heffernan et al. 2010b). For example, industrial and agricultural contaminants are emerging as an area of concern for the health of aquatic fauna (Evans-White and Lamberti 2009, McMahon et al. 2012), and

many contaminants have been shown to trigger trophic cascades and overabundant primary producers (Fleeger et al. 2003). Aquatic gastropods have so far received little conservation or legal attention in the southeastern US, despite the fact that it is a hotspot for gastropod diversity (Neves et al. 1997, Lydeard et al. 2004, Brown et al. 2008a, Lysne et al. 2008). Because *Elimia spp.* live as long as 10-11 years, and start breeding only after they reach 1-2 years of age (Hill et al. 1992, Johnson and Brown 1997, Powles 2000), their populations may be particularly sensitive to disturbance and slow to recover. Recognition of the central role of grazers as regulators of primary producer composition and abundance should suggest broader approaches to restoration and future research.

Table 3-1. Factorial ANOVAs for the four response variables for experiment 1 (left column) and experiment 2 (right column). Post-hoc analyses (Tukey's HSD) significant at the  $p < 0.05$  levels are listed for the effects without interaction terms. The site\*treatment effects for AFDM, %R, and RR are displayed in Figure 3-5.

Variable		F	p	Among factor difference (Tukey HSD)	F	P	Among factor difference (Tukey HSD)	
		Experiment 1: Low initial algae				Exp 2: High initial algae		
Algae Chl a	Intercept	121.5	$p < 0.001$		1364.5	$p < 0.001$		
	Site	19.3	$p < 0.001$	HS,MP>ST,GF	33.2	$p < 0.001$	ST>GF>HS	
	Treatment	37.7	$p < 0.001$	1>2>3	22.6	$p < 0.001$	1,2,3>4	
	Site x Treatment	3.9	0.008		8.3	$p < 0.001$		
Algae AFDM	Intercept	554.7	$p < 0.001$		2969.9	$p < 0.001$		
	Site	39.0	$p < 0.001$	HS,MP>GF>ST	51.2	$p < 0.001$	GF>HS>ST	
	Treatment	49.3	$p < 0.001$	1>2>3	13.4	$p < 0.001$	1,2,3>4	
	Site x Treatment	5.1	0.002		3.8	0.008		
% Removed	Intercept	1537.3	$p < 0.001$		539.2	$p < 0.001$		
	Site	2.5	0.099		0.5	0.608		
	Treatment	4.2	0.057		3.2	0.065		
	Site x Treatment	0.7	0.589		1.5	0.252		
Removal Rate	Intercept	28.5	$p < 0.001$		37.2	$p < 0.001$		
	Site	14.3	$p < 0.001$	HS,GF,MP>MP,ST	6.5	0.008	GF, HS>HS,ST	
	Treatment	0.4	0.552		2.4	0.12		
	Site x Treatment	0.1	0.941		0.6	0.661		

Table 3-2. Daily accumulation rates of filamentous algae (AFDM  $\text{g m}^{-2} \text{ day}^{-1}$ ) per experimental treatment ( $\text{g m}^{-2}$  wet weight snail biomass) at each experimental site, calculated as the slope of the regression line of algae AFDM by date, categorized by treatment. Note that a few treatments have final AFDM < initial AFDM, yet show positive (but negligible) accumulation rates – this is due to small fluctuations of AFDM in intermediate dates.

<b>Treatment</b>	<b>HS (Headspring)</b>	<b>GF (Grassy Flats)</b>	<b>MP (Mill Pond)</b>	<b>ST (South Takeout)</b>
Experiment 1. Low initial algae: 4 February 2011 – 3 March 2011				
Initial AFDM $\text{g m}^{-2}$ (average)	3.36	1.46	2.03	1.65
Final AFDM in $306 \text{ g m}^{-2}$ (average)	2.72	1.41	7.53	1.08
$0 \text{ g m}^{-2}$	1.42	0.79	0.55	0.09
Ambient	0.67	0.08	0.40	0.02
$306 \text{ g m}^{-2}$	0.05	0.03	0.27	0.00
Experiment 2. High initial Algae: 13 May 2011 – 26 May 2011 (5 June 2011)				
Initial AFDM $\text{g m}^{-2}$	27.26	31.83	-	17.34
Final AFDM $\text{g m}^{-2}$ in $330 \text{ g m}^{-2}$	7.06	29.88 (41.07)	-	7.32
$0 \text{ g m}^{-2}$	0.96	1.33 (0.82)	-	0.16
$110 \text{ g m}^{-2}$	0.12	0.53 (0.49)	-	-0.47
$220 \text{ g m}^{-2}$	0.62	-0.47 (0.21)	-	-0.72
$330 \text{ g m}^{-2}$	-1.80	-0.01 (0.35)	-	-0.92

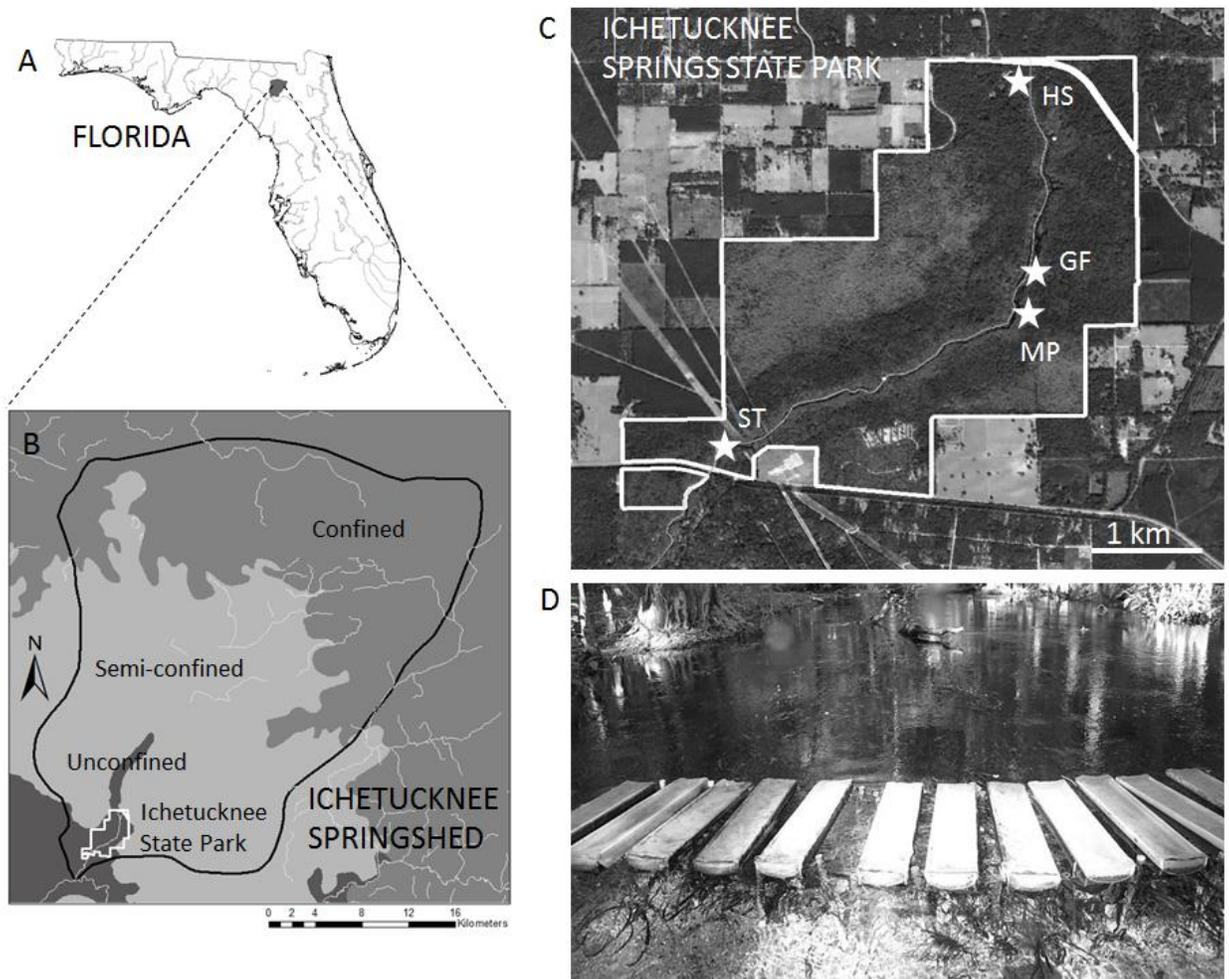


Figure 3-1. A) Location of the Ichetucknee springshed in north Florida; B) map of the springshed showing confining layers above the karst aquifer, with the Ichetucknee Springs State Park boundary drawn in white; C) Ichetucknee Springs State Park, with the four experimental sites marked with stars (Head Spring (HS), Grassy Flats (GF), Mill Pond (MP), and South Takeout (ST)); D) photograph of the experimental apparatus at Mill Pond (MP) before being lowered under the water surface.

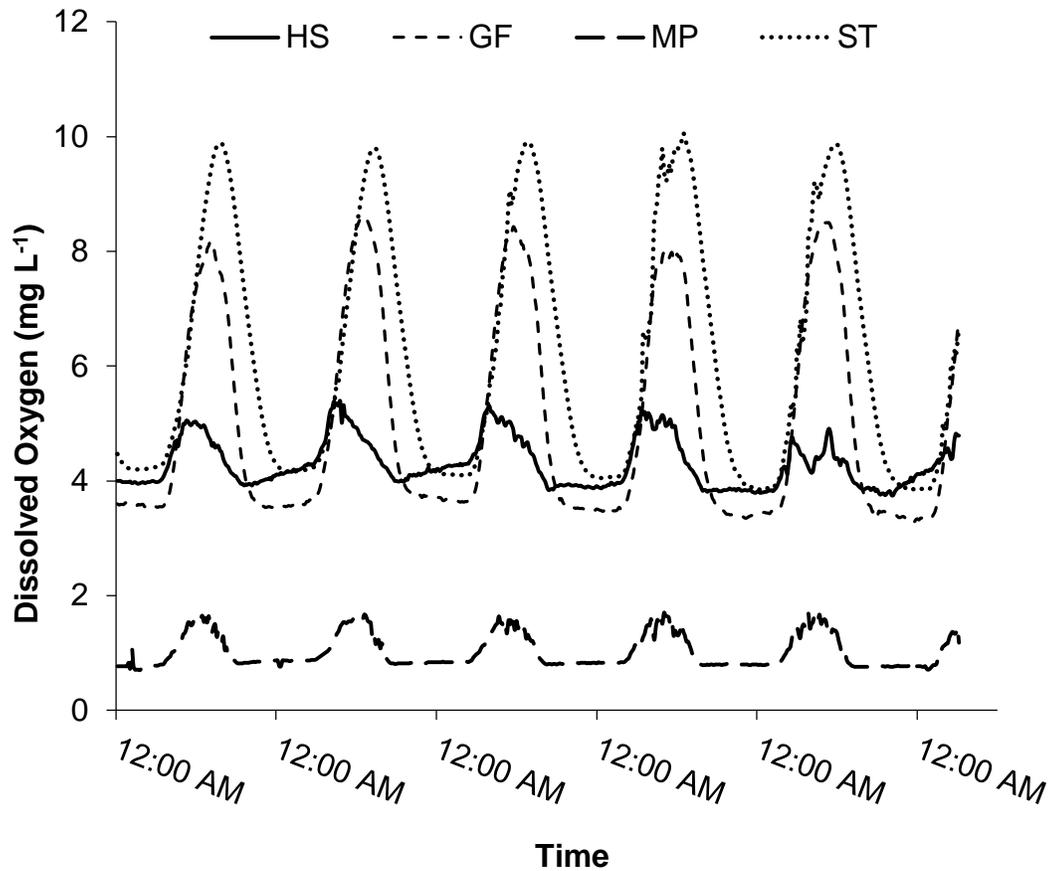


Figure 3-2. Five day dissolved oxygen profiles for the four sites with experimental installations, measured between 21 February 2011 and 30 March 2011. HS (Headspring) and MP (Mill Pond) are spring vents and exhibit relatively stable oxygen concentrations. In contrast, GF (Grassy Flats) and ST (South Takeout) are in the main stem of the river, subject to higher fluctuations in DO concentration as a consequence of diurnal fluctuations in benthic primary productivity and respiration.

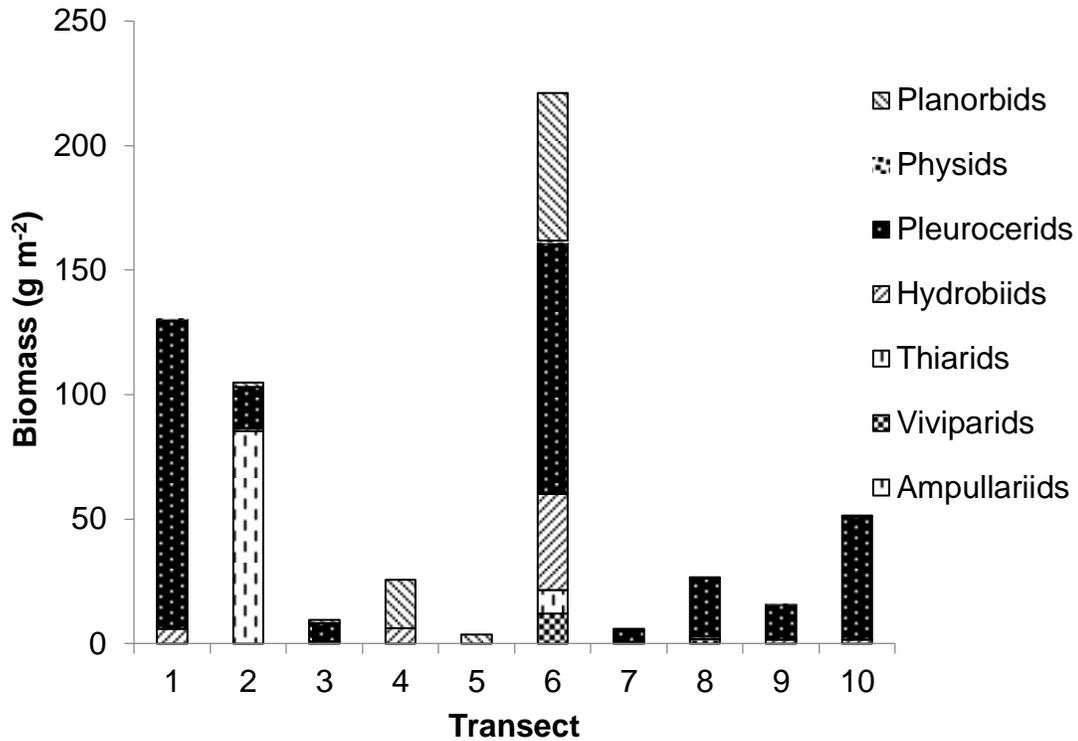


Figure 3-3. Gastropod distribution and composition along the length of the Ichetucknee River on 13 June 2011, beginning at HS (Headspring, transect 1) and ending at ST (South Takeout, transect 10). Each transect represents average values from three biomass samples collected at points evenly distributed along the cross-section of the river.

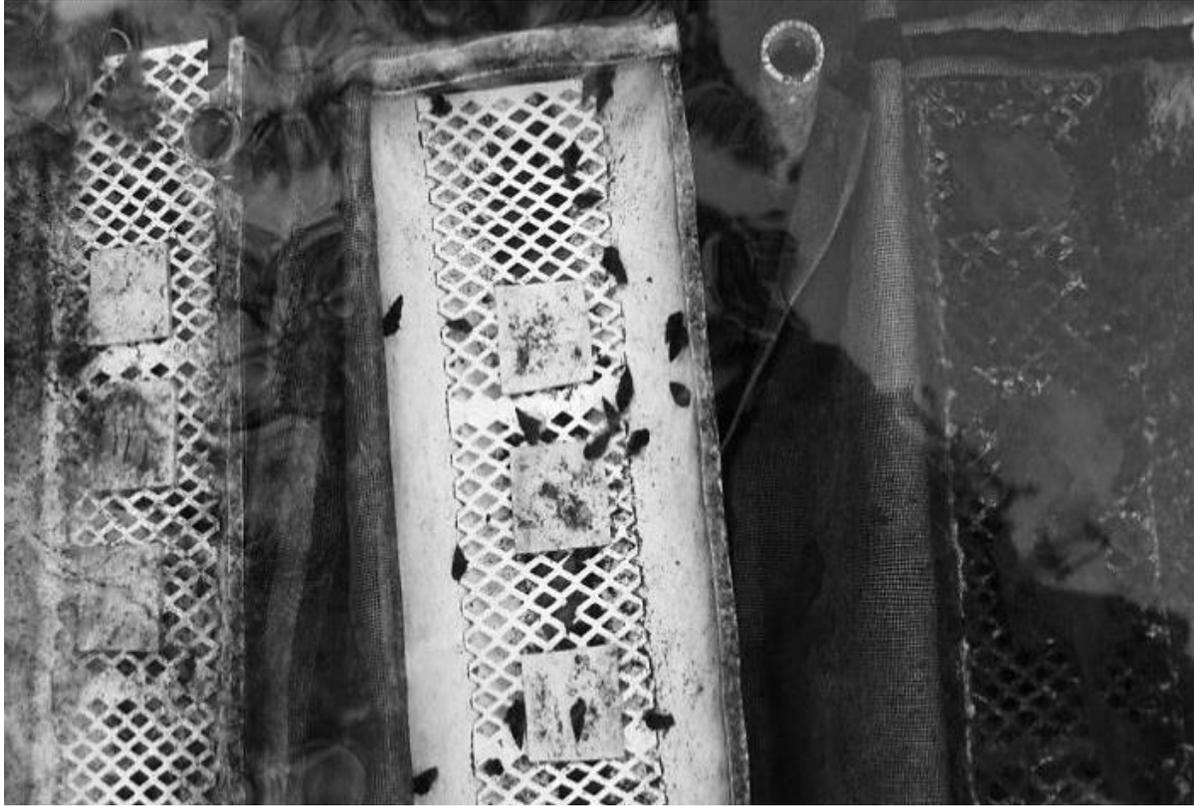


Figure 3-4. Three flumes in experiment 1, at HS (Headspring) on 3 March 2011. The center flume shows the high *Elimia floridensis* treatment ( $\sim 306 \text{ g m}^{-2}$ ), the left flume shows the ambient level, and the right shows the dark algae mat in the 0 snail exclosures

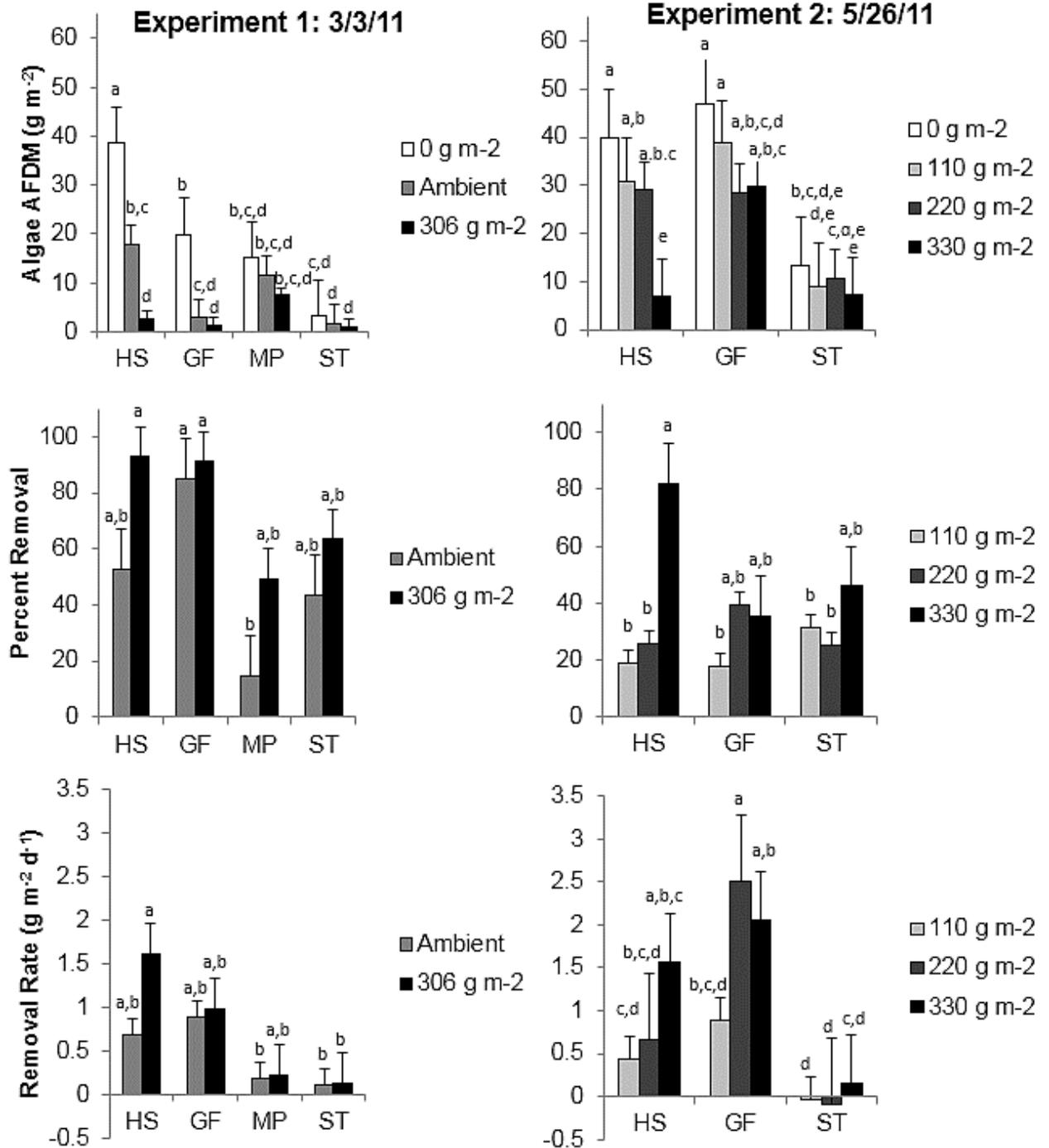


Figure 3-5. Experiment 1 (left column, low initial algae) and Experiment 2 (right column, high initial algae) means and standard error bars for AFDM (top row), percent removal (%R) (middle row), and removal rate (RR) (bottom row), for the listed grazer treatments at the four locations for Experiment 1 (HS (Headspring), GF (Grassy Flats), MP (Mill Pond), and ST (South Takeout)) and three locations for Experiment 2 (HS, GF, ST).

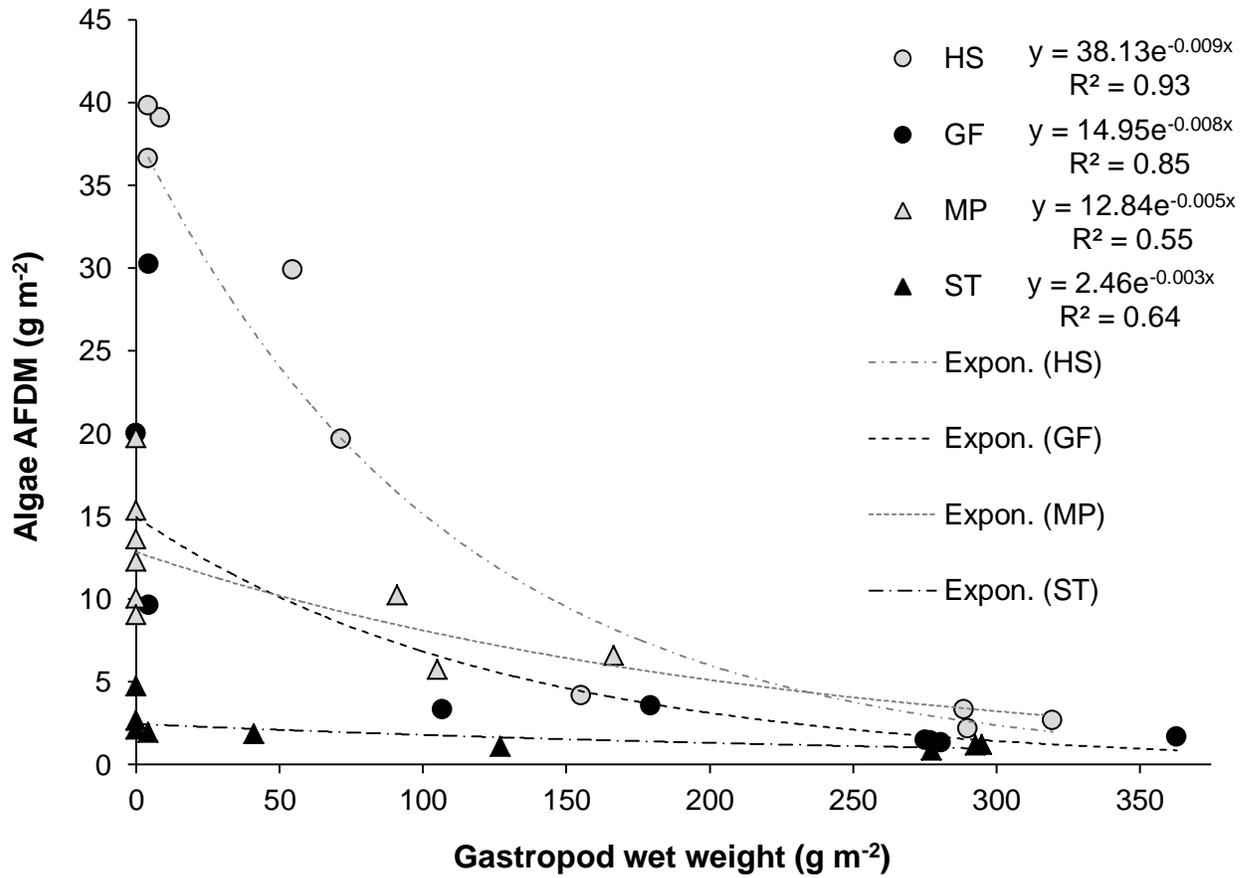


Figure 3-6. Plot of algal AFDM by gastropod wet weight for Experiment 1, categorized by location (HS (Headspring), GF (Grassy Flats), MP (Mill Pond), and ST (South Takeout)). The weight of snail biomass was measured on 3 March 2011 for enclosures, any snails that infiltrated the enclosures, and snails found within the ambient grazer treatments. All fits are significant at  $p < 0.05$  level.

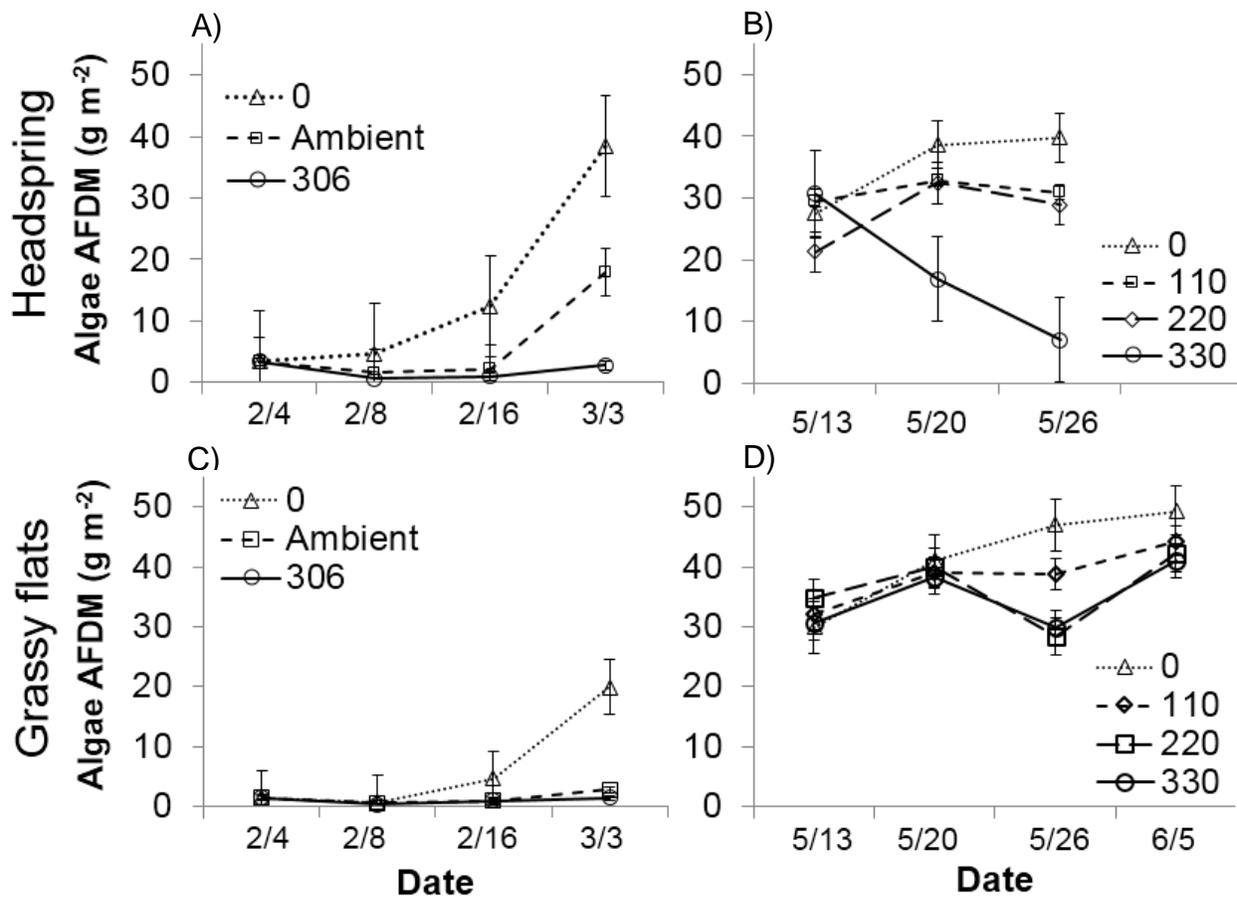


Figure 3-7. Algae AFDM g m<sup>-2</sup> accumulation over time, plotted by collection date and categorized by snail treatment (g m<sup>-2</sup> wet weight snails), for A) Headspring (HS) Experiment 1 (low initial algae); B) HS Experiment 2 (high initial algae); C) Grassy Flats (GF) Experiment 1; and D) GF Experiment 2. Note that GF has a supplemental 10 days duration, allowing an additional collection date on 5 June 2011.

CHAPTER 4  
DISSOLVED OXYGEN IMPACTS ON ALGAL GRAZING BY *ELIMIA FLORIDENSIS* IN  
STREAM MESOCOSMS

**Introduction**

Herbivory is a dominant control on the biomass and composition of primary producers (Cyr and Pace 1993, Estes et al. 2011, Poore et al. 2012). Across a wide array of aquatic systems, grazers regulate algal biomass (Cyr and Pace 1993, Heck and Valentine 2007, Gruner et al. 2008, Estes et al. 2011), even where nutrient enrichment might otherwise lead to nuisance algal accumulation. That is, where grazers are unaffected by environmental or anthropogenic stressors, increased primary production can be transferred into secondary production, leading to conditions of high algal productivity and high grazer biomass, but low standing crops of algae (Rosenzweig 1971, Feminella and Hawkins 1995, Borer et al. 2005). Indeed, grazer exclusion studies indicate that consumers reduce algal standing crops by 59-79% yearly (Cyr and Pace 1993, Hillebrand 2009). However, this top-down control varies as a function of both consumer abundance and consumer efficiency, both of which are susceptible to external stressors (Breitburg et al. 1997). Thus, external factors that affect grazers, including sub-lethal effects, can induce dramatic effects on both the biomass and composition of the autotrophs.

**Hypoxic Stress**

Hypoxic conditions have driven widespread degradation of aquatic ecosystems across the globe (Diaz 2001, Rabalais et al. 2002, Steckbauer et al. 2011). The effects of severe hypoxia can be obvious (most notably in the Gulf of Mexico “dead zone”), yet enumerating thresholds for mortality and sublethal effects on the biota can be challenging (Diaz and Rosenberg 1995, Diaz 2001, Steckbauer et al. 2011). Grazer

abundance and distribution is controlled by many factors in addition to dissolved oxygen, including predation, parasites, invasive species, food resources, flow regime, and pollutants, confounding consistent predictive associations between DO and biota, particularly in flowing waters (Hanley and Ultsch 1999, Garvey 2007). Despite these confounding factors, studies have found that mean lethal oxygen concentrations ( $LC_{50}$ ) for freshwater lotic invertebrates range from a 2-day  $LC_{50}$  of  $0.27 \text{ mg L}^{-1}$  for an ephemeropteran to a 30 day  $LC_{50}$  of  $4.8 \text{ mg L}^{-1}$  for a plecopteran (Landman et al. 2005). Gastropods are generally more tolerant of low DO, with 28-day  $LC_{50}$  values as low as  $0.51 \text{ mg L}^{-1}$  (Das and Stickle 1993). Conventional thresholds of hypoxia ( $< 2 \text{ mg L}^{-1}$ ) and severe hypoxia ( $< 0.5 \text{ mg L}^{-1}$ ) emerge from comprehensive reviews of marine hypoxia studies (Diaz and Rosenberg 1995, Diaz 2001), but may still underestimate sublethal effects. Vaquer-Sunyer and Duarte (2008) suggest that conventional levels underestimate both lethal and sublethal effects in marine systems, underscoring the considerable uncertainty that remains in less well-studied freshwater systems.

Sublethal effects are far less visible than lethal effects, but can impose strong limitations on individual behavior that scale up to community-level impacts if the animals cannot escape the hypoxic zones. Highly mobile species such as fish are generally less resistant to hypoxia, and most respond by leaving the hypoxic environment (Diaz and Rosenberg 1995, Connolly et al. 2004). Less mobile species can respond with behavioral changes and small scale movements, such as gastropod migration up the water column to access increased partial pressure of  $O_2$  near the air-water interface (Hanley and Ultsch 1999, Vaquer-Sunyer and Duarte 2008), a behavior that may ameliorate DO stress, but increase predation risk (Breitburg et al. 1997, Saloom and

Duncan 2005). And while many species can survive transient hypoxic stress through mobility leaving the ecosystem relatively unimpacted, long term effects of hypoxic stress can change ecosystems via dramatic decreases in secondary production, which lead to the loss of higher trophic levels as well (Baird et al. 2004, Sturdivant 2011). This loss of production in the faunal community is likely due to the shifting of energy allocation. Short-term metabolic compensation includes anaerobic respiration or reduced metabolic rates, which decreases most activity (Stickle et al. 1989), and leads to the slowing or cessation of feeding (Kapper and Stickle 1987, Forbes and Lopez 1990, Das and Stickle 1993) and reproduction (Wu 2002, Cheung et al. 2008). Hypoxia can additionally act as an endocrine disruptor, further inhibiting reproduction (Wu 2002), or weakening resistance to other stressors such as pesticides and other contaminants (Evans-White and Lamberti 2009) .

Surveys of sublethal DO thresholds for different species have found effects at DO levels in freshwater as high as 10.9 mg L<sup>-1</sup> for cod, but 90% of the surveyed experiments found sublethal effects below 5 mg L<sup>-1</sup> (Vaquer-Sunyer and Duarte 2008). This coincides well with the US EPA freshwater regulations, which accepted a 5 mg L<sup>-1</sup> DO threshold, finding it sufficient to allow only “slight” to “moderate” production impairment for nonsalmonid fauna (Chapman 1986).

### **Florida’s Springs**

Florida has more than 700 named karst aquifer springs, many of which have experienced a shift in primary producers from submerged aquatic vegetation to nuisance filamentous algae blooms. While historic data on gastropod population densities are scarce, data from the Ichetucknee River, an entirely spring fed river in Columbia County FL, indicate that populations of the native gastropod *Elimia spp*, a

long lived pleurocerid snail, (Huryn and Denny 1997, Huryn et al. 2013) have declined dramatically ( $315 \pm 161 \text{ g m}^{-2}$  in 1979 vs.  $63 \pm 70 \text{ g m}^{-2}$  in 2012; DuToit 1979, Liebowitz Chapter 3), and that contemporary populations across numerous systems are generally low (Chapter 2). Recent *in situ* experiments have illustrated that native gastropods can inhibit filamentous algal accumulation in well oxygenated areas, but not in hypoxic sites, due to high mortality (Dormsjo 2008, Liebowitz Chapter 3).

DO levels are generally thought to be constant in springs over months or even years, yet significant declines have been noted on longer time scales (mean across 42 springs of  $3.03 \pm 0.29 \text{ mg DO L}^{-1}$  in 1972 vs.  $2.00 \pm 0.24$  in 2002, with 18 of 42 springs  $< 1 \text{ mg L}^{-1}$ ; Heffernan et al. 2010). Moreover, because of low-frequency water quality monitoring, short-term low DO pulses in response to spring flow reversals as downstream waters flood (Martin and Dean 2001) could easily go undetected. Because *Elimia* in particular are long lived (Huryn et al. 1994), the impacts of even short term lethal events could have long term implications for population recovery and algal reduction via grazing.

We sought to test four linked hypotheses about DO effects on *Elimia* grazing. First, we hypothesized that overall relationships between grazer effectiveness and DO would be positive, with low DO levels diminishing gastropod control of algal biomass. This effect could arise via three mechanisms: decreased survival, decreased per capita grazing rate, and decreased export from bioturbation. Second, we hypothesized that initial algae conditions impact grazer efficiency, with the specific prediction that grazers will be able to reduce more absolute biomass in the high algae conditions, but will be less efficient and unable to reduce the algal biomass to low levels. Third, we

hypothesized that the percent of algae removed is positively associated with gastropod density, with gastropods exerting a stronger overall grazing impact at high densities. Fourth, we hypothesized that pulse hypoxia events can have lasting impacts, with the specific prediction that return of grazing rates will lag behind return of oxic conditions. Together, these hypotheses explore the overarching question of whether changes in DO can contribute, via a trophic cascade, to the changes in algal biomass observed in many springs.

## **Methods**

### **Stream Mesocosms**

Experiments were conducted using nine recirculating stream mesocosms (Fig. 4-1) installed in a greenhouse on the University of Florida campus. The greenhouse received natural, shaded light conditions, with photosynthetically active radiation (PAR) ranging from 0-150  $\mu\text{mol photons m}^{-2} \text{ second}^{-1}$ , depending on weather and time of day. Mesocosms were constructed of 15 cm diameter 100 cm long PVC flumes. Each flume contained approximately 2.5 L of water; experimental flow of approximately 1 L  $\text{min}^{-1}$  resulted in a residence time of 2.5 min. Perforated clear plexiglass partitions were inserted in each flume to separate biomass treatments (longitudinally), maintain snails in the appropriate compartments (grazed vs. ungrazed, lateral), and evenly distribute flow (Fig. 4-1). DO levels were manipulated using four oxygen stripping towers constructed using 15 cm diameter 150 cm tall PVC pipes filled with bio-filter media and capped on both ends. Water was pumped in at the top and  $\text{N}_2$  gas fed in the bottom; this DO stripping method avoids potentially confounding effects on biota associated with other methods (Connelly et al. 2004). Water temperature was maintained between 19 and 23.5 °C, a range typical of the system from which the snails were collected.

Unfiltered spring water was collected from the main spring vent at Blue Springs in Gilchrist County ( $\text{NO}_3^- = 990$  ppb, SRP = 18 ppb), and seeded with an algal slurry sourced from the same location.

Three replicates each of three experimental DO treatments were established ( $\pm 0.25$  mg L<sup>-1</sup>): 1) low, 0.5 mg L<sup>-1</sup>, 2) medium, 1.5 mg L<sup>-1</sup>, 3) high, 5.5 mg L<sup>-1</sup> (YSI 6920 sonde with 6150 ROX optical DO probe, YSI, Inc.). Water was recirculated in a manner that mixed both high- and low-DO waters in Basin 1 before redistributing them (Fig. 4-1) to avoid confounding variation in nutrient availability through the experiment. Water for the high-DO treatment was pumped from Basin 1 to Basin 2, and gravity distributed with flow controlled by valves to the flumes. Water for the low-DO flumes was pumped from Basin 1 into the top of one DO stripping tower, suspended above, and feeding into, Basin 3, which was sitting at the same elevation as Basin 2. Three additional suspended stripping towers drew water from and returned water to Basin 3, allowing a greater quantity of water to be rapidly deoxygenated. Low-DO water from Basin 3 was gravity distributed via valves to the low-DO flumes. Medium-DO flumes received water from Basins 2 and 3, blended using flow control valves to yield water at the target DO level. During the 2-day hypoxia event, all flumes were covered by clear plexiglass lids to inhibit re-aeration; lids were open at the bottom of the flumes to allow venting of excess heat.

### **Grazing Impacts**

Three concurrent biomass treatments were evaluated, using a fractional factorial design using two initial algae biomass levels and two snail densities, which, along with ungrazed controls, were subjected to three DO levels (Fig. 4-1). Biomass treatment 1 (BT 1) was located at the head of the flume, using tiles that had been grown in the

mesocosms for 1.5 months prior to the experiment (mean = 26.6 g m<sup>-2</sup> dry mass). This treatment contained 10 snails per enclosure (approximately 0.5 grams wet weight each), yielding a density of 333 g m<sup>-2</sup> snail biomass on a wet weight bases. The second treatment (BT 2) contained a lower initial algal biomass (mean = 6.0 g m<sup>-2</sup> dry mass, grown for 2 weeks prior to experimental initiation) and the same snail biomass. The third treatment (BT 3) contained the same low algae biomass as BT 2 (mean = 5.8 g m<sup>-2</sup>, 2 weeks of growth) and 10 snails, but double the enclosed area, creating a lower snail density of 167 g m<sup>-2</sup>. Using an empirical conversion for *Elimia* wet:dry weight ( $y = 0.64x - 0.11$ ,  $R^2 = 0.98$ ), we divided gastropod dry mass by algae dry mass to get the following gastropod to algae biomass ratios: BT 1 = 8, BT 2 = 36, BT 3 = 16. At the conclusion of the experiment, total algal export (bioturbation) was collected from the downstream end of each BT 1 section by removing the mesh partition and using tweezers to collect biomass, which was then dried and weighed.

### **Gastropod Behavior and Survival**

Grazing activity, emergence, torpor, and mortality were monitored 2-3 times throughout the day for each treatment. Data are reported as a proportion of individuals exhibiting each behavior (Kolar and Rahel 1993). Snails were considered actively grazing if their tentacles were visible and their head was observed sweeping back and forth across the substrate. Snails breaking the water surface were considered emerging, and those with their operculum closed, or unmoving (but attached to the surface), were considered inert. Inert snails suspected of being dead were noted, and if they did not move from their location in 24 hours, were recorded and removed from the flumes.

## Sample Collection and Processing

Gastropods were collected from Blue Springs in Gilchrist County. *Elimia* were added to the base of the flumes (segregated from algae growth tiles) and acclimated at high DO levels for 24 hours prior to being distributed among the treatments at the start of the experiment on the morning of 13 June 2012. DO was decreased slowly to one of the three experimental levels (0.5, 1.5, and 5.5 mg L<sup>-1</sup>) over a 2 hour period, kept at these levels for 48 hours, and then turned off following tile sampling on 15 June 2012; at that time, DO levels returned to 5.5-6.5 mg L<sup>-1</sup>. One week after the 2-day low DO pulse was ended (on 21 June 2012), the second set of tiles was collected. Each collection involved obtaining one tile from each compartment in each of the three replicate flumes. Tiles were placed in re-sealable bags, kept in the dark and on ice, and processed within 72 hours. Algae biomass was scraped off the tile with a razor, diluted with DI to a volume of 200 mL, subsampled by half to allow successful filtration of high biomass samples, and filtered through pre-weighed Whatman GF/F 0.45 µM filters. Filters were then dried at 60°C for 24 hours and weighed, subtracting the filter weight to obtain the dry mass (DM).

## Data Analysis

Three metrics were used to analyze grazer effects on algae responses: 1) dry mass (DM), 2) percent removal (%R), and 3) removal rate (RR). DM is a direct measure of dry algal biomass, and %R is a measure of grazing efficiency in comparison to the ungrazed control:

$$\%R = (1 - DM_G/DM_U) * 100 \quad (4-1)$$

where DM<sub>G</sub> is algal dry mass in the grazed treatment and DM<sub>U</sub> is algal dry mass in the ungrazed control. Finally, RR denotes snail-specific removal rate, computed as:

$$RR = (\Delta DM_U - \Delta DM_G) / (t * N) \quad (4-2)$$

where  $DM_U$  and  $DM_G$  are as in Eq. 1, but evaluated with respect to initial and final biomass estimates,  $t$  is the number of days between initial and final estimates and  $N$  refers to the number of live snails at the beginning of the time step.

To assess the overall effect of DO levels on algal accumulation, we used factorial ANOVA, specifically evaluating the interactive effects of DO and snail density on algal biomass for each initial biomass treatment and for biomass export at the final time step (21 June 2012). ANOVA was conducted for RR and %R over both time steps (13-15 June and 15-21 June) to assess DO treatment effects following the 2-day hypoxia stress, and also after a week of recovery. The %R variable allows comparison of relative grazer impact across initial algal biomass treatments (Hillebrand 2009). ANOVA was used to assess differential impacts of high versus low initial algae biomass (BT 1 vs. BT 2) and high versus low gastropod biomass (BT 2 vs. BT 3) as a function of DO, and analyzed separately for effects during and following the 2-day induced hypoxia. Pairwise treatment comparisons were evaluated using Tukey's honestly significant differences (HSD) multiple comparison tests. Behavioral data were examined using ANOVA, comparing DO effects on snail grazing, emergence, torpor, and death at the end of the hypoxia pulse (15 June) and the experiment (21 June).

## Results

Dissolved oxygen treatments had a statistically significant effect on all algal response variables (Dry Mass, % Removal, and Removal Rate) within each biomass treatment, with the exception of RR for BT 3 (Table 4-1). Average algal dry mass (on 21 June) was significantly lower in all snail treatments than in snail-free controls. High-DO treatments were significantly lower than medium and low-DO treatments, which were

not significantly different from each other (Fig. 4-3). The same pattern was observed for %R, which was greater in the high-DO treatment than in the medium and low-DO treatments; the latter were not significantly different from each other. Removal rate in the medium-DO treatment was intermediate between high- and low-DO, but was not significantly different from either (Fig. 4-5).

While the low-DO treatment had the highest mortality, snails in both hypoxic treatments had significantly higher mortality rates than in the high DO treatments (Fig. 4-6). Additionally, snails in the hypoxic treatments were more inert, were observed breaking the surface far more frequently, and were observed actively grazing less frequently. DO also significantly affected the per capita grazing rate for BT 1 and BT 2, but not BT 3 (Table 4-1). Mean export rates were higher in the higher DO treatments, but these differences were not statistically significant; the presence of snails was the only factor that significantly affected biomass export (Table 4-1, Fig. 4-4).

Initial algae biomass and gastropod density did not influence the percent removal of algae (Table 4-2). The comparison of BT 1 vs. BT 2 (high initial algae vs. low initial algae, same snail density) showed that BT 2 had a significantly higher percent removal on 6/15, but the two biomass treatments were no longer significantly different by 21 June. The percent of algae removed by high vs. low gastropod density on low initial algal biomass showed no significant differences on either date.

During the two days of hypoxic stress, behavioral metrics of grazer health (active grazing, breaking the water surface, torpor, and mortality) were significantly different between the high DO and both of the hypoxic treatments, though low and medium DO were not significantly different from each other (Fig. 4-6). During the subsequent

recovery week in which high DO was restored to all treatments, some indicators of grazer health and capacity to control algae showed improvement. However, while medium DO treatments recovered (showing significantly higher % grazing, and lower % inert, and lower death rates), low DO treatments did not.

## **Discussion**

### **Impacts of DO on Grazing Potential**

This study supports the hypothesis that DO can strongly influence the accumulation of algal biomass through both lethal and sublethal effects on grazers. In the high-DO treatments of this study, the percent removal of algal biomass approximated global averages, however removal fell far below average in the medium and low-DO treatments. The high-DO treatments ranged from 60-74% removal across all three biomass treatments by 21 June (no significant differences among biomass treatments). Our observation of a consistent percentage of algae biomass removed, despite large differences in gastropod:algae dry mass ratios (ranging from 8 to 36), is congruous with multiple meta-analyses of herbivore removal experiments which found that herbivores removed an average of 59-79% of annual algae biomass, with no significant differences among ecosystem types or productivity gradients (Cyr and Pace 1993, Hillebrand 2009). Notably, however, the medium and low-DO treatments in this study produced much lower removal percentages, ranging from 14-47% removal in medium-DO ( $1.5 \text{ mg L}^{-1}$ ), and 1-15% in low-DO ( $0.5 \text{ mg L}^{-1}$ ) by the end of the experiment. Multiple mechanisms may explain reduced grazing effects in low DO treatments, including snail mortality as well as changes in behavior inhibiting active grazing and bioturbation. This suggests that the native gastropod *Elimia floridensis* exerts typical grazer effects on algal accumulation under high-DO circumstances, but

that these impacts are dramatically reduced to near negligible removal under DO stress. Because gastropods are the dominant invertebrate grazers, and *E. floridensis* is often the dominant gastropod, this provides strong mechanistic evidence that hypoxia limits the potential for native biological control of algal blooms in Florida spring systems.

### **Lethal and Sublethal Mechanisms of Hypoxia**

Multiple concurrent mechanisms appear to explain declining control of algae under hypoxic stress. Direct mortality was a main factor, with 70% mortality in the 0.5 mg L<sup>-1</sup> and 52% mortality in the 1.5 mg L<sup>-1</sup> treatment over the 8-day study. This strongly supports the overarching hypothesis that *Elimia floridensis* grazing pressure is sensitive to hypoxia. We note, however, that 12% mortality in the 5.5 mg L<sup>-1</sup> treatment indicates other unidentified factors that influence survival in these mesocosms. These results are consistent with field experiments that show a strong, but variable effect of DO on mortality (Dormsjo 2008; Liebowitz Chapter 3).

Meta-analyses of lethal and sublethal hypoxia levels indicate that gastropods are among the most hypoxia tolerant taxa, with mean LC<sub>50</sub> levels of 0.89 ± 0.11 mg L<sup>-1</sup> DO (Vaquer-Sunyer and Duarte 2008), with documented 28-day LC<sub>50</sub> values of 0.79 mg L<sup>-1</sup> (Kapper and Stickle 1987), and 0.51 mg L<sup>-1</sup> (Das and Stickle 1993). While these studies employed different methodologies and are therefore not directly comparable, the near 50% mortality at 1.5 mg L<sup>-1</sup> in this study suggests that *Elimia* may be more sensitive than other tested gastropods. However, extrapolation of laboratory DO responses to field behavior can be difficult due to the high variability of DO in the field, as well as compensatory mechanisms for short-term hypoxic exposures (Berg and Ockelmann 1959, Hanley and Ultsch 1999, Garvey 2007).

Sublethal impacts can be subtle and difficult to detect, but have strong effects by reshaping the trophic interactions and energy flows in a system (Breitburg et al. 1997, Evans-White and Lamberti 2009, Sturdivant 2011). The behavioral responses that we observed in response to low DO included snails spending more time at the air-water surface or in torpor, and less time actively grazing. Together, these behavioral adjustments help explain lower per capita grazing rates. *Elimia* exhibited behavioral modifications that reallocate energy from feeding to basic survival strategies, as was seen in the gastropod genus *Nassarius* (Cheung et al. 2008). These strategies may aid in short-term survival, but could eventually lead to lower fecundity and population growth, and indirectly to the accumulation of algal biomass. However, despite behavioral modifications, 48% mortality of *Elimia* in response to a two-day pulse at 1.5 mg L<sup>-1</sup> DO indicates that the threshold for sub-lethal effects must be higher than that.

### **Effects of Grazer:Algae Biomass Ratios**

The effects of contrasting initial biomass ratios were smaller than expected, suggesting that both algal density and grazer density had little impact on potential biomass removal within the ranges we tested. While this result is consistent with other studies that show consistent grazer effects across wide productivity gradients (Feminella and Hawkins 1995, Hillebrand 2009), it may also be due to low biomass accumulation in the flumes overall which limited differentiation among biomass treatments. We did observe that algal percentage removal was higher in BT 2 than BT 1 at the end of the 2-day hypoxia event (15 June; Table 4-2), which likely follows from the higher snail to algae biomass ratio (36 versus 8). However, by 21 June the difference had disappeared. There was no evidence of herbivore escape densities of algae, which would have been manifest as a persistent low percentage of algal biomass grazed in BT

1, where algae biomass started with 27 g m<sup>-2</sup> dry mass. We note, however, that this biomass is well below the EPA “nuisance bloom” threshold of 50 g m<sup>-2</sup> ash free dry mass. As a result, this experiment was unable to test the effects of what would be considered large bloom formations. Also, the effect of high versus lower gastropod biomass was not significant within the range of gastropod densities tested (Table 4-2). While *in situ* experiments generally find positive relationships between snail density and algal consumption, lab experiments often find no effects or negative effects of snail densities on algal consumption. Feminella and Hawkins (1995) speculated that this may be a result of overcrowding in laboratory treatments, though the range of grazer densities used in this laboratory experiment corresponded with the ranges in the *in situ* experiments in Chapter 3, in which higher grazer densities showed higher algal consumption.

### **Recovery and Implications for Florida’s Springs**

The week of recovery period saw modest increases in grazing behavior and less surfacing in the 1.5 mg L<sup>-1</sup> DO treatments, suggesting slight recovery, however the percent of algae removed from each biomass treatment did not increase significantly in the hypoxia treatments after the recovery week. So, although snail behavior showed fewer signs of stress, their capacity to graze efficiently did not fully return. Additionally, the 0.5 mg L<sup>-1</sup> DO treatments did not exhibit signs of recovery, and snails continued to perish, suggesting that even a two-day pulse of severe hypoxia can have lasting population level effects. Due to time constraints, we were unable to run multiple iterations of the hypoxia trial to identify sublethal DO levels between 1.5 and 5.5 mg L<sup>-1</sup>, or cross those levels with varying pulse-durations, therefore further studies of longer

duration will be required to determine environmental DO needs for snail populations to thrive and maintain high grazing capacities.

Table 4-1. ANOVA table of four response variables (DM, %R, RR, and Export) by experimental treatments (DO, Date, Snail presence) for each Biomass Treatment. Significant effects show differences between treatment levels using Tukey's HSD at  $p < 0.05$  for univariate effects, and significant interaction effects are labeled in Figure 4-2 and 4-4.

Response Variable	Effect	F	p	Among factor diff. (Tukey HSD)	F	P	Among factor diff. (Tukey HSD)	F	P	Among factor diff. (Tukey HSD)
		BT 1: High Algae, High Snails			BT 2: Low Algae, High Snails			BT 3: Low Algae, Low Snails		
Algae	Intercept	378.16	<0.0001		556.68	<0.0001		588.49	<0.0001	
Dry Mass	Snails	15.1	0.002	0 > 1	43.42	<0.0001	0 > 1	15.61	<0.0001	0 > 1
	DO	3.56	0.061		13.59	0.001	1,2 > 3	16	0.002	1,2 > 3
	Snails*DO	5.85	0.017		5.55	0.02		3.07	0.084	
% Removed	Intercept	56.15	<0.0001		94.52	<0.0001		19.13	0.001	
	Date	3.29	0.095		2.64	0.13		3	0.109	
	DO	16.72	<0.0001	3 > 2,1	19.83	<0.0001	3 > 2,1	7.48	0.008	3 > 1,2
	Date*DO	4.05	0.045		0.84	0.457		0.44	0.656	
Removal Rate	Intercept	22.45	<0.0001		24.03	<0.0001		4.37	0.059	
	Date	5.57	0.036	1 > 2	8.91	0.011	1 > 2	0.21	0.655	
	DO	4.22	0.041	3 > 1	6.68	0.011	3 > 1	1.44	0.275	
	Date*DO	0.06	0.945		2.9	0.094		1.24	0.325	
Export	Intercept	25.22	<0.0001							
	Snails	7.04	0.021	1 > 0						
	DO	0.61	0.560							
	Snails*DO	0.52	0.609							

Table 4-2. ANOVA comparisons of Percent Removal (%R) by biomass treatments, categorized by date and DO. The column on the left shows high initial algae (BT 1; mean = 26.6 g m<sup>-2</sup> algae DM) vs. low initial algae (BT 2; mean = 5.9 g m<sup>-2</sup> DM algae) during hypoxic stress (15 June) and after a week of recovery (21 June), with high snail biomass (333 g m<sup>-2</sup>) in both treatments. The column on the right compares biomass treatments with high snail biomass (BT 2) versus low snail biomass (BT 3, 167 g m<sup>-2</sup> snails), with low algae biomass initial conditions in both treatments.

Percent Removal (%R)	Effect	F	p	Among factor diff. (Tukey HSD)	F	P	Among factor diff. (Tukey HSD)
		BT 1 vs. BT 2			BT 2 vs. BT 3		
6/15	Intercept	79.9	<0.001		19.5	< 0.001	
	BT	4.9	0.047	2 > 1	3.4	0.09	
	DO	17.2	<0.001	3 > 2, 1	7.2	0.009	3 > 2,1
	BT*DO	2.9	0.092		0.85	0.45	
6/21	Intercept	77.2	<0.001		97.6	<0.001	
	BT	2.3	0.16		3.5	0.09	
	DO	20.5	<0.001	3 > 2 > 1	19.4	<0.001	3 > 2,1
	BT*DO	0.5	0.63		2.0	0.18	

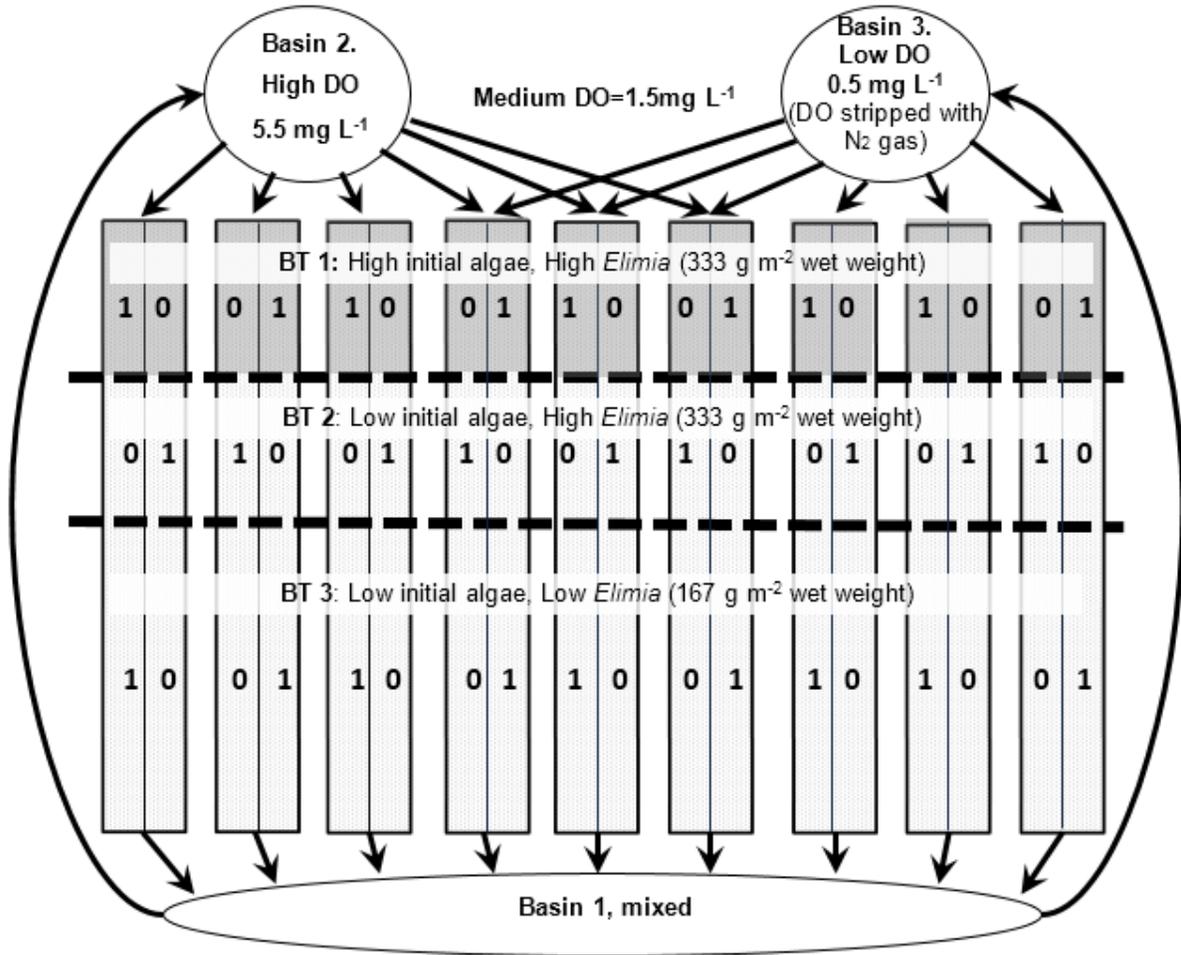


Figure 4-1. Model of the artificial stream mesocosm and experimental design. Each vertical rectangle represents one of nine flumes, each with a vertical partition separating snail treatments (1) from snail-free controls (0). Biomass treatments (BT 1-3) are aligned vertically, separated by mesh barriers indicated by dashed lines. Water flow follows the direction of the arrows and feeds the high-, medium-, and low-DO treatments. The dry biomass of algae was 27 g m<sup>-2</sup> for “high algae” and 6 g m<sup>-2</sup> for “low algae” treatments. The dry biomass ratios of gastropods (converted from the wet weights via empirically derived conversion factor of 0.64x – 0.11 ) to algae for each biomass treatment are: BT 1 = 8, BT 2 = 36, and BT 3 = 18 (see text).

A)



B)



Figure 4-2. Photo of flumes for A) three high-DO treatment replicates, and B) three low-DO treatment replicates on 15 May 2011, showing visual impacts of snails (black dots) versus no snails on algae standing crop. Treatment key is shown in figure 4-1. Note the effects of snails on algal cover (darker brown = more algae) in high DO-treatments, but not in low-DO treatments.

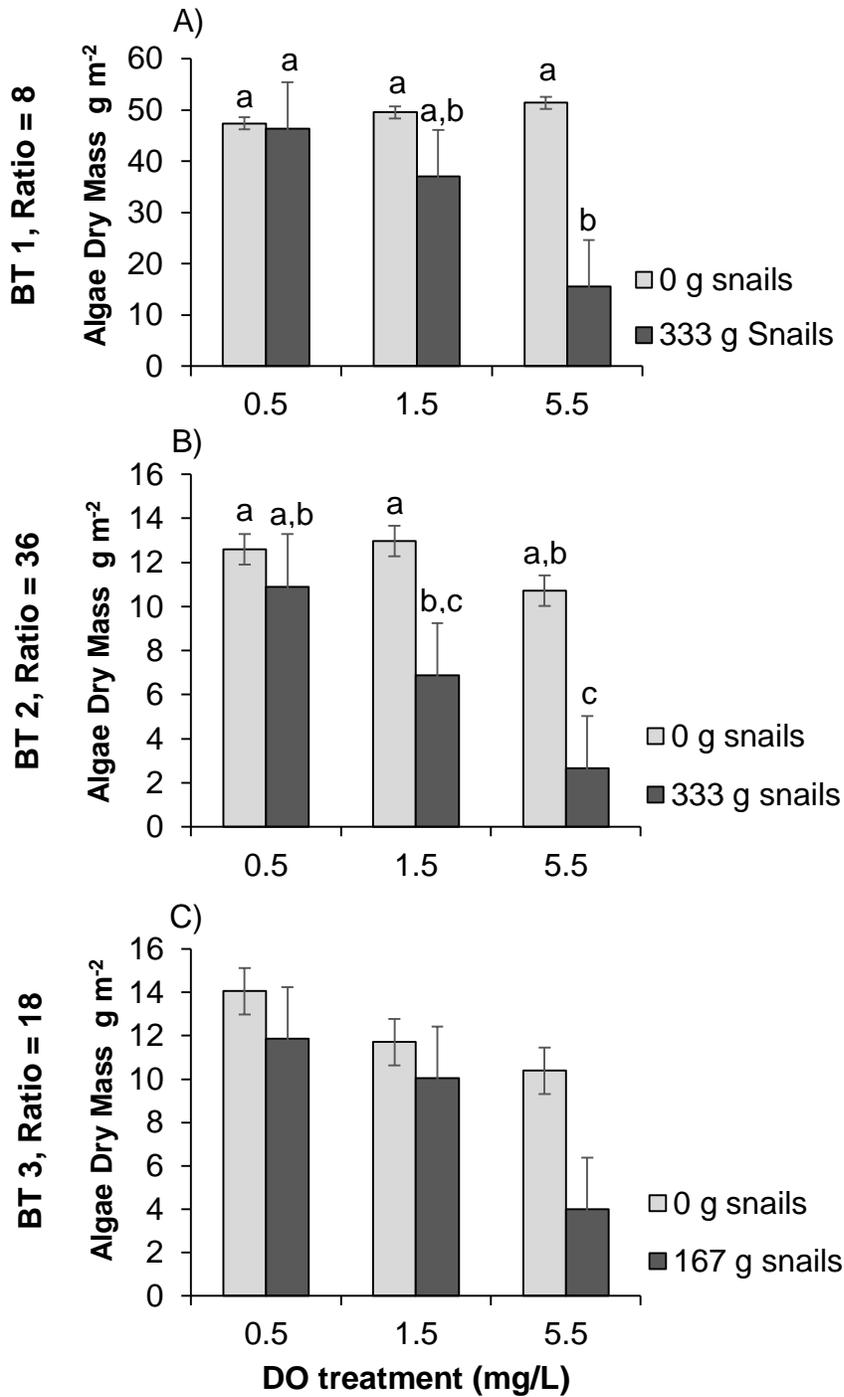


Figure 4-3. Mean algae dry mass ( $\pm$  standard error) categorized by DO treatment and presence (snail biomass in  $\text{g m}^{-2}$ ) or absence of snails for biomass treatments with varying ratios of gastropod:algae biomass. A) BT 1 (biomass ratio = 8), B) BT 2 (biomass ratio = 36), and C) BT 3 (biomass ratio = 18) on the final collection day, 21 June 2012. Significant interactive effects within each biomass treatment were followed by Tukey's HSD ( $p < 0.05$ ) and marked on the graph), and univariate effects per treatment are shown in Table 4-1.

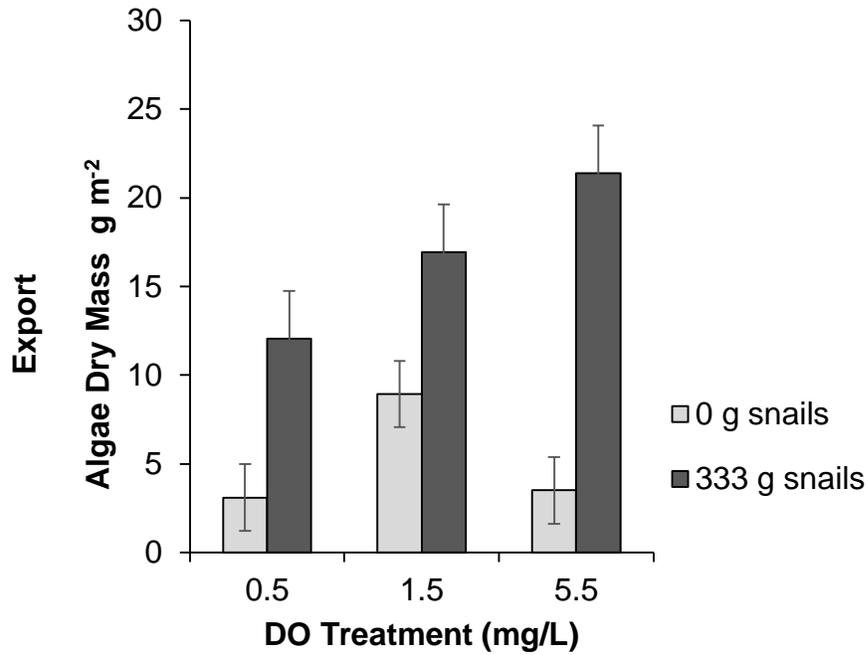


Figure 4-4. Mean algae dry mass ( $\pm$  standard error) categorized by DO and presence (333 g m<sup>-2</sup>) or absence of snails, for biomass export collected downstream of BT 1 on the final collection day, 21 June 2012. Interactive effects were not significant, and univariate effects per treatment are shown in Table 4-1.

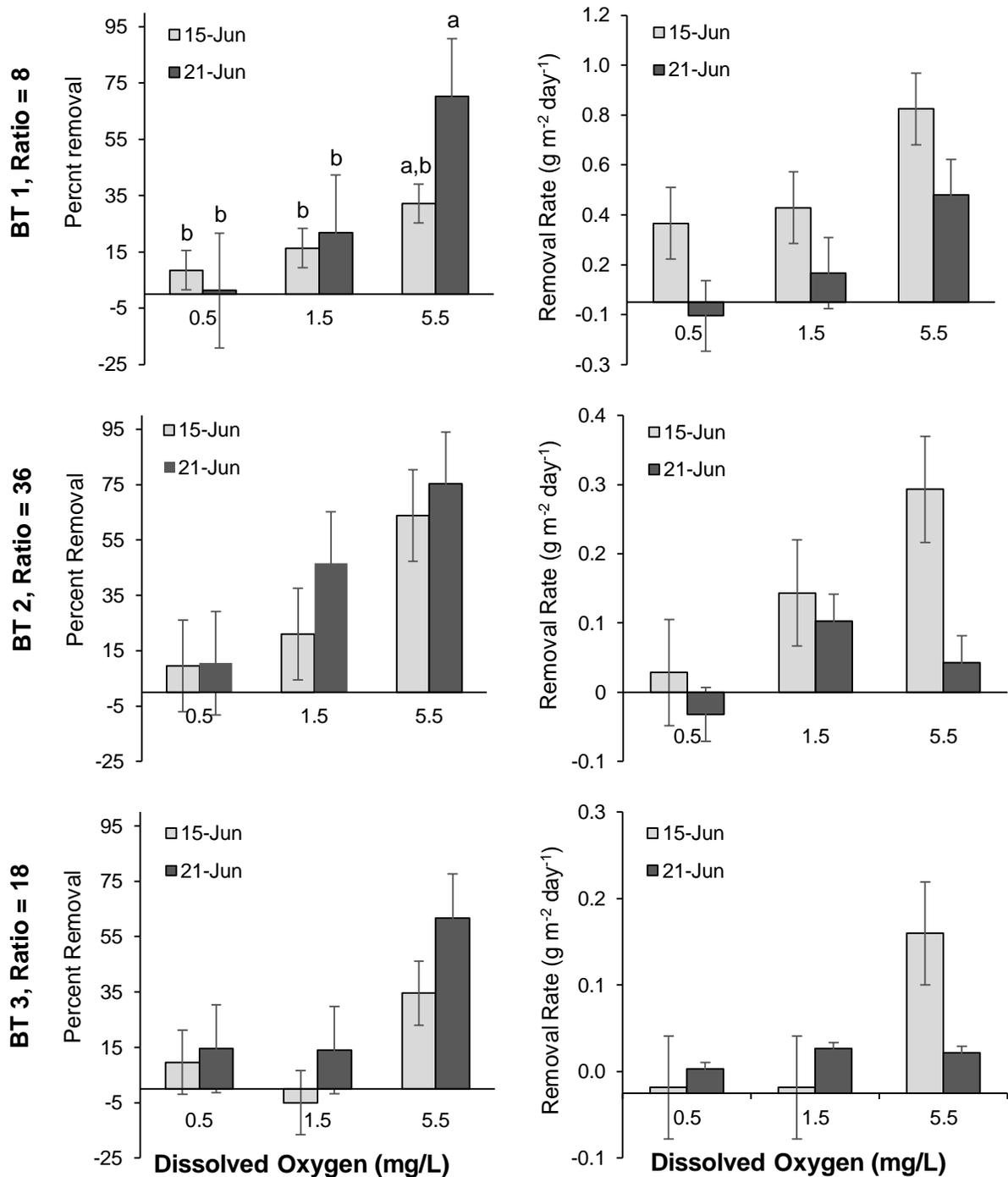


Figure 4-5. Mean Percent Removal and Removal Rate ( $\pm$  SE), separated by Biomass Treatment ((BT), and corresponding gastropod:algae biomass ratio (Ratio)), categorized by collection date (15 June and 21 June 2012) and DO level. Significant interactive effects within each biomass treatment (Tukey's HSD  $p < 0.05$ ) are marked on graph, and univariate effects are shown in Table 4-1.

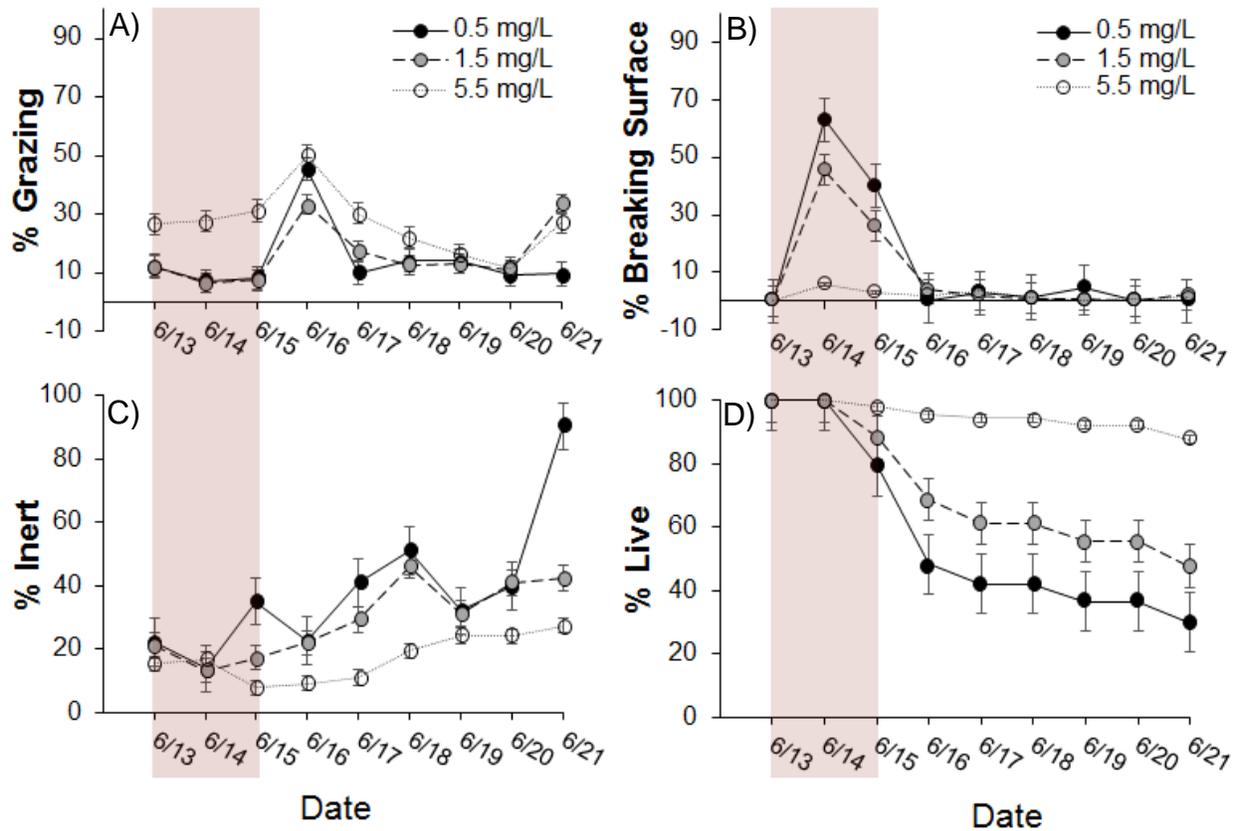


Figure 4-6. Means  $\pm$  standard error for behavioral data averaged by day of the experiment (13-21 June): % Grazing, % Breaking the surface, % Inert, and % Living. ANOVA for DATE (during hypoxia on 15 June, and after a week of recovery on 21 June) by DO treatment for each behavioral variable: A) % Grazing ( $F(2, 128) = 6.9, p = 0.002$ ), B) % Breaking the surface ( $F(2, 128)=11.5, p < 0.001$ ), C) % Inert ( $F(2, 128)=5.8, p=.004$ ), and D) % Live ( $F(2, 128)=10.2, p < 0.01$ ). Grey bar indicates duration of hypoxia treatments.

## CHAPTER 5 CONCLUSION

### **Grazer Biomass Thresholds and Alternative States**

This study provides multiple lines of evidence to support the hypothesis that high densities of grazers have the potential to inhibit algal blooms in Florida's springs, but that they are less able to reduce algal biomass once a bloom has formed. The observational field study found a potential grazer biomass threshold at about 30 g m<sup>-2</sup> of wet weight of gastropods, below which algal biomass was uniformly high, and above which algae could be high or low; above 428 g m<sup>-2</sup> gastropod wet weight, algae were uniformly low. This is a wider set of thresholds than were found in the *in situ* studies, in which ~100 g m<sup>-2</sup> of *Elimia* were needed to maintain low algal biomass when starting from initially low algae conditions, and > 330 g m<sup>-2</sup> *Elimia* were necessary to reduce a pre-existing algal bloom. Although there is an apparent discrepancy in the threshold values, it is common for surveys and experimental findings to arrive at different values, potentially due to differences in temporal scale, historical contingencies, and the myriad interactions that occur in the field, but which are reduced in controlled experiments (Leibold et al. 1997).

The bimodal residuals from the field surveys, along with the different grazer densities needed to decrease algae in high versus low algal conditions in the *in situ* experiments, suggest the presence of an herbivore escape density and alternative states. While true alternative stable states are difficult to demonstrate definitively (Schroder et al. 2005), these two lines of evidence build a strong case that the system is hysteretic at the least. The implication for restoration is that even if gastropod populations were restored to high levels, areas with high algal standing stocks may not

revert to low-algal states unless the system is reset in some manner, making the system resistant to switching back to an herbivore dominated state. Additionally, as there are few locations that currently have the high levels of gastropods that demonstrated control on algal accumulation, the causes of grazer declines must be well understood before attempting biological restorations.

### **Gastropod Conservation**

Although most attention to the global biodiversity extinction crisis focuses on tropical ecosystems or megafauna, declines in less charismatic groups such as gastropods can have large impacts. Florida's springs are specifically listed as hotspots of global hydrobiid diversity, with 84 species of hydrobiids (43 of which are endemic), as well more than 100 species of the genus *Elimia* (Brown et al. 2008a, Strong et al. 2008), a genus which appears to be particularly important in controlling algal proliferation in Florida's springs (Chapter 2). Gastropod populations can be a dominant invertebrate presence in springs and streams of the southeastern US, but their populations are declining severely throughout the region (Lydeard et al. 2004, Brown et al. 2008a). There is little documented historic data about the abundance or declines of gastropods in Florida, however declining populations in Ichetucknee Springs (DuToit 1979) and anecdotal reports from biologists and park managers suggest that Florida is experiencing similar trends to those noted throughout the region. Therefore, causes of gastropod declines should be discerned and addressed.

As there have been noted changes in DO levels in the springs, with many sites dipping below  $1 \text{ mg L}^{-1}$  (Heffernan et al. 2010b), this study tested the effects of hypoxia on *Elimia* mortality and grazing rates to see how such changes may impact the ecosystem. We found that, while there was a weak correlation between gastropods and

DO concentrations in the field, our experimental studies showed high mortality and reduced grazing under hypoxic conditions. The weak relationships in the field may have been due in part to the fact that DO is not constant; surveys such as these necessarily must rely on snapshots of environmental conditions, which may miss pulse events that can restructure ecosystems but leave no trace of the stressor itself. Additionally, sublethal effects can cause asynchrony in the biomass signal in response to DO stress, making trends difficult to discern. Gastropods have higher tolerance for hypoxia than most invertebrates, with  $LC_{50}$  values as low as  $0.5 \text{ mg L}^{-1}$  (Vaquer-Sunyer and Duarte 2008), however we found severe mortality and sublethal effects at and below  $1.5 \text{ mg L}^{-1}$ . This makes DO an important factor for management attention.

Hypoxia explains a portion of gastropod distributions in the field, yet additional factors are likely at play. The multivariate models found a variety of expected factors predicting gastropod distribution, such as light and flow, but there still remained a measure of unexplained variability. Though beyond the realm of this study, we suggest that the emerging body of work in the field of aquatic contaminants such as fungicides, pesticides, pharmaceuticals, and industrial pollutants (Fleeger et al. 2003, Phelps et al. 2006, Brown et al. 2008b, Evans-White and Lamberti 2009, McMahon et al. 2012) may help explain regional gastropod declines and constitute an important focus for future research. Additionally, the habitat metrics of flow and sediment regimes have been shown to severely impact gastropod distribution (Stewart and Garcia 2002), as have invasive species (Riley et al. 2008) and salinity (James et al. 2003, Ramakrishnan 2007). All these factors together help define the habitat needs of gastropod populations,

to allow them to thrive and maintain their roles as powerful drivers of healthy ecosystems.

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## BIOGRAPHICAL SKETCH

Dina Liebowitz graduated from the University of Pennsylvania with a B.S. in Biology and Environmental Studies, and a thesis exploring land-use and fire patterns in Bolivia. After spending a year volunteering with human rights and environmental NGOs in Israel, she worked as a field biology coordinator for Harvard University's Department of Organismic and Evolutionary Biology. In 2007 she completed an M.S. in wildlife ecology and conservation from the University of Florida, with a concentration in tropical conservation and development, focusing on marine resource use patterns and stakeholder assessments for marine protected areas in the Bahamas. During that time, she had the opportunity to spend five weeks in a Pacific Islands with a field training course exploring community based natural resource management in the Solomon Islands. She was an NSF IGERT Fellow in the Adaptive Management of Water, Wetlands, and Watersheds program during her doctoral training, and now plans to combine her interdisciplinary experiences to work towards the goals of natural resource management and conservation.