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

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ABSTRACT

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

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

INTRODUCTION

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

Hydrobiids are prosobranch gastropods, occur worldwide in fresh and brackish water (Mladenka &

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

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

Cyanobacteria such as *Lyngbya* are generally considered to be nutritionally inadequate food sources for

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

Vaucheria sp. belongs to the Xanthophyceae, a group of yellow-green algae that reproduce asexually and sexually (via zoospores). In Florida, *Vaucheria* sp.

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

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

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



FIGURE 1

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

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

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

MATERIALS & METHODS

Study Organism

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

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

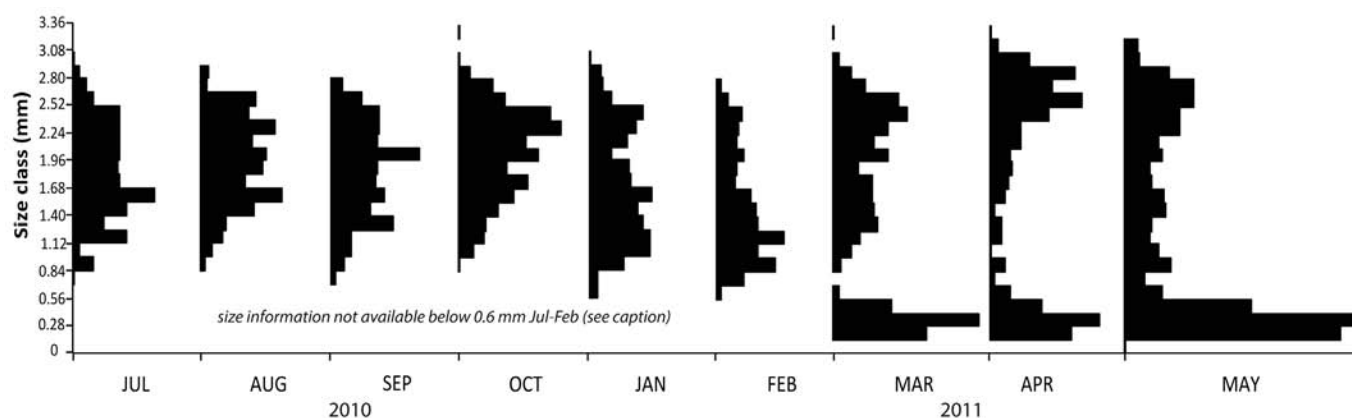


FIGURE 2

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

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

The treatments (n=12 for each treatment) consisted of various diets of filamentous algae that occur naturally in Blue Spring 1) *Vaucheria*, which served as a non-cyanobacteria control, 2) *Lyngbya*, a cyanobacteria, or 3) a mixture of *Vaucheria* and *Lyngbya*. Containers were assigned to groups using a randomized block design. Before the study began, snails were fed *Lyngbya* sp. *ad libitum* for three weeks and then starved for 48 h. *Lyngbya* sp. was used for the initial stage of the experiment because it was easily

grown in large amounts. Water was replaced every three weeks, and limestone rocks were autoclaved and containers replaced every six weeks to limit bacterial and algal growth in the containers. The study was terminated after four months due to snail reproduction; all data shown here are from measurements taken prior to first reproduction.

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

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

equal carbon content was added weekly to each treatment.

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

Although there were no significant differences in initial sizes among treatments (Fig. 3), we used relative

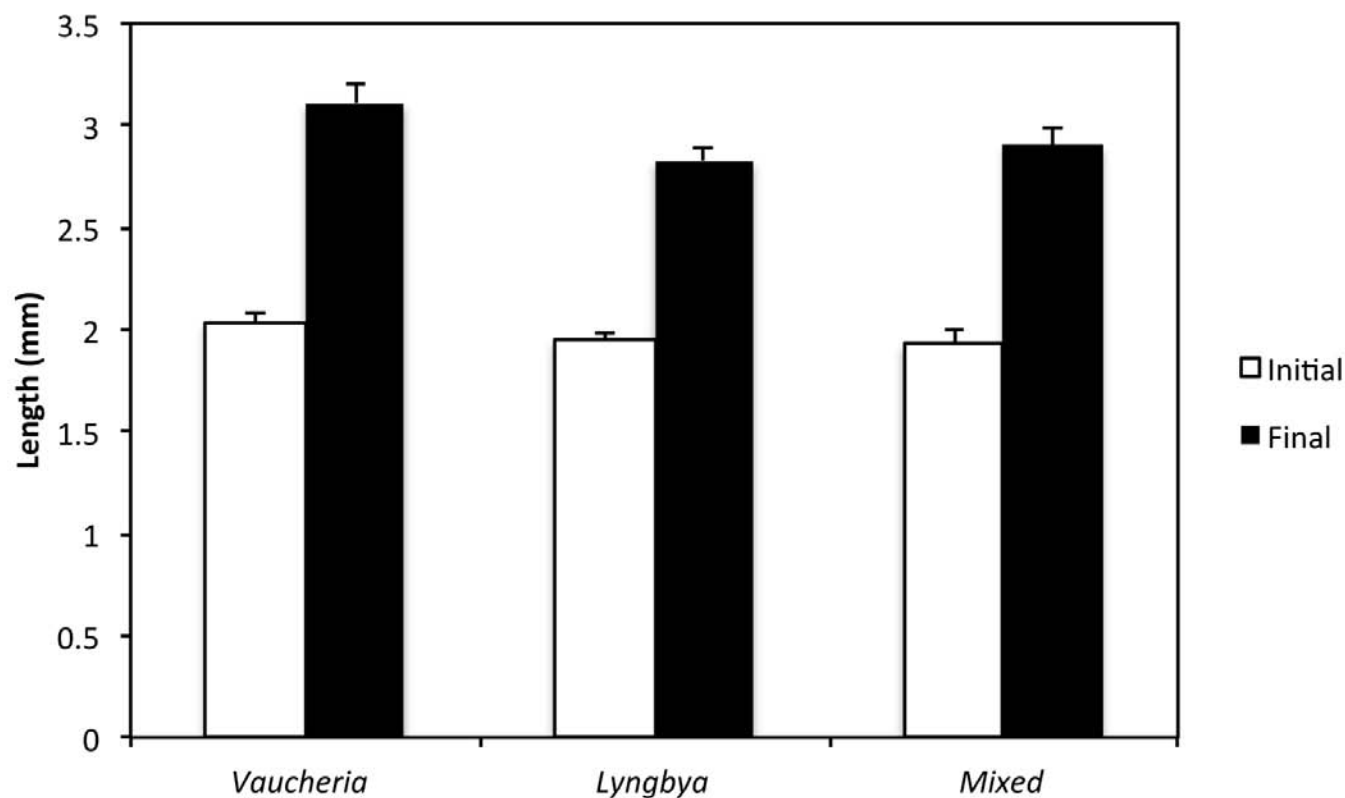


FIGURE 3

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

growth rate to account for differences in size. Relative growth was calculated as:

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

where t = duration of experiment in days

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

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

RESULTS

Snails in all treatments increased in size over the course of the study (Fig. 3). There were no significant differences in initial ($p = 0.23$) or final absolute length

($p=0.052$) across treatments. However, snails in the *Lyngbya* treatment had the lowest overall absolute growth rate ($K = 0.011$, 0.008 , and 0.01 mm/d for *Vaucheria*, *Lyngbya* and mixed diets respectively). Also, at the end of the study, the mean relative growth rate of snails fed *Lyngbya* or *Vaucheria* was lower than snails fed the mixed diet ($p = 0.0002$; Fig. 4). Mortality was very low overall and did not differ significantly across treatments (0.88% , 1.2% , 0.86% , for *Vaucheria*, *Lyngbya*, and mixed diet, respectively).

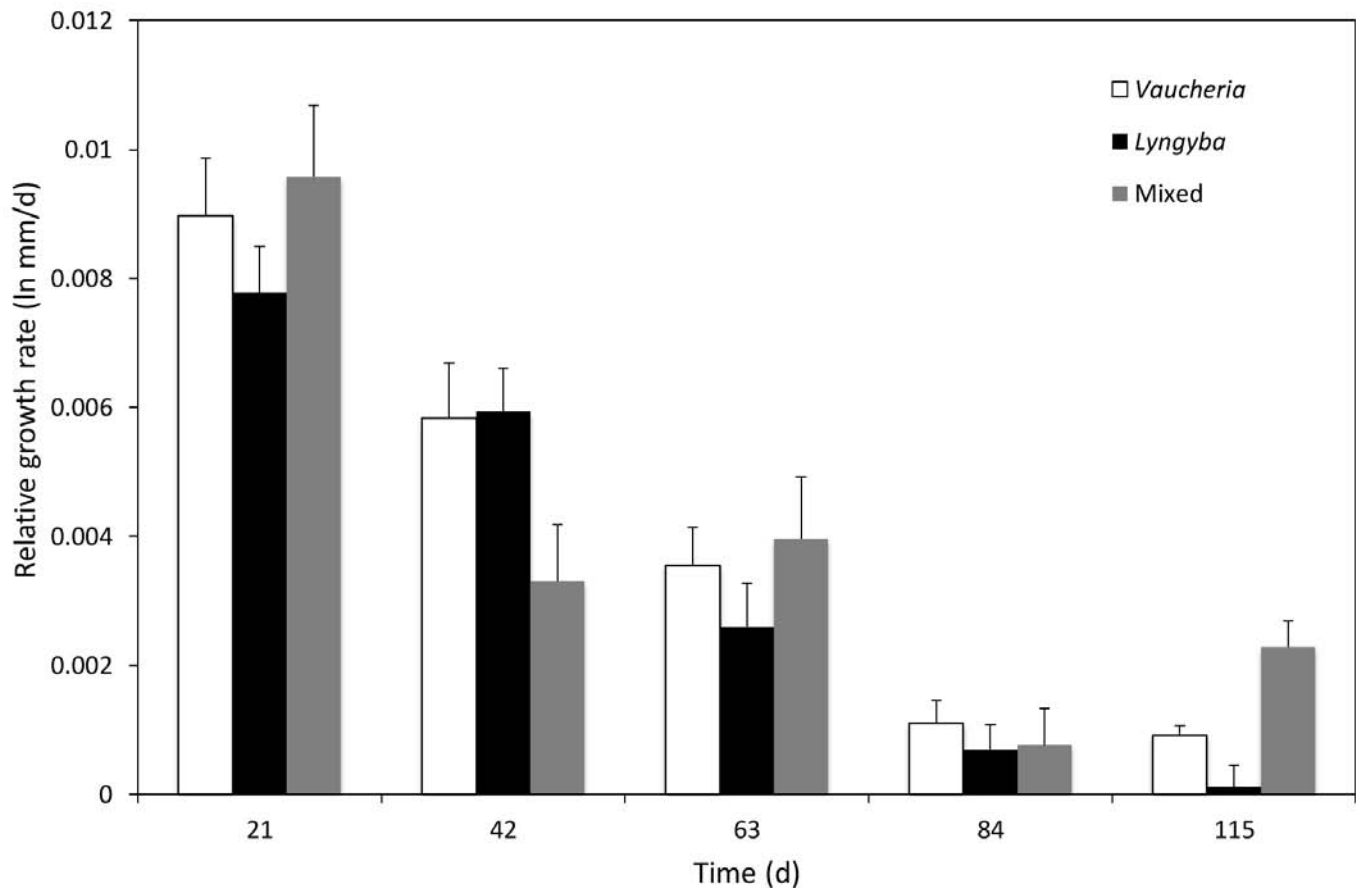


FIGURE 4

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

DISCUSSION

Consistent with our hypothesis, a uni-algal diet of the cyanobacteria *Lyngbya* sp. or the yellow green *Vaucheria* sp. negatively affected relative growth rate of *Floridobia floridana* compared to the mixed diet. This effect was only seen during the final study period, when snails fed a mixed diet continued to grow and those on the uni-algal diet either did not grow (*Lyngbya*) or grew at a reduced rate (*Vaucheria* sp.) The mixed diet may have provided additional nutrients absent in a diet of *Vaucheria* or *Lyngbya* alone. The negative effects of

uni-algal diets are well-documented: the growth rate of *Villosa iris* was highest when fed two green algae and a diatom as compared to a uni-algal diet (Gatenby *et al.*, 1997). Similarly, post-settlement growth of *Dreissena polymorpha* was higher on a mixed diet of four algal species compared to one species of cyanobacteria (Wacker & von Elert, 2002). Growth rates of the marine snail *Turbo sarmaticus* were also highest when fed a mixture of three marine algae (Foster *et al.*, 1999). However, is it interesting that in our study a mixed diet that contained cyanobacteria would lead to higher relative growth rate,

as cyanobacteria, particularly *Lyngbya* (see below), are expected to provide little additional nutrition (Brett *et al.*, 1997; but see Schmidt & Jónasdóttir, 1997).

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

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

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

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

In contrast with our predictions, mortality was similar across all treatments. The occurrence of mortality may indicate that none of the diets we provided were

of particularly high quality; studies of other freshwater gastropods exposed to cyanobacteria displayed no mortality during an 8-wk study period (Lance *et al.*, 2007; 2008).

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

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

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

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CLEARANCE RATES OF *VILLOSA IRIS* (BIVALVIA: UNIONIDAE) FED DIFFERENT RATIONS OF THE ALGA *NEOCHLORIS OLEOABUNDANS*

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ABSTRACT

We investigated effects of algal cell concentration and mussel size (shell length) on the clearance rate (CR) of the rainbow mussel, *Villosa iris*. Mussel were either batch-fed a single ration of algae for 24h, or were fed three different algal rations that were replenished every hour for 3 hours. Mean CR of *V. iris* batch fed a single ration (1.3×10^6 c/mL, 8.8 mg/L) of algae (*Neochloris oleoabundans*) decreased with time and the concomitant decline in cell concentration, but never reached zero. As length increased, so did clearance rate ($p < 0.0001$). Pseudofeces were produced by all individuals in the first three hours of feeding, and were irregularly produced as algal cell concentration dropped later in the test.

Mussels fed the lowest ration (0.34 mg dry wt/L) maintained elevated CRs over time with no production of pseudofeces; CR of mussels fed the middle ration (1.02 mg dry wt/L) decreased with time, and produced pseudofeces – intermittently. CR's of mussels fed the high ration (3.4 mg/L) increased with time, and produced a large amount of pseudofeces throughout the test. Following the premise that the optimum ration yields greatest net particle ingestion without incurring sorting costs of pseudofecal production, we estimated that *V. iris* would require 2.8 mg dry wt of algae (4.2×10^8 cells of *N. oleoabundans*) on a daily basis, based upon CR's measured for the middle ration in this study.

KEY WORDS Freshwater mussels, clearance rate, algal ratio

INTRODUCTION

Freshwater mussels of the Unionacea are among the most widespread bivalves (Banarescu, 1990). Where mussels are present, they often comprise a significant proportion of the benthic biomass (Strayer et al., 1994; Newton et al., 2011), and play important roles in particle removal, nutrient cycling, and in structuring benthic species assemblages in lakes and streams (Howard & Cuffey, 2006; Vaughn & Spooner, 2006; Atkinson et al., 2010; Allen et al., 2012). Thus, their decline may be adversely affecting aquatic ecosystem integrity. To improve our understanding of the effects of suspension-feeding populations of freshwater mussels on aquatic ecosystems, we need quantitative information on various feeding processes. Additionally, conservation efforts to restore populations through propagation and culture require a better understanding of freshwater mussels' feeding physiology and the effect of particle concentration on feeding rates in order to develop cost-effective feeding regimes that meet the animals metabolic demands.

The bulk of information on bivalve feeding physiology has been collected on commercially significant marine bivalves (clams, mussels, and oysters). In marine bivalves, clearance rate (CR) generally increases with increasing particle concentration (number of cells or dry weight of cells per unit volume) to a maximum and then progressively declines (Foster-Smith, 1975; Bayne et al., 1976; Riisgard, 1991); thereby, regulating particle retention rate and the amount of material available for ingestion (Winter, 1978; Navarro & Winter, 1982; Navarro et al., 1992) as well as avoiding excessive pseudofecal production and energy costs associated with sorting (Jorgensen, 1990). Paterson (1984) observed this pattern in the freshwater mussel, *Elliptio complanata*, as did Roper and Hickey (1995) in *Hyridella menziesi*. Pusch et al. (2001) and Vanderploeg et al. (1995) found that the natural seston concentrations in their studies, however, did not saturate the clearance rate capacity of their unionid mussels. Bricelj and Malouf (1984) suggested that a bivalve's success in maximizing its energy gain in a turbid environment depends on a combination of two features: a high selection efficiency pre-ingestion which may prevent significant loss of nutritious food material in pseudofeces (Kiorboe & Mohlenberg, 1981), and producing copious amounts of pseudofeces to reject bulk excess or irritating material and to preferentially reject undesirable particles to improve quality of material ingested. These bivalves would be better adapted to cope with high suspended loads than other species, which control ingestion mainly by reducing clearance rate (Winter, 1970; Foster-Smith, 1975). Indeed, Ward and MacDonald (1996) showed that some bivalve species demonstrate high plasticity in how they respond to a broad range of suspended particle concentrations, by

maintaining both high and low CR, high pseudofecal production, and utilizing pre-ingestion selection capabilities to select for desired food items otherwise diluted by non-nutritive material in turbid environments. They suggested that the ability of a species to compensate for increased suspended particle concentrations depends on the capacity adaptations of the species. For example, species that typically reside in low turbid environments were unable to compensate for increased particle concentrations and demonstrated high mortality and poor growth (Cranford & Gordon, 1992). Gascho Landis et al. (2013) recently reported that high suspended particle concentrations resulted in reproductive failure for freshwater mussels. Indeed, researchers have looked at several factors known to affect clearance rate in bivalves, such as flow rate, temperature, particle size and concentration, body size and reproductive phase (Kryger & Riisgard, 1988; Tankersley & Dimock, 1993; McCall et al., 1995; Vanderploeg et al., 1995; Spooner & Vaughn, 2008). Clearance rates of freshwater mussels exposed to a variety of particle concentrations, however, needs further examination.

The rainbow mussel, *Villosa iris*, is a small-sized mussel (< 70 mm) commonly found in small rivers in riffle-glide environments, and has a wide distribution in the St. Lawrence, upper Mississippi, Ohio, Tennessee and Cumberland River basins. It is bradyctictic (long-term brooder), which generally spawns in late summer. Gravid females hold their glochidia (larvae) over the winter in their marsupial gill area until spring when the glochidia are released to encyst on a suitable host-fish, where metamorphosis into a juvenile mussel is completed. While the conservation status of the *V. iris* is presently of no concern, the Tennessee River system contains a significant number of endangered freshwater mussel species. Many of these endangered species also are small-sized, long-term brooders that inhabit similar environments as *V. iris*. Differences in feeding physiology and the ability of a suspension-feeder to adapt to changes in seston concentrations may contribute to niche partitioning within a bed of mussels (Vanden Byllaardt, 2011), and may explain why one species is imperiled and the other is not within the same drainage. Nevertheless, until empirical data on feeding requirements of endangered species are available, we propose to use data from this study of *V. iris* as a guide for the development of captive care protocols for endangered *Villosa sp.* and *Epioblasma sp.* of freshwater mussels. Our objectives were to evaluate the clearance rate of *V. iris* over 24 h from a single batch-feeding, investigate the effect of algal cell concentration (ration) on clearance rate, and estimate the algal cell concentration to feed mussels on a daily basis that could meet their presumed energy balance in captivity.

METHODS

Clearance rates of *V. iris* were measured in two experiments. In the first experiment (Single Ration Test, SRT), mussels were fed a single ration of algae, and clearance rates (mL/h) and algal cell concentrations (c/mL) were monitored for 24 h. In the second experiment (Multiple Ration Test, MRT), clearance rates (mL/h/g dry tissue weight (dtw) of our standard-sized mussel) were measured for mussels fed one of three algal rations; these rations were maintained for three 1-h feeding periods. Calculation of clearance rates is described later in this paper. Although clearance rate, filtration rate, and pumping rate are sometimes used interchangeably, they measure different physiological functions. According to Bayne et al. (1993) clearance rate is the "rate at which water pumped by the animal is cleared of particulate matter by filtration (mL/h)"; filtration rate is the "rate at which seston or particles are removed from suspension (mg/h)"; pumping rate (mL/h) is the total volume of water that is pumped through the gills and is usually higher than the CR. In this paper we determined the clearance rate of *V. iris* from the clearance of suspended material according to Coughlan (1969).

Mussel acclimation and algae culture

Twelve male *V. iris* (shell length 37-52 mm, mean $\pm$ SD = 43.4 ± 3.8 mm) were collected from Copper Creek, Scott Co., Virginia, U.S.A. in June, 1997 for use in the single ration test. We collected 30 male mussels (shell length 37-51 mm) in February, 1998 for the multiple ration test. We measured shell length from the anterior to posterior ends of the shells. Mussels were transported to the laboratory in 10L of aerated river water in a cooler. Mussels were then acclimated from field temperatures (17°C in summer and 12°C in winter) at $1.2^\circ\text{C}\cdot\text{h}^{-1}$ to laboratory temperatures of 20°C. Mussels collected in the summer (SRT) were acclimated overnight at ambient temperatures of 20°C; they also were batch fed 1×10^6 c/mL (6.8 mg dry wt/L) of *Neochloris oleoabundans* and allowed to feed for 24h. Mussels collected for the MRT were collected at river temperatures of 11°C. After a 3h transport to the laboratory, temperatures in the cooler of mussels reached 14°C. Mussels then were held individually in 250 mL containers without food, and acclimated from 14 to 20°C at $1.2^\circ\text{C}\cdot\text{h}^{-1}$ for 5 h to the new temperature regime.

We selected the green alga *N. oleoabundans* for this study because it was shown to be suitable for rearing mussels (Gatenby et al., 1997; Patterson, 1998). Algae were grown in *Neochloris* media (Gatenby et al., 1997) under continuous white fluorescent light (photon flux: $35 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) at $20 \pm 1^\circ\text{C}$. Some mussels clear particle sizes of 5-10 μm more efficiently than smaller particles ($< 5 \mu\text{m}$) (Paterson, 1986; Miura & Yamashiro, 1990; Tankersley & Dimock, 1993). We harvested *N. oleoabundans*

during log phase growth when the algae ranged 5-10 μm (average ca. 6.2 μm) in diameter. Ten 100 mL aliquots of algae were dried for 8 h at 90°C to calculate dry weight.

Experimental procedure

Single ration test

Following acclimation, mussels were transferred to individual aerated chambers containing 250 mL (pH 8.0) of a 1:1 mix of well water and dechlorinated city water. They were then batch fed 1.3×10^6 c/mL (8.8 mg dry wt/L) of *N. oleoabundans*. We collected 10 mL water samples after 1, 2, 3, 5, 8, and 24 h, and reduction in particle concentration was determined. Algal cells were not replenished after each sampling interval, simulating an aquaculture practice of feeding mussels only once per day. We determined particle concentration at each sampling using a Coulter Counter, Model ZM, aperture 100 μm . The production of feces and pseudofeces was visually observed and noted for each mussel during the first 8 h of the test.

Clearance rate calculation

Clearance rates (CR) in mL/h for the single ration test were calculated using the following equation (Coughlan 1969):

$$\text{CR} = \{V \cdot (n \cdot t)^{-1}\} \cdot [\ln(\text{conco} \cdot [\text{concf}]^{-1})]$$

where V = volume (mL) of feeding chamber, n = 1 mussel in each feeding chamber, t = duration of sampling interval, and conco and concf are cell concentration at the beginning and end of each sampling interval, respectively.

Because we intended to return these test animals to the creek, we did not calculate CR on a dry weight basis.

Statistical analyses

Clearance rate data were log-transformed prior to analysis to stabilize the variance. We used repeated measures analysis of variance (rmANOVA) to examine clearance rates over time (Proc Mixed, SAS version 8.2, SAS Institute, Cary, North Carolina), with shell length included as a covariate. This allowed us to determine if there was an effect of mussel size on CR, while also examining the CR trend in time. The least squares (LS) means for clearance rates (i.e., the CR means adjusted for mussel size) were compared.

Multiple ration test

In preliminary tests, we observed decreased feeding activity (reduced valve gape, closed apertures) with increased time in the laboratory. We began the multiple ration test, therefore, immediately upon acclimating the mussels to laboratory temperatures. Following acclimation, mussels were transferred to individual aerated chambers containing 250 mL (pH 8.0) of a 1:1 mix of well

water and dechlorinated city water. Ten individuals (per ration) were fed one of three rations (cell concentration) of *N. oleoabundans*: ration A was 5.0×10^4 c/mL (0.34 mg dry wt/L), ration B was 1.5×10^5 c/mL (1.02 mg dry wt/L), and ration C was 5.0×10^5 c/mL (3.4 mg dry wt/L). The experiment lasted 3 h and was initiated when each mussel exhibited shell gape and apertures were visible. Particle concentrations were measured at the end of each 1 h period using a Coulter Counter (Model ZM), and any algal cells that were cleared were replaced. The production of feces and pseudofeces was not quantified by dry weight; however production of pseudofeces and feces were visually observed and noted for each mussel.

Clearance rate calculation

In the multiple ration test, we calculated hourly weight-specific clearance rates as mL/h/dry tissue weight of standard mussel used in this test (see CR calculations below); hereafter, referred to as mL/h/g_{std}. All clearance rates were adjusted for animal weights using an allometric relationship because the magnitude of most physiological responses is dependent on the size of the animal. Thus, clearance rate was expressed in terms of the average weight of the animal used in this experiment to avoid extrapolating to sizes not adequately represented in our investigation (Kreeger & Newell, 2001). This allometric relationship between size and clearance rate was derived by least squares regression of the log_e-transformed dry tissue weight and the loge-transformed raw clearance rates. All mussels were randomly assigned to each treatment. We assumed that there was equal distribution of mussel sizes in each treatment as was represented in the total sample size. The average dry tissue weight for all of the mussels was 0.289 g, and this was considered to be the standard animal weight. The calculation of CR, therefore, was as follows:

$$CR = \text{antilog} \left[(\ln CR_{\text{raw}}) + \{b \times ((\ln dtw_{\text{std mussel}}) - (\ln dtw_{\text{rep}}))\} \right]$$

where Cr_{raw} = raw clearance rates (mL/h); b = the allometric weight exponent (0.854), which was generated by regression of the pooled CR and size data used in the MRT; $dtw_{\text{std}} = 0.289$ g, mean dry tissue weight of standard-sized mussel (standard-sized mussel = mean dry weight of 30 mussels used in the MRT); dtw_{rep} = dry tissue weight (g) of replicate mussel. Three algae-only controls demonstrated that no algae settled out of suspension ($p < 0.01$).

Statistical analyses

We compared mussel clearance rates between the 3 hourly feeding periods within each treatment and among treatments. First, we re-examined the relationship between shell length (ranging 37–51 mm) and clearance rate, expressed as both mL/h and mL/h/g_{std} (0.289 g standard-sized mussel), using simple linear regression. We examined whether the effect of shell length on clearance rate was sufficiently removed when clearance rates were

expressed on a per weight basis (for all rations combined). Having determined that the size effect was removed, we analyzed the effect of algal ration (cell concentration) and time on CR that were weight-corrected by allometry (see CR Calculations; $CR = \text{mL/h/g}_{\text{std}}$). We used a two-factor repeated measures analysis of variance (two-way rmANOVA) to determine the time (within subjects) and treatment (between subjects) effects on Ln-transformed CR data (Proc Mixed, SAS, version 8.2, SAS Institute, Cary, NC). The Tukey-Kramer honestly significant difference (HSD) multiple comparison test was used to identify treatment differences within time periods. In order to estimate the amount of algae to feed mussels on a daily basis, the effect of algal ration on total amount of organic material cleared in 3 hours was evaluated using One-way ANOVA followed by Duncan's Multiple Range Test (DMRT) that tested for between treatment differences. The number of algal cells was log-transformed to stabilize the variance.

RESULTS

Single ration test

Repeated measures analysis of variance (rmANOVA) showed that clearance rates decreased with time ($p = 0.0001$) (Figure 1); there also was a significant covariate effect of shell length on clearance rate ($p = 0.0001$), indicating that the relationship between CR and time was not independent of shell length. Further examination of the adjusted (LS) means indicated a significant non-linear relationship for CR with time ($p = 0.001$). This relationship is described by the following equation (Figure 1; the predicted points from the relationship are represented by Ps and the actual observed values are represented by Os.):

$$LS_CR = \beta_1 + \beta_2 / \beta_3 + \text{time}$$

where $\beta_1 = 2.78$, $\beta_2 = 1.63$, and $\beta_3 = 0.33$.

Thus, CR slowed down with time and the concomitant decline in cell concentration, but never reached zero (Figure 1). The covariate estimate for length was 0.1042 ($p < 0.0001$), indicating that as length increased, however, so did clearance rate. For example, mean algae concentration had dropped over ten-fold in the chambers with the three largest mussels (54–57 mm), and the concentration dropped seven-fold in the chambers holding the smaller-sized (37–44 mm) mussels. Pseudofeces were produced by all individuals in the first three hours of feeding, and were irregularly produced as algal cell concentration dropped later in the test. Clearance rates at 24 h ranged 18.2 – 24.8 mL/h and approximately 6.5 mg dry wt of algae was cleared in 24 h.

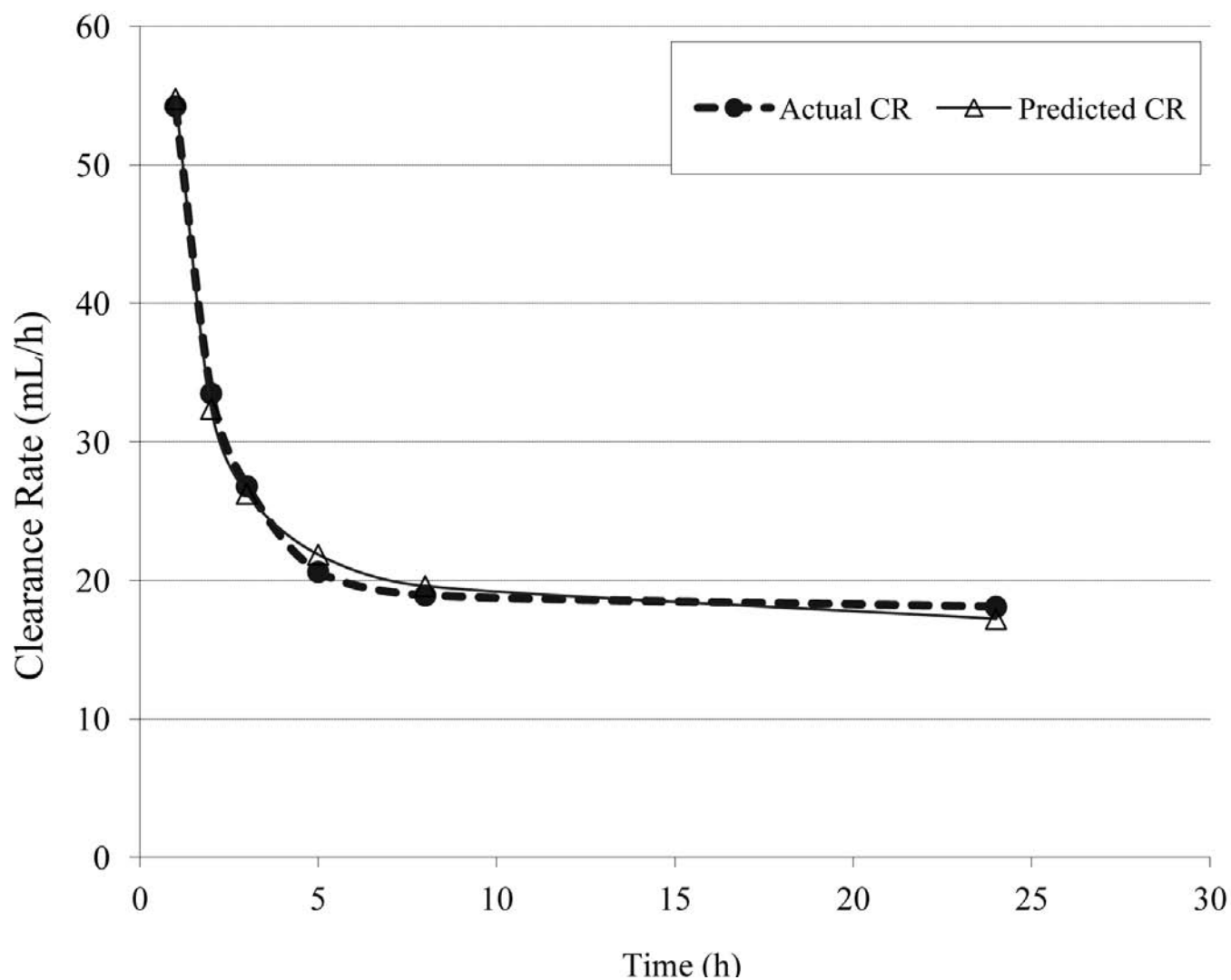


FIGURE 1

Change in clearance rate over time for *Villosa iris* fed a single ration (1.3×10^6 algal c/mL) of algae. This relationship is described by the following equation:

$$LS_CR = \beta_1 + \frac{\beta_2}{\beta_3 + time};$$

the predicted points from the relationship are represented by Δ 's and the actual observed values are represented by •'s.

Multiple ration test

Linear regressions showed that clearance rates expressed as mL/h were dependent on shell length ($p = 0.012$); however, clearance rates that were weight corrected by allometry (expressed as mL/h/g_{std}) showed no significant relationship with shell length ($p = 0.448$). The effect of length on clearance rate was, therefore, removed when clearance rates were expressed on a weight-specific basis, and subsequent analyses were performed on these values.

The rmANOVA showed that there was a significant interaction effect of treatment with feed hour (time) ($p=0.0035$), which then precluded the main effects of either factor alone. In other words, the main effect of time was not independent of the main effect of treatment. So, we looked at the interaction of the two rather than the effect of either alone.

The Tukey-Kramer HSD indicated that in hours 1 and 2 there was a significant difference between ration C and rations A and B (Table 1). Three hours after initiation there was a difference between ration A and B, but there was no longer a difference between rations B and C, nor between A and C. Mussels fed ration A maintained elevated clearance rates through time. Mean clearance rate of mussels fed ration B significantly decreased with time, and mean clearance rate of *V. iris* fed ration C significantly increased with time. In addition, the total number of cells cleared by *V. iris* during the entire 3h feeding period was significantly different among treatments (Table 2; $p<0.05$). Mussels fed the highest ration (C) cleared the greatest amount of algae followed by mussels fed ration B and then ration A. A greater percentage of the available algae, however, was cleared from ration A than from rations B and C (Table 2). All mussels in ration C produced pseudofeces during all 3 feeding times. We did not observe production of pseudofeces by mussels fed ration A; however, we observed intermittent production of pseudofeces in ration B, which also varied between mussels during the 3 h test. In other words, not all mussels in ration B produced pseudofeces all the time. We estimated an average ingestion rate during the 3 h period at 0.05 mg/h for Ration A and 0.15 mg/h for Ration B based upon CR and total number of cells removed from suspension. We could not estimate ingestion rate for Ration C because we did not quantify pseudofeces.

DISCUSSION

Bivalve clearance rates are a function of physiological and environmental factors including gill type, body size, body condition, temperature, current speed, particle size, particle type and concentration (Winter, 1970, 1978; Walne, 1972; Way et al., 1989; Vanderploeg

et al., 1995; Spooner & Vaughn, 2008). The interplay between clearance rate and ingestion also depends on the digestibility and nutritional value of the diet. For example, in Willows' (1992) model for optimal digestive investment, energy costs for particle removal, sorting, and digestion are balanced by the energy gained from the food type. He cautions that at low concentrations of food, energetic costs of filtration and digestion become a significant component of the overall energy budget, and will determine the upper limit for sustained clearance rate. At very high particle concentrations, bivalve clearance rates may decline which would reduce excessive pseudofecal production and energy costs associated with sorting (Jorgensen 1990). Indeed, suspension-feeding marine bivalves have been shown to be highly adaptive with the ability to regulate clearance rates within a range of suspended particle concentrations (Ward & MacDonald, 1996). Our work with *V. iris* suggests that freshwater mussels are equally as adaptive as their marine counterparts.

Single ration test

Villosa iris fed a dense suspension of algae (1.3 x 10⁶ c/mL, 8.8 mg/L), produced an abundance of pseudofeces within the first hour, with pseudofecal production declining over time with the decrease in algal cell concentration. *Villosa iris* initially cleared at a high rate, lowered clearance rate by over 56 % within 2 h, and between 8 and 24 h, clearance rates appeared to level off presumably to maintain particle ingestion rate. Greater clearance during the first feeding hour may have reflected an empty gut, with all or most food from the previous acclimation period having been assimilated or passed through the gut. Higgins (1980) reported an increase in clearance rate when unfed oysters were re-introduced to food. The provision of food to starved *Mytilus edulis* also stimulated an increase in filtration and O₂ consumption, followed by a reduced clearance rate upon satiation of the digestive system (Thompson & Bayne, 1974; Bayne et al., 1976; Riisgard, 1991). Alternatively, *V. iris* may have lowered its clearance rate to avoid excessive sorting costs as demonstrated by other suspension feeding bivalves.

Multiple ration test

Very low food concentrations can lead to shell closure, reduced clearance rate, and reduced metabolism (Riisgard & Randlov, 1981; Jorgensen et al., 1986). Indeed, several species of marine bivalves have been shown to reduce their feeding activity in the laboratory when particle concentrations dropped by 50% of initial concentration (Bricelj & Malouf, 1984; Higgins, 1980; Navarro & Winter, 1982). In ration A, *V. iris* maintained elevated CRs such that on average, 59% of the particles were removed each hour. The moderate decrease in CR

in the third feeding hour could indicate that feeding activity was altered in response to the particle concentration dropping by over 50% of the starting concentration. It is unclear whether these elevated CRs (mean CR=145.5 mL/h/g) at low rations would be maintained beyond 3 h as high CR's have added energy costs and could result in lower scope for growth. We estimated that mussels in ration A ingested an average of 0.05 mg/h.

In ration B, we suspect that *V. iris* maximally cleared (160.2 mL/h/g) in the first hour also as a result of food deprivation during acclimation, which was followed by a decrease in clearance rate presumably to regulate particle ingestion in accordance with the replenished ration. This relationship between clearance rate and ingestion rate following gut satiation is common among bivalves (Hornbach et al. 1984; Way, 1989; Riisgard, 1991). Virtually no pseudofeces were produced by mussels fed Ration B; therefore, we estimated their average ingestion rate during the 3 h period at 0.15 mg/h, which presumably yielded a positive and balanced energy rate.

Paradoxically, clearance rates of *V. iris* fed at the highest concentration (3.4 mg/L) increased over time. Copious amounts of pseudofeces were produced over time, but they were not quantified. It is plausible that *V. iris* regulated ingestion following the strategy proposed by Bricelj and Malouf (1984) when exposed to high particle concentrations, by increasing clearance rate and pseudofecal production. Thompson and Bayne (1972) concluded that *M. edulis* suffered nutritive stress when feeding for long periods in high concentrations of suspended particles. *Villosa iris* was fed on a highly nutritious diet; thus, it is unclear whether they would suffer nutritive stress over a longer period of high ration maintenance (of a nutritious diet). The energetic gains or losses of this ration would depend on the costs of maintaining high CR and high pseudofecal production over a long period.

Winter (1978) suggested that the greatest cell density found to not produce pseudofeces was the optimum food concentration at which the costs of filtration activity were reduced to a low-energy consuming level and all algal cells cleared were ingested. For *V. iris*, this "pseudofeces threshold" appeared to be near 1.02 mg/L of *N. oleoabundans* where only a small amount of pseudofeces was observed. We estimated the average ingestion rate during the 3 h period for mussels at 0.15 mg/h (or 3.6 mg dry wt per 24h). Thus, a lower CR combined with more energy (mg dry wt of organic material) gained (cleared) by mussels fed 1.02 mg/L than mussels fed 0.34 mg/L presumably yielded the better energy investment. We believe that ration C is suboptimal to the lower rations because mussels could have greater energetic costs associated with high CR, sorting and pseudofeces

production. As well, the continuous production of pseudofeces implied an excess of food was provided, and this would not be cost-effective for a hatchery. Similarly, mussels fed the single ration (8.8 mg dry wt/L) cleared approximately 6.5 mg dry wt of algae in 24 h, but they too produced an abundance of pseudofeces early on and then intermittently throughout the test. We assumed that excess algae allocated to the production of pseudofeces were not ingested. Therefore, 6.5 mg dry wt of algae in 24 h would exceed that which is necessary to support this mussel's condition in the lab, and not economical for a hatchery.

We concluded that 1.02 mg/L of *N. oleoabundans* would best balance the energy needs of *V. iris* in a laboratory-setting. At the hourly CR's measured for the middle ration in this study, *V. iris* would require 3.6 mg dry wt of algae (4.2 x 10⁸ cells of *N. oleoabundans*) on a daily basis. These results were consistent with latter findings on the effect of particle concentration on the carbon budget (unpublished data), and our estimates of total particles ingested based upon four components that we found accounted for all of the ¹⁴C activity ingested by mussels. In our study of the C-budget, ingestion went down with increasing cell concentration, and we hypothesized that excess material removed from suspension and directed to the production of pseudofeces was not ingested.

Besides animal size and species, differences in CR of suspension-feeding bivalves also have been attributed to the quality of the test diet (Newell & Jordan, 1983; Kreeger & Newell, 2001). Kryger and Riisgard (1988) suggested that algal diets are higher in quality than natural seston quality. We compared our results with results for other freshwater mussels regardless of whether seston, laboratory algae, or bacteria was the food source (Table 3). Because food quality is known to affect CR, we only included studies that had an organic component in their "test diets", such as algae or natural seston. We extrapolated CR data reported as mL/g/min to mL/g/h and data reported as mL/h we converted to mL/g/h if the bivalve dry tissue weight was reported. As expected, *V. iris* fed at any algal ration had a total mean CR greater than another small-sized mussel, *Toxolasma texasense* fed bacteria at 1-2 x 10⁷ c/mL. The CR of *V. iris* fed algae was similar, however, to the CR of larger-sized (>80 mm) mussels, *Anodonta anatina* and *Unio tumidus*, fed a high concentration of seston (Table 3). In addition, *A. anatina*, *U. crassus*, *U. pictorum*, *U. tumidus* and *Lampsilis siliquoidea* fed algae showed CRs ten-fold greater than similar sized mussels fed natural seston, and nearly ten-fold greater than the *V. iris* fed algae in this study (Patterson, 1984; Kryger & Riisgard, 1988; Vanderploeg et al., 1995; Pusch et al., 2001) (Table 3). We attributed these differences in CR to differences in diet quality (animals feed at a higher rate on

higher quality food), the size and physiological status of the animal, and the environmental tolerances of the species (Silverman et al., 1997). Clearance rates in bivalves can be underestimated due to experimental conditions that do not simulate the habitat of infaunal bivalves, and methodology used to quantify CR (Riisgard, 2001). Thus, the clearance rates reported here could very well be different than that which would be observed under natural conditions. The complex relationships between diet quality, ration, mussel species, physiological or reproductive status and CR needs further examination in order to understand the effect that suspension-feeding mussels have on aquatic ecosystems, and in order to design a feeding regime that is appropriate to the nutritional needs of a suite of freshwater mussel species.

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TABLE 1

Mean hourly clearance rates ($\text{mL}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$) of *Villosa iris* for 3 feeding periods and at 3 algae rations: Ration A = 5×10^4 c.mL⁻¹, Ration B = 1.5×10^5 c.mL⁻¹, and Ration C = 5.0×10^5 c.mL⁻¹. Mean CR + SD (of mean LN CR) in columns (Ration) followed by the same letter are not statistically different by Tukey-Kramer HSD; means within feed hours followed by the same upper case letter also are not significantly different by Tukey-Kramer HSD, $\alpha = 0.05$.

Time Interval (h)	Ration A	Ration B	Ration C
1	108.6 ± 0.9 a, A	160.2 ± 0.5 a, A	29.9 ± 1.4 a, B
2	181.1 ± 0.4 a, A	143.4 ± 0.6 a, A	56.6 ± 0.6 a, b, B
3	146.9 ± 1.2 a, A	42.4 ± 1.8 b, B	86.2 ± 0.9 b, A, B

TABLE 2

Mean total algal cells (mg dry wt) cleared, and the percent of available algae cleared by *Villosa iris* at three different algal rations during all three feeding hours. Algal rations: Ration A = 5×10^4 c/mL, Ration B = 1.5×10^5 c/mL, and Ration C = 5.0×10^5 c/mL. Means + SD (LN cells cleared) within a column followed by the same letter were not significantly different ($\alpha = 0.05$).

Ration	Mean Algal Cells (mg/h) Cleared per mussel	Mean Percent of Total Available Algae Cleared
A	7.3×10^6 cells ± 0.38 (0.05 mg/h)	59.2%
B	2.2×10^7 cells ± 0.43 (0.15 mg/h)	54.9%
C	3.9×10^7 cells ± 0.57 (0.26 mg/h)	30.7 %

TABLE 3Comparison of clearance rates (CR) from *Villosa iris* (this study) with those of other unionid mussels fed organic diets.

Species	Food Source	Ration	CR (mL h ⁻¹ g ⁻¹)	Bivalve Dry wt (g)	References
<i>Anodonta anatina</i>	<i>Chlorella vulgaris</i>	1.2 x 10 ⁴ c/mL	827-923	3.141	1
<i>Unio crassus</i>	<i>C. vulgaris</i>	1.2 x 10 ⁴ c/mL	1233-1532	2.7	1
<i>Unio pictorum</i>	<i>C. vulgaris</i>	1.2 x 10 ⁴ c/mL	1060-1525	3.017	1
<i>Unio tumidus</i>	<i>C. vulgaris</i>	1.2 x 10 ⁴ c/mL	875	2.4	1
<i>Anodonta anatina</i>	seston	8-19 mg/L	170-620	3.5	2
<i>Unio tumidus</i>	seston	8-19 mg/L	150-581	2.5	2
<i>Villosa iris</i>	<i>N. oleoabundans</i>	5.0 x 10 ⁴ c/mL (0.34 mg/L)	181.1	0.289	3
<i>Villosa iris</i>	<i>N. oleoabundans</i>	1.5 x 10 ⁵ c/mL (1.02 mg/L)	143.3	0.289	3
<i>Villosa iris</i>	<i>N. oleoabundans</i>	5.0 x 10 ⁵ c/mL (3.4 mg/L)	56.6	0.289	3
<i>Villosa iris</i>	<i>N. oleoabundans</i>	1.3 x 10 ⁶ c/mL	18.2 – 24.8 mL/h	na	3
<i>Villosa lienosa</i>	bacteria	1-2 x 10 ⁷ c/mL	393.6	0.913	4
<i>Toxolasma texasense</i>	bacteria	1-2 x 10 ⁷ c/mL	33.8	0.454	4
<i>Cyclonaias tuberculata</i>	bacteria	1-2 x 10 ⁷ c/mL	1150	0.67	4
<i>Lampsilis ovata</i>	bacteria	1-2 x 10 ⁷ c/mL	354	1.180	4
<i>Elliptio dilatata</i>	bacteria	1-2 x 10 ⁷ c/mL	459.6	1.080	4
<i>Elliptio complanata</i>	5 µm beads, lake water	1 x 10 ³ c/mL	ca. 300	6-7cm (length)	5
<i>Elliptio complanata</i>	2.02 µm beads in 0.45 µm filtered seston	1-3 mg/L	10.9 mL/h	na	6
<i>Lampsilis siliquioidea</i>	<i>Chlamydomonas</i> sp.	2.5 mm ³ /L	1450	1.5-2.1	7
<i>Actinonaias ligamentina</i>	green and diatom algal mix	89.7 mg C/L	ca. 3*		8*
<i>Lampsilis cardium</i>	green and diatom algal mix	89.7 mg C/L	ca. 4		8
<i>Truncilla truncata</i>	green and diatom algal mix	89.7 mg C/L	ca. 8		8
<i>Quadrula pustulosa</i>	green and diatom algal mix	89.7 mg C/L	ca. 4		8
<i>Amblema plicata</i>	green and diatom algal mix	89.7 mg C/L	ca. 3		8
<i>Fusconaia flava</i>	green and diatom algal mix	89.7 mg C/L	ca. 3		8
<i>Megalonaias nervosa</i>	green and diatom algal mix	89.7 mg C/L	ca. 1		8
<i>Obliquaria reflexa</i>	green and diatom algal mix	89.7 mg C/L	ca. 4		8

(1) Kryger & Riisgard (1988).

(3) This study.

(5) Paterson (1984).

(7) Vanderploeg et al. (1995).

(2) Pusck et al. (2001).

(4) Silverman et al. (1995 and 1997).

(6) Leff et al. (1990).

(8) Spooner & Vaughn (2008).

*Clearance rates of mussels acclimated to 25°C were reported in bar graphs; thus, we approximated CR values based on unit scales in graphs

EFFECT OF SMALL DAMS ON FRESHWATER MUSSEL POPULATION GENETICS IN TWO SOUTHEASTERN USA STREAMS

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ABSTRACT

The global imperilment of freshwater mussels is strongly linked to widespread habitat destruction by dams, but more subtle mechanisms by which dams impact mussels are not well studied. For example, dams fragment populations in free-flowing reaches, potentially leading to low survival probability due to genetic effects, but few studies have addressed the genetic effects of fragmentation on mussel populations. We examined patterns of genetic variation in the mitochondrial CO1 and ND1 genes in populations of two mussel species that were fragmented by >175 y old small dams. We found that only a few rare haplotypes were restricted to reaches either upstream or downstream of the dams, and an array of genetic parameters showed little differentiation among upstream and downstream reaches. These results can be interpreted in one of two ways. First, gene flow across these dams may remain high, resulting in little genetic fragmentation. Alternatively, the apparent lack of population differentiation could be a historical artifact of high, pre-dam gene flow, and the genetic markers we used may not yet reflect relatively recent population isolation.

KEY WORDS stream; dam; conservation; biodiversity; invertebrates

INTRODUCTION

Dams are responsible for many freshwater mussel population declines and extinctions (Williams et al., 1992; Neves et al., 1997; Vaughn & Taylor, 1999). Streams are impacted by dams through alterations in habitat, modifications in river hydrology and temperature, and blocked migration routes of host fishes (Waters, 1996; Lessard & Hayes, 2003; Graf, 2006). These effects may reduce freshwater mussel distribution, egg fertilization, infection of host fishes, and juvenile settlement particularly downstream of large dams (Fisher & LaVoy, 1972; Layzer & Madison, 1995; Moles & Layzer, 2008). Small dams (<5 m) also have negative effects, but mussel density, species richness, and growth rates can be higher immediately downstream of small dams compared to other parts of some watersheds (Gangloff et al., 2011; Singer & Gangloff, 2011). Dam age and height, stream physiochemistry, and watershed land use may be key factors responsible for the observed benefits of these small dams (Gangloff et al., 2011). In contrast to the effects of dams on physical stream habitats, more subtle effects such as genetic population fragmentation are not well known for mussels.

Genetic evidence for population fragmentation by dams has been documented in highly mobile fishes such as white-spotted charr (*Salvelinus leucomaenis*) and bull trout (*Salvelinus confluentus*) (Neraas & Spruell, 2001; Yamamoto et al., 2004) and in less mobile fishes such as logperch darters (*Percina caprodes*; Haponski et al., 2007). Conversely, populations of other fishes, including greenside darters (*Etheostoma blennioides*) and black redhorse (*Moxostoma duquesnei*), as well as crayfishes, showed no genetic differentiation between populations upstream and downstream from dams (Haponski et al., 2007; Reid et al., 2008; Hartfield, 2010). The few studies of mussel genetic population structure provide similarly mixed results, but none show evidence of reduced gene flow or isolation by dams. Populations of several mussel species showed little or no detectable genetic population structure despite the presence of dams that separate these populations (Berg et al., 1998; Grobler et al., 2006; Szumowski et al., 2012), but other populations show evidence of significant structure apparently unrelated to recent dam effects (Hughes et al., 2004; Elderkin et al., 2008; Grobler et al., 2011). These studies suggest that dam-induced genetic effects on aquatic

organisms are highly situation-specific and may depend on factors such as dam porosity, the number and proximity of dams within a river system, and the mobility and life history of species. In addition, because mussels are dependent on fish hosts and host fish use varies among species, genetic structure of mussel populations is highly influenced by differences in mobility and life history traits among fish species.

In this study, we examined the genetic structure of *Elliptio arca* and *Elliptio complanata* populations located upstream and downstream from two >175 y old small dams that impound short reaches of the streams (< 2 km). *Elliptio arca* is endemic to the Mobile Basin and has declined substantially, making it a species of high conservation concern (Mirarchi et al., 2004). Primary fish hosts of *E. arca* are darters (Haag & Warren, 2003). Darters are known for their low mobility that can be greatly reduced or blocked by stream barriers (Warren & Pardew, 1998; Schaefer et al., 2003). *Elliptio complanata* is widespread in Atlantic slope drainages and is considered stable (Williams et al., 1993). *Elliptio complanata* is reported to use members of the Centrarchidae, Percidae, and Fundulidae as host fishes, but recent evidence suggests that American eels (*Anguilla rostrata*) may also be an im-

portant host (Lellis, 2001; Cummings & Watters, 2004). Juvenile eels are able to climb wet dam faces directly, and adults can move short distances over-land, allowing them to circumvent stream obstructions such as dams (Sorensen & Bianchini, 1986; Tesch, 2003). We predicted that if the dams are acting as barriers for these two mussel species and their host fishes then we should find unique haplotypes restricted to reaches either upstream or downstream from the dams, low gene flow values, population structuring values that suggest no interbreeding, and statistically significant genetic differentiation values.

METHODS AND MATERIALS

We studied genetic diversity in *Elliptio arca* (Alabama Spike) in Sandy Creek, a third-order tributary of the Tallapoosa River (Mobile River Basin), in east-central Alabama, and *Elliptio complanata* (Eastern Elliptio) in the upper Tar River (Pamlico River Basin), a fourth-order stream in north-central North Carolina (Fig. 1). Both of the study streams drain largely forested catchments in rural, sparsely-populated sections of the southern Appalachian Piedmont and are fragmented by historic mill dams (height <5 m), which impound short reaches of the streams (<2 km).

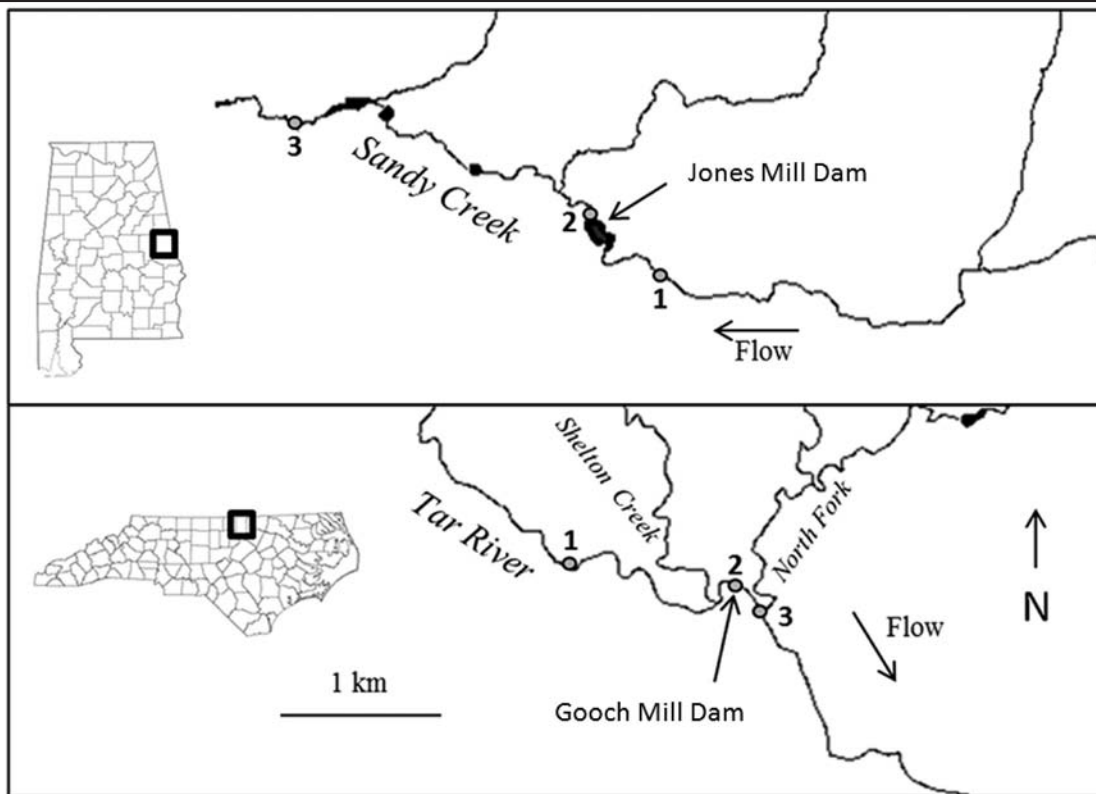


FIGURE 1

Mussel collection sites on Sandy Creek, Chambers Co., Alabama, and the Tar River, Granville Co., North Carolina. On both streams, site 1 is upstream of the mill dam impoundment, site 2 is immediately downstream of the dam, and site 3 is 0.5 km (Sandy Creek) or 5.0 km (Tar River) downstream of the dam. On Sandy Creek, the darkened area in the stream channel upstream of site 2 represents the mill dam impoundment; darkened areas downstream of site 2 are artifacts of the map and do not represent impoundments.

Sandy Creek is impounded by Jones Mill Dam (c. 1836) in Chambers County, Alabama, and the Tar River is impounded by Gooch Mill Dam (c. 1797) in Granville County, North Carolina. Both dams are structurally intact and do not have obvious routes for upstream fish passage. The sluiceways that powered the millworks of both dams are now sediment-filled and have not been operational for at least 50 y. Water moves over the top of these dams, except in periods of low flow, and seasonal high flow events create substantial flow over the dams. We collected mussels in free-flowing reaches upstream, immediately downstream, and farther downstream of the dams (Fig. 1). Large populations of the study species exist in all of these reaches (Gangloff et al., 2011; McCormick, 2012).

We excised fresh tissue (adductor muscle) in the lab and stored it in TE buffer in a -20°C freezer. We sampled adductor muscle to reduce the possibility of sampling male mitotypes, because unionid reproductive tissues can exhibit doubly uniparental mtDNA inheritance (Breton et al., 2007). Any male mitotypes that were sequenced were omitted from the data set. DNA was extracted using a Qiagen DNeasy® kit and animal tissue extraction protocol and stored in a -20°C freezer. We examined fragments of the mitochondrial NADH dehydrogenase 1 (ND1) and cytochrome c oxidase 1 (CO1) genes. These markers were chosen due to their widespread use in freshwater mussel phylogenetic and phylogeographic studies (Serb & Lydeard, 2003; Campbell et al., 2005, 2008; Elderkin et al., 2008).

Approximately 600 base pairs of the CO1 gene and 700 base pairs of the mitochondrial ND1 gene were amplified with polymerase chain reaction (PCR) using available primers (Serb et al., 2003; Campbell et al., 2008). PCR product was then sent to Retrogen, Inc. (San Diego, CA) for sequencing with an ABI 3730 DNA Analyzer (Applied Biosystems, Grand Island, NY). Forward and reverse sequences were compiled and edited in Sequencher (Gene Codes Corporation, Ann Arbor, Michigan) and aligned in MEGA5 (Tamura et al., 2011). CO1 and ND1 sequences for each specimen were concatenated in order to create a single sequence for each individual and one haplotype network for each species. TCS was used to construct haplotype networks (Clement et al., 2000). Reference individuals in these haplotype networks are concatenated *Elliptio arca* sequences from the Black Warrior Drainage (GenBank Accession Number AY655093) and the Coosa Drainage (AY654995) and *Elliptio complanata* from the Connecticut River (AY158780) and the James River (EU448173; Serb et al., 2003; Campbell et al., 2005; M. Gangloff et al., unpublished data). Individual sequences within our dataset that represented unique haplotypes (when not concatenated) were uploaded to GenBank (Accession numbers KC708454 – KC708480). DnaSP was used for population genetics analyses (Rozas et al., 2003).

We computed several standard population genetics statistics, including nucleotide diversity, haplotype diversity, population structuring, gene flow, and genetic differentiation. Nucleotide diversity (π) is defined as the mean number of nucleotide differences between any two sequences and was calculated using equation 10.5 from Nei (1987). Values of nucleotide diversity range from 0 (low) to 0.2 (high) in animals (Daniels et al., 2002; Marko, 2004). Haplotype diversity (H_d) reveals haplotype richness within a subpopulation and was calculated using equation 8.4 from Nei (1987). Values of haplotype diversity range from 0 (low) to 1 (high) in animals (Barber et al., 2002; Cross et al., 2007). The population structure statistic (F_{st}) calculates the genetic variation among subpopulations, with values ranging between 0 and 1, with values closer to 1 suggesting less interbreeding (Hudson et al., 1992, equation 3). Gene flow (N_m) is an estimate of the effective number of migrants exchanged between subpopulations per generation (Hudson et al., 1992, equation 4). Values between 0 and 1 are considered low and those greater than 1 high. Negative F_{st} and N_m values are a result of more diversity within subpopulations than between populations. Genetic differentiation (S_{nn}) determines the probability of haplotype recovery from the same location (Hudson, 2000). Values near 1 are indicative of highly-differentiated populations, and values near 0.5 suggest populations are panmictic.

RESULTS

The population of *E. arca* in Sandy Creek had eight haplotypes, two of which were found only upstream of the dam (haplotypes 3 and 4) and two were found only downstream (haplotypes 2 and 8; Fig. 2A). Two of the most common haplotypes were found in all three reaches (haplotypes 1 and 5), and two were shared between only two reaches (haplotypes 6 and 7). Nucleotide diversity was low for all populations, ranging from 0.00179 to 0.00196, and haplotype diversity was relatively high, ranging from 0.68 to 0.79 within reaches (Table 1). Population structuring was low ($F_{st} = 0.00683$), and gene flow between reaches was high ($N_m = 72.69$). Genetic differentiation was closer to 0.5 than to 1 ($S_{nn} = 0.38$), suggesting that *E. arca* populations separated by Jones Mill Dam are one panmictic population.

The population of *E. complanata* in the Tar River had nine haplotypes, one of which was found only upstream from the dam (haplotype 6) and two were found only immediately downstream of the dam (haplotypes 2 and 4; Fig. 2B). Three of the most common haplotypes were shared between all three reaches (haplotypes 1, 5, and 7), and three haplotypes were shared between the upstream reach and at least one downstream reach (haplotypes 3, 8, and 9). Nucleotide diversity was also

low in this species, ranging from 0.01011 and 0.01047, and haplotype diversity was high, ranging from 0.69 to 0.83 (Table 1). Population structuring was low ($F_{st} = -0.06181$), and gene flow was high between the three

populations ($N_m = -8.59$). Genetic differentiation was closer to 0.5 than to 1 ($S_{nn} = 0.31$), suggesting that *E. complanata* populations separated by Gooch Mill Dam are one panmictic population.

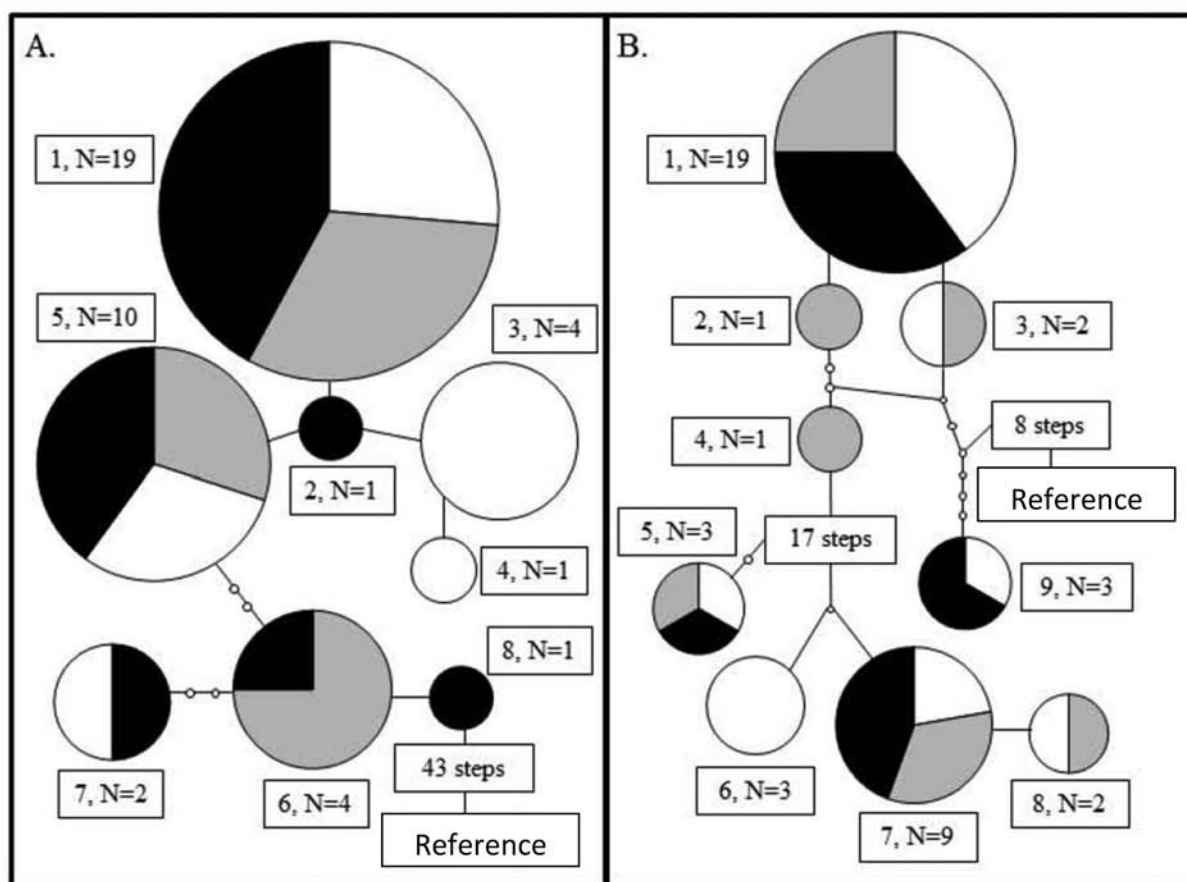


FIGURE 2

Parsimony network of mtDNA haplotypes for the concatenated CO1 and ND1 genes in (A) *Elliptio arca* and (B) *Elliptio complanata*. Each pie chart represents a unique haplotype with connecting lines representing one nucleotide difference (step) between haplotypes, except where otherwise noted. Observed haplotypes are labeled with an identifying number followed by the number of individuals having that haplotype (N). Colors represent the proportion of individuals from each reach having a particular haplotype (white, upstream of the dam; grey, immediately downstream of the dam; black, farther downstream of the dam; see Fig. 1). Pie charts with only one color are haplotypes unique to a particular reach. See Methods for information about reference individuals.

DISCUSSION

We found no strong evidence of genetic isolation in either *Elliptio arca* or *E. complanata* as a consequence of stream fragmentation by mill dams. Although we did observe unique haplotypes upstream (3 out of 17 haplotypes) and downstream (4 out of 17 haplotypes) from both dams, this small number of unique haplotypes does not conclusively suggest that isolation is occurring (Grobler et al., 2006; Perrin et al., 2008). If occurring, isolation would also be evident from low gene flow, high population structuring values, and significant genetic differentiation parameters (Hamilton, 2009).

Because evidence for isolation is weak, either unidirectional or bidirectional gene transfer may be occurring across these small dams. Downstream gene transfer for both species could happen easily by sperm drift or during high flow events when infected host fishes are washed over the top of mill dams. Upstream gene transfer is more difficult to envision, especially for *E. arca*. Darters, host fishes for *E. arca*, are known for their low mobility that can be greatly reduced or blocked by stream barriers (Schaefer et al., 2003). Darters would have a difficult time moving upstream over Jones Mill Dam during the flooding events when there is substantial flow over the dam. On the other hand, the American eel, a likely host fish for *E. complanata* in the Tar River, is well-known for its ability to circumvent stream obstructions such as dams (Sorensen & Bianchini, 1986; Tesch, 2003). This ability provides a plausible mechanism for upstream movement of glochidia and gene flow. Although eels were not found by recent surveys at Gooch Mill Dam, they have been found upstream of other small mill dams in the Tar River (J. Holcomb, unpublished data). Other potential host fishes for *E. complanata* present at Gooch Mill Dam, *Lepomis cyanellus*, *Lepomis gibbosus*, *Lepomis macrochirus*, and *Micropoterus salmoides*, would likely have a difficult time getting upstream of Gooch Mill Dam (Ellis, 1974; J. Holcomb, unpublished data). In contrast to downstream gene flow, upstream dispersal is probably less frequent and highly context-specific, and we are unable to propose mechanisms of upstream gene flow for *E. arca* in Sandy Creek. Nevertheless, the distribution of unique haplotypes and other genetic measures do not support a primarily downstream mode of gene flow in either population.

Although we found no evidence of genetic isolation or unidirectional gene flow, a number of factors need to be considered when assessing the extent to which populations upstream and downstream of the dams are isolated. More rapidly evolving genetic markers such as microsatellites might detect population structure that was not evident from mtDNA, which may not show evolutionary changes over the 177-216 year existence of these dams and the relatively small number of mussel

generations during this time. Similarly, because population sizes of both species remain large at all of our sites, they may retain a large percentage of historical genetic diversity such that our measures reflect signatures of former, pre-dam gene flow rather than contemporary gene flow (see Grobler et al., 2011). Our relatively small sample size may also have limited our ability to detect rare haplotypes or other patterns of genetic variability and structuring in these populations. Future work could take advantage of non-lethal DNA collection techniques such as viscera, mantle, and foot swabbing to allow increased sample size without sacrificing more individuals (Henley et al., 2006).

Our study provides a first look at the extent to which small dams might fragment freshwater mussel populations. Dams are a pervasive component of stream ecosystems with > 2.5 million small dams in the United States (National Research Council, 1992), and stream fragmentation by dams poses serious demographic risks to isolated populations in addition to potential genetic consequences (Morita & Yamamoto, 2002; Schick & Lindley, 2007). Dam removal projects are an increasingly important tool for re-establishing biological connectivity and ecosystem function and may provide benefits to numerous aquatic species, but they may also have substantial negative short-term impacts (Stanley et al., 2002; Stanley & Doyle, 2003; Sethi et al., 2004). In Sandy Creek and the Tar River, dense, species-rich mussel assemblages occur immediately downstream from these dams (Singer & Gangloff, 2011; McCormick, 2012). Although more research is needed to determine patterns and mechanisms of gene flow, the lack of strong evidence for genetic isolation in our study suggests that, at least in the short-term, removing Jones and Gooch mill dams should be considered low priority objectives relative to other habitat restoration projects in these watersheds.

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TABLE 1

Nucleotide diversity (π), haplotype diversity (H_d), population structuring (F_{st}), gene flow (N_m), and genetic differentiation (S_{nn}) for the concatenated genes (CO1 and ND1) in *Elliptio arca* and *Elliptio complanata*. Reaches represent populations in the vicinity of mill dams on Sandy Creek, AL, (*E. arca*) and the Tar River, NC, (*E. complanata*). The upstream reaches were upstream of the mill dam impoundments, the mill dam reaches were immediately downstream of the dams, and the downstream reaches were 0.5 km (Sandy Creek) or 5.0 km (Tar River) downstream of the dams (see Fig. 1).

Species	Reach	n	π	H_d	F_{st}	N_m	S_{nn}
<i>Elliptio arca</i>	Upstream	14	0.00186	0.79			
	Mill dam	12	0.00179	0.68			
	Downstream	16	0.00196	0.72			
	Overall	42	0.00188	0.73	0.00683	72.69	0.38
<i>Elliptio complanata</i>	Upstream	17	0.01047	0.76			
	Mill dam	14	0.01011	0.83			
	Downstream	13	0.01036	0.69			
	Overall	44	0.00993	0.75	-0.06181	-8.59	0.31

VERTICAL MIGRATION AND REPRODUCTIVE PATTERNS OF A LONG-TERM BROODING FRESHWATER MUSSEL, *VILLOSA CONSTRICTA* (BIVALVIA: UNIONIDAE) IN A SMALL PIEDMONT STREAM

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ABSTRACT

We delineated a permanent 15 m by 9 m reach of a mussel bed in a small piedmont stream in the Cape Fear River Basin of North Carolina, USA. A total of 14 surveys were conducted at the study site from May 2005 to September 2006 at time intervals ranging from 2 weeks to 3 months. The study area was divided into fifteen 1-m-wide transects, and each transect was thoroughly searched twice during each survey event for any mussels on the substrate surface. We recorded species identification, length, gravidity (for known females) and replaced the mussel in the exact spot it was found. A pilot study was conducted to determine detection success with one, two, and three passes per transect and detection success was monitored on all transects throughout the study. We estimate that two passes over these transects yielded approximately 90% of the mussels on the sediment surface. Vertical migration patterns of *Villosa constricta*, and in particular females, were highly seasonal. Additional within-season variation could not be explained by seasonal patterns alone. Larger individuals were recaptured more frequently. Female mussels became gravid from August through March indicating that spawning and glochidial release took place over an extended period. In 2005, glochidial release was 1-2 months later than in 2006 and lasted through June. In 2006, glochidial release began before 7 February in 2006 and lasted through April. Smaller *V. constricta* (23-28 mm) were more likely to be gravid, and about half of the individual females were observed to spawn in consecutive years.

KEY WORDS burrowing, surveys, spawning, reproductive timing, glochidial release

INTRODUCTION

The sessile nature of freshwater mussels (Unionidae) makes them among the easiest group of animals to collect for research. However, correctly interpreting survey results and quantifying mussel populations are much more difficult. Unionids burrow into, and emerge from, the substrate in response to reproductive cycles and environmental cues (Balfour & Smock, 1995; Waters, O'Dee & Chordas, 2001; Schwalb & Pusch, 2007). While burrowed, they are unavailable to capture through visual and tactile surveys of the substrate surface.

Because they burrow, excavation and sorting of the substrate is necessary to fully evaluate a mussel community with a single survey (Miller & Payne, 1988). Richardson & Yokley (1996) demonstrated that excavation was necessary to find juvenile mussels for documentation of recruitment. Smith and coworkers (2000) and Strayer & Smith (2003) also presented convincing evidence that mussel population numbers and demographics could not be accurately understood without substantial substrate excavation. Despite the need for quantitative assessments of population abundance and

density, surficial surveys remain in widespread use. Limited human and financial resources or the potential for habitat destruction through excavation may preclude subsurface techniques. In areas inhabited by federally endangered species, excavation may be prohibited. Visual surveys are sufficient for some survey objectives, such as determining species presence (Strayer & Smith, 2003), or collecting individuals for propagation and laboratory studies. As mussel species become increasingly rare (Williams et al., 1993; Lydeard et al., 2004), understanding their vertical movement within stream substrate (vertical migration) will become increasingly important when attempting to find reproductively active individuals or attempting to document that the species is still extant. Regardless of the objectives, visual and tactile surveys can be more effectively planned and interpreted when the vertical migration patterns of freshwater mussels are considered (Strayer & Smith, 2003).

Vertical migration patterns have been linked to reproductive behavior (Amyot & Downing, 1998; Watters et al., 2001) and studying these behaviors in concert is likely more fruitful than observing them separately.

Indeed, the National Strategy for the Conservation of Freshwater Mussels (NNMCC, 1998) called for significant research into mussel reproductive biology. Early researchers grouped mussels into two basic categories of summer and winter brooders (Ortmann, 1909; Lefevre & Curtis, 1910), but more recent research (Watters & O'Dee, 2000) has shown that mussel reproduction patterns are more variable than originally viewed. Changing water temperature and daylight length have been associated with vertical movement of mussels (Watters et al., 2001; Perles et al., 2003). In one study, water velocity proved to be a key driver of burrowing activity (Schwalb et al., 2007). To fully understand recruitment and population dynamics, natural resource managers need more than a general understanding of basic reproductive patterns in mussels. They need species-specific and even population-specific data to fully grasp the variation and nuances that affect recruitment in different mussel populations. Accordingly, we monitored a mussel bed in a small piedmont stream of North Carolina using visual survey techniques combined with a mark-recapture strategy to follow the vertical migration and reproductive patterns of multiple species. We focused our analysis on *Villosa constricta* (Conrad, 1838), a small sexually dimorphic unionid that rarely exceeds 40 mm in length. *Villosa constricta* prefers clean sand and gravel substrate in small streams (Fuller 1977). It is considered to be a long-term brooder with a brooding season recorded from August through June (Johnson, 1970). It ranges along the mid-Atlantic slope from the Santee-Cooper basin north to the James River basin in Virginia (Johnson, 1970).

METHODS

Study Site

The study site was located in the Cape Fear River Basin on New Hope Creek in Orange County, North Carolina (N 35.9921, W 79.0473). This site and its upstream watershed were characterized by a relatively stable stream channel and forested riparian zones. We delineated a permanent 15-m-long by 9-m-wide study area at the end of a shallow pool using rebar driven into the stream banks. The study area was divided into fifteen 1-m-wide transects, and each transect was subdivided into three 1-m by 3-m sections. The stream channel was approximately 12 m wide at this point, and depth ranged from 20 – 50 cm at normal base flow. Substrate consisted of primarily mixed sand and gravel with some embedded cobble and a varying amount of light silt cover. The relatively fine substrate and lack of vegetation made visual detection of mussels on the substrate surface relatively easy.

Survey techniques

A pilot study was conducted to assess the difference in detection success obtained when conducting one, two or three survey passes of each transect. Surveys were conducted on six transects at the main study site in May 2005. Three passes with three different surveyors were conducted at 6 transects and used to compare the number of mussels found during each pass. Following the pilot study, 14 mussel surveys were conducted at the study site at time intervals ranging from 2 weeks to 3 months during May 2005 and September 2006. Surveys were not conducted during October and November of 2005 due to low water and high amounts of leaf litter on the stream bottom.

All surveys were conducted at base flow conditions. The day prior to each survey, we laid white chains on the bottom of the stream to delineate the borders of the transects. On the day of the survey, two complete passes were made over each transect using two different surveyors with view scopes (buckets) to visually locate as many mussels on the substrate surface as possible. The number of mussels found on both passes was recorded for all transects as a measure of detection success.

During each survey, mussels were initially left in place when found and their locations were marked by inserting a survey flag into the substrate. After both passes were complete, we picked up all flagged mussels, recorded appropriate data and placed them back into substrate next to the flag marking their location. We recorded species, gender (of sexually dimorphic species), state of gravidity (for known females), and location in the study grid. Shell length, width, and height were recorded when the mussels were first found. Gravidity was classified as either not gravid (no marsupial swelling), early gravid (marsupial swelling beginning), fully gravid (marsupia fully swollen), or partially released (parts of the marsupia fully swollen and parts fully deflated). Unique alphanumeric marks were etched in the left valve of each mussel found with a Dremel™ tool when that individual was first located.

In April and September 2006, we conducted two searches (approximately 2 person-hours each) in the 75 meters immediately downstream of the study area to attempt to locate any marked mussels that had emigrated from the study site.

Stream flow data was acquired from a USGS gauging station (USGS 02097314) several kilometers downstream on New Hope Creek. All surveys were conducted at base flow conditions. A HOBO temperature recorder (model H08-001-02, Aquatic Ecosystems, Apopka, FL) in a clear protective case underwater at the study site recorded water temperature at two-hour intervals.

Statistical analysis

Statistical analysis was conducted using the statistical software packages Minitab 13.30 (Minitab Inc., State College, PA) and JMP (Version 10, SAS Institute, Cary, NC). Detection success (DS) was calculated as $DS = N1/(N1+N2)$, where N1 was the number of mussels detected on the surface during the first pass, and N2 the number of mussels detected during the second pass. Detection success data were arcsine transformed, and general linear models (GLM) were used to compare detectability between survey dates and between transects. A Mann-Whitney U test was used to test whether individual males or females were recaptured more often. A P value of < 0.05 was considered statistically significant.

RESULTS

Pilot Study

In our pilot study that provided an initial assessment of detection success, the first pass yielded 69.8-88.5% of the total mussels found in two passes (median = 80.4%, quartiles = 71.0, 86.4%) and 64.4-85.7% (median = 73.7, quartiles = 66.1-79.1%) of the total mussels found in three passes. Two passes yielded 87.5-100% (median = 91.0%, quartiles = 81.2, 97.6%) of the total found after the third pass. Assuming detection success remained equal between passes, the number of mussels found in a fourth pass would have been negligible and insufficiently useful to warrant the time and expense of additional survey effort.

Primary Mussel Surveys

During the 14 surveys, we found and marked 1,381 mussels representing 9 different species (Table 1). *Villosa constricta* comprised 17.7% (244 individuals) of all mussels found throughout the study. As determined by shell shape, there were 114 female (46.7%) and 130 male (53.3%) *V. constricta* collected. Males ranged from 20-50 mm long (median = 35.5 mm, quartiles = 32.0, 39.0 mm) and females ranged from 21-38 mm long (median = 28 mm, quartiles = 27.0, 32.0 mm). Size class distribution throughout the study period was similar between survey dates, and there was no apparent association between season and length of epibenthic *V. constricta*.

The ratio between the number of mussels found in the first and second passes during the 14 site surveys was similar to what was observed during the initial pilot detection success trial. Out of 142 transects monitored for detection success throughout the study, the first pass yielded a median of 78.1% (quartiles = 71.4, 84.8%) of the total number of *V. constricta* found in both passes. There was no difference in detection success

between dates ($P = 0.681$, GLM) or between transects ($P = 0.857$, GLM).

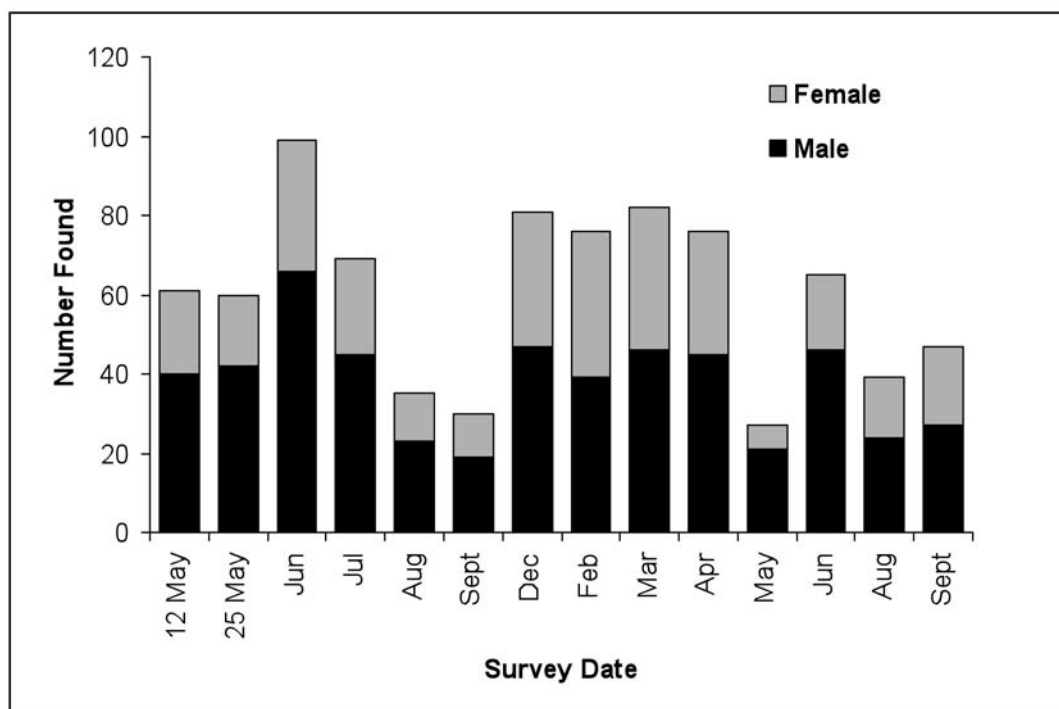
Horizontal and Vertical Movement

Horizontal movement either within or out of the study area was minimal. Of the 609 individual recapture events for *V. constricta*, 459 of those (75.4%) were recaptured in the same transect in which they were previously found. There were 133 recaptures (21.8%) in the transect immediately adjacent to the one in which they were previously found. Only 17 recaptures (2.8%) indicated movement of more than 1 meter by an individual. Nine of those moved upstream and eight moved downstream. No individual *V. constricta* was detected to move more than 6 meters. No marked individuals of any species were seen downstream of the study area in the two searches conducted.

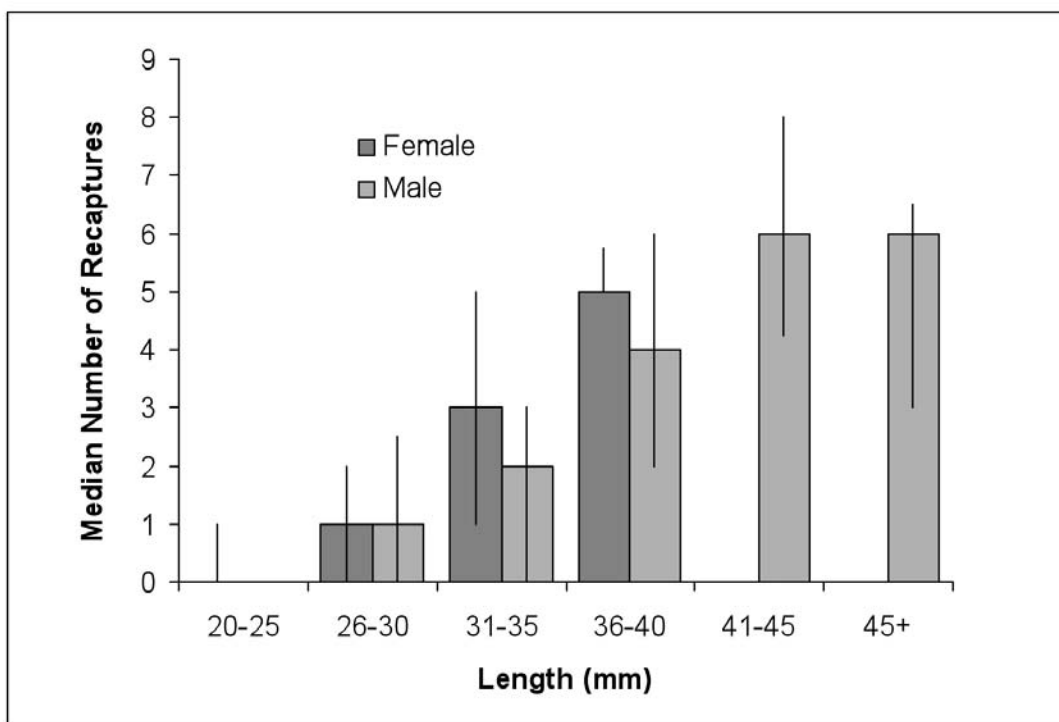
Survey results for *V. constricta* varied greatly over time (Figure 1), and a majority of the population was burrowed at all survey events. In individual survey events, we found between 11.1% (27 individuals) and 40.6% (99 individuals) of all *V. constricta* marked over the course of the study (median = 25.8%, quartiles = 15.6, 31.7%). Relative abundance of this species ranged from 7.5 - 22.2% (mean = $13.3 \pm 4.4\%$) of the total mussel catch in individual surveys. When all individuals from all species marked throughout the study were considered, the relative abundance of *V. constricta* compared to other species was lower than the relative abundance in all but two of the 14 surveys.

The number of recaptures for individual *V. constricta* was positively correlated with length for both males and females (Figure 2). Females were burrowed more often than males; consequently, the relative proportion of the population observed to be female was under-represented in individual surveys. During only one survey event (February 2006) was the proportion of female *V. constricta* (48.7%) greater than the overall proportion seen throughout the entire study (46.5%). The percentage of *V. constricta* that were female during individual surveys ranged from 22.2 to 48.7% (median = 35.8%, quartiles = 32.5, 42.2%). Out of the 14 surveys, individual males were found a median of four times (quartiles = 2, 6) while individual females were found a median of only two times (quartiles = 1, 4). This difference was statistically significant ($P < 0.001$, Mann Whitney U).

The individual brooding females, identified by mark, that were on the surface at a given time was variable. There were 15 gravid females found in March 2006 and 13 found one month later, but these surveys had no gravid individuals in common. In contrast, 24 of the 57 males (51.1%) found in April 2006 were also found in March 2006.

**FIGURE 1**

Number of male and female *Villosa constricta* found during each survey from May 2005 - September 2006.

**FIGURE 2**

Median number of recapture events for individual *Villosa constricta* of varying size during 14 surveys in New Hope Creek, Cape Fear River basin. Error bars represent 25th and 75th percentiles.

The number of individuals found on the substrate surface appeared to, at least in part, follow a seasonal pattern. We consistently found a high number of individuals during the colder months from December 2005 - April 2006 (Figure 1). This was primarily due to females emerging from the substrate during this time period while the number of males found did not substantially increase. In contrast, late summer surveys in both study years produced fewer individuals than earlier in the year.

There was also substantial variation in vertical migration patterns within seasons (Figure 1). Though both May 2005 surveys were almost identical in the number of *V. constricta* observed, there was a 55% increase in the number of mussels on the surface over the course of only three weeks from 25 May to 15 June 2005. In another 3 weeks (7 July), we found a reduction of the number of mussels on the substrate surface back to levels observed in May. In the spring of 2006, there was substantial variation in numbers of *V. constricta* found from month to month, with the lowest number being found in May. That May survey yielded only 35.5% of the number of individuals found just one month earlier (April) and 40.3% of the number found just one month later (June).

Spawning and glochidial release

Sixty-seven females (58.8%) were found were found

to be gravid at some point during the study. Females from 25-28 mm were most likely to be gravid (Figure 3). From August 2005 – April 2006, we recorded an entire spawning and brooding season represented by 53 separate individuals found to be gravid. Thirty-three females were not observed to be gravid at any point during this time period. Neither spawning nor glochidial release were single events; both were spread out over several months and a wide range of temperatures. We observed individuals initially becoming gravid in August, and all individuals found gravid in August and September were in the early gravid stage (Figure 4). In December, approximately 30% of gravid females were in the early gravid stage while the rest were considered fully gravid. The percentage of gravid individuals in the early stage remained approximately the same in February, but glochidial release had already begun. One individual was found to have partially released its glochidia, and another initially found gravid in August had fully released. Recapture in February of two individuals that had been in the early stages of gravidity in December showed development of those two broods to full maturity during that cold winter time period. In March, 93.3% of gravid individuals were found to have partially released their broods. In April, 76.9% of gravid females had partial broods remaining, and one individual was still in the early gravid phase. No gravid females were seen in

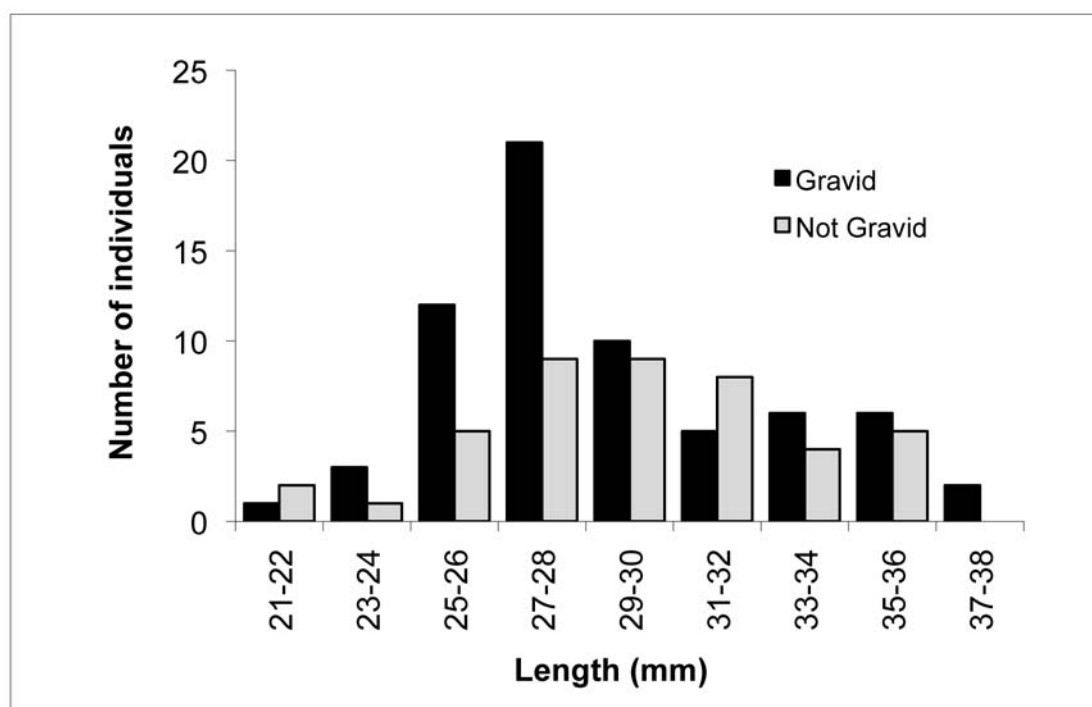
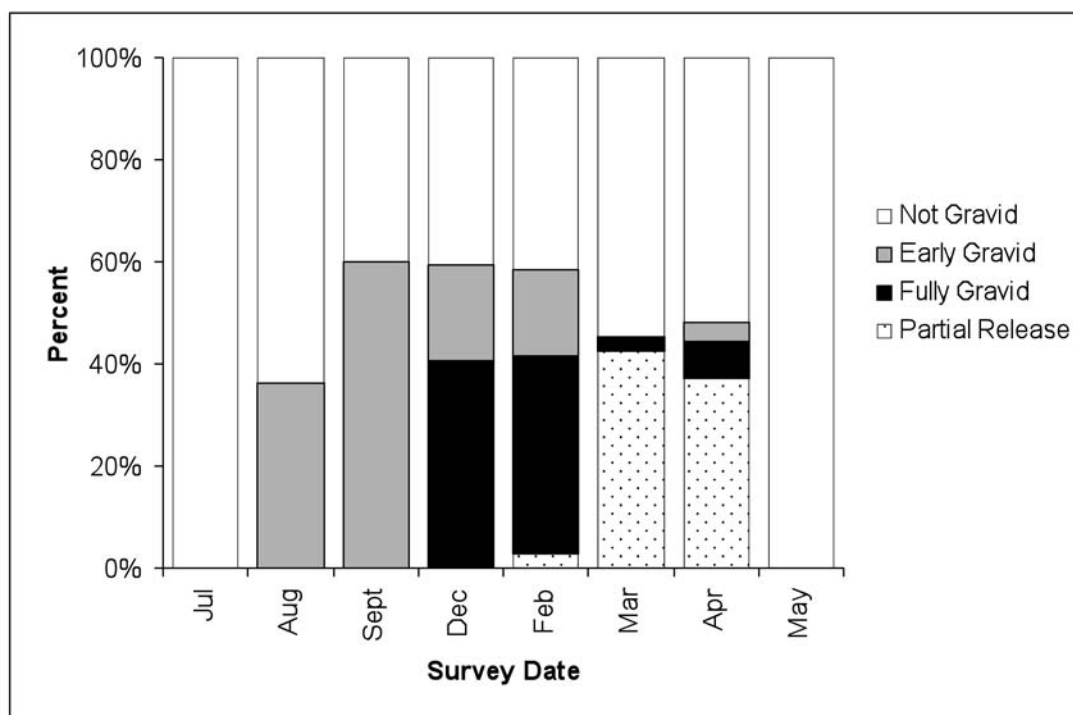


FIGURE 3

Length frequency of female *Villosa constricta* in a given length class that were found gravid or not gravid over the course of the study.

**FIGURE 4**

Percent of *Villosa constricta* females found in various stages of gravidity from July 2005 - May 2006.

May 2006.

The period of glochidial release for *V. constricta* varied substantially between study years. This seemed to be due to differences in flow between years. In 2006, glochidial release had already begun by 7 February when stream temperatures were near 5°C. No gravid females were observed after 13 April of that year when temperatures had reached approximately 15°C. From 13 April – 18 May, when the last releases of glochidia would have occurred in 2006, stream temperatures fluctuated between 15 and 20°C. In 2005 gravid individuals were observed as late as 15 June at a stream temperature of 24.8°C. Flow data from the downstream USGS indicated nine storm events of varying intensity between mid-February to mid-April in 2005 compared to only three smaller ones during the same time period in 2006 (Figure 5).

Results from following individual mussels over time also supported the idea of an extended spawning time. One female that was not gravid in December was recaptured in a gravid state in April. Another individual was not gravid in February but was found with a partially released brood in March. In addition, we found one female that was not gravid in March but was in the early gravid stages in April.

We found no evidence of individuals having multiple broods in a year. Of the 14 females found gravid in May and June 2005, six of those were gravid and six were not gravid again the following brooding season (August 2005 - April 2006). The other two were not found again. Of the 11 found gravid in September 2006, five of those were found gravid the previous brooding season. One of those five was also found gravid the initial year of the study, representing three consecutive years of successful spawning.

DISCUSSION

Evaluation of survey data

We found that two passes through a well delineated, 1-meter-wide transect were generally effective for finding the mussels on the substrate surface. The use of only a single pass was less effective and highly variable because it was more susceptible to a lapse in vigilance by an individual surveyor. Smith and coworkers (2000) found differences in efficiency between individual mussel surveyors, and this differed by site as well as substrate type. However, a second pass over a given area will naturally decrease survey error as the total number of mussels found approaches 100% of the mussels actually on the substrate surface. Based on these studies,

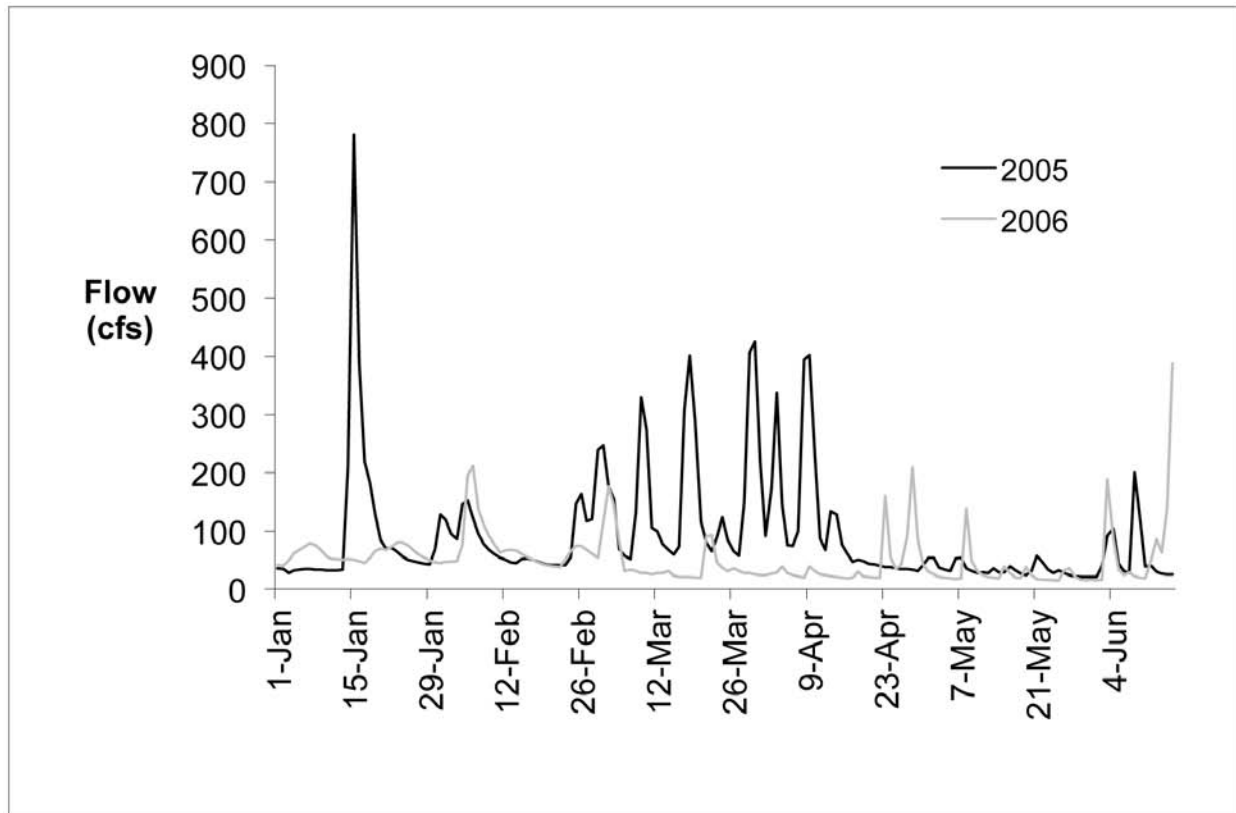


FIGURE 5

Flow data from USGS gauge 02097314 on New Hope Creek at Blands, NC approximately 20 km downstream from the study site.

we believe mussel surveys that use visual transect data should employ at least two passes to reduce this error. While a third pass likely would have yielded a few additional mussels in most transects, we estimate that two survey passes yielded approximately 90% of the mussels on the substrate surface. This detection rate should be sufficiently representative for tracking vertical migration patterns over time. Although increased field experience may reduce the likelihood of a surveyor missing mussels, the detection success of field crew members should be periodically evaluated to sustain reasonable confidence in survey results.

It is possible that detection efficiency varied slightly between species, but we were unable to measure that due to our survey protocol. We only marked the locations of mussels after each pass rather than picking them up because we wanted to be able to place the mussel back in its exact location after each collection. Because mussels were not immediately picked up for identification when they were found, we could not evaluate detection efficiency for a single species. Detection of smaller species is thought to be less efficient than that of larger species (Van Cleave, 1940).

Horizontal and Vertical Migration

There was minimal horizontal movement of study animals within the study site and no marked animals were found during two surveys within 75 m downstream. Taken together, this suggests that the mussel bed was highly stable and that the proportion of animals that were not observed appeared to have been associated with vertical rather than horizontal movement.

We found that a majority of the *V. constricta* were burrowed throughout the year with different individuals coming to the surface at different times. Even during peak times of emergence for glochidial release in March and April 2006, many individuals seen during one survey, were burrowed during the next survey. This is somewhat in contrast with other studies of vertical migration where most of the species studied have had at least one time of the year in which a majority of the population was epibenthic. Amyot & Downing (1991) found up to 96% of *Elliptio complanata* (Lightfoot, 1786) on the sediment surface at one time. In another study with *E. complanata*, Balfour & Smock (1995) found up to 80% on the sediment surface. Up to 80% of mussels were epibenthic during summer months in the River Spree in

Germany (Schwalb & Pusch, 2007). Watters and co-workers (2001) conducted a laboratory study with eight species and found close to 100% of six of those species emerged at times throughout the study. But two species in that study, *Obliquaria reflexa* (Rafinesque, 1820) and *Quadrula pustulosa* (Lea, 1831), did not exhibit the same synchronous emergence and were more likely to be burrowed. This observation that individual animals in a population may spend most of their life burrowed has important implications for the monitoring of extremely rare species. As a species declines in abundance, a significantly greater amount of effort would be required to detect its presence or absence or quantify populations, as it neared extirpation from a site (Smith, 2006). Prior knowledge of when a species is most likely to emerge becomes of heightened importance as populations decline.

We collected only a small number of mussels less than 25 mm. In other studies, larger mussels were more likely to be captured at the sediment surface (Amyot & Downing, 1991; Smith et al., 2000; Schwalb & Pusch, 2007) or recaptured in mark-recapture studies (Vilella et al., 2004). This may be due to detection bias or actual differences in the behavior of large and small mussels. It is well known that juvenile mussels are primarily burrowed (Hochwald & Bauer, 1990; Richardson & Yokley, 1996), but Schwalb & Pusch (2007) demonstrated that even smaller adults were more likely to burrow than larger ones. Our observations of *V. constricta* concur with these findings. Negishi and coworkers (2010) observed that the vertical distribution of different size classes of *Pronodularia japonensis* (Lea, 1859), in central Japan, varied seasonally. Small juveniles (<20 mm) were more abundant on the surface in the spring, while adults were predominantly burrowed. However, both juveniles and adults were observed on the sediment surface in the summer, and both size-classes were predominately burrowed in the winter. In contrast, we did not observe any consistent relationship between time of year and the size-class distribution of epibenthic *V. constricta* throughout the study period in New Hope Creek in NC.

Female *V. constricta* were recaptured approximately half as often as males in our study. Extensive visual surveys of streams in the upper Neuse River Basin from May-August 2001 yielded roughly a 3:1 male:female ratio of this species (Eads et al., 2006). The same study found a 2:1 male:female ratio in a muskrat midden used for age and growth analysis. While males usually outnumbered females by about 2:1 in most of the individual surveys done in our study on New Hope Creek, mark-recapture techniques revealed that the actual ratio at the site was essentially 1:1. Rogers, Watson & Neves (2001) followed a population of *Epioblasma florentina walkeri* (Wilson & H.W. Clark, 1914) with mark-recapture on a monthly basis and saw far greater variation

in male:female ratios within individual months than was seen in our study. In total, females outnumbered males almost 2:1 over the course of their study, but males were predominantly found from August-October and females were predominantly found in February-July.

Vertical movement of females was notably seasonal. They tended to be visible on the surface in the latter half of the brooding period, and apparently burrowed soon after the release of their brood. Even though spawning began in August and September, that time of year consistently yielded the fewest epibenthic mussels. While vertical migration patterns associated with reproduction have been documented previously (Amyot & Downing, 1998; Rogers et al., 2001; Watters et al., 2001; Perles, Christian & Berg, 2003), a significant finding of this study was the discovery of a large number of *V. constricta* on the sediment surface during the winter. While most mussel surveys occur during warmer months to better suit the biologists in the water, our results show that winter and early spring surveys may be quite productive for some species. In fact, our late summer surveys in August and September were the least productive. Dependent on research objectives and brooding behavior of the target species, those searching for mussels should consider the potential benefits of cold weather surveys.

In addition to broad seasonal patterns in vertical migration, we seem to have observed significant variations within seasons that greatly affected our survey results. Schwalb & Pusch (2007) found a sharp decline in the number of epibenthic mussels during one week in the middle of their reproductive season that coincided with increased stream flows. We saw a marked rise in the number found in June 2005 and a similarly marked decline in May 2006 relative to the surveys preceding and succeeding these unusual events. Even though none of our surveys occurred during the rise and fall of the hydrograph around a storm event, perhaps weather patterns leading up to these surveys played a role in the number of mussels we would find on the surface. If these events had occurred as individual surveys rather than as a part of a longer study, we would have had formed drastically different opinions of the mussel population at that site. This result further supports the idea that the results of surficial mussel surveys should be interpreted with care. Simple catch-per-unit-effort data can vary drastically even within a few weeks at a given site.

Spawning and glochidial release

Freshwater mussels certainly demonstrate baseline seasonality in their reproductive efforts, but this behavior is modified each brooding season based on responses to changing environmental conditions. If we had conducted our study only during 2005, we would

have concluded that *V. constricta* release glochidia in May and June (and perhaps earlier). If we had done the work only in 2006, we would have concluded the species released glochidia only from February through April. This demonstrates the need to follow populations over multiple years to fully understand their reproductive habits. This is especially true of mussels that spawn or release glochidia in spring. Because spring weather patterns and stream conditions can vary greatly from year to year, the reproductive behavior driven by these variables is altered as a result. The timing of spawning and glochidial release by the freshwater pearl mussel *Margaritifera margaritifera* (Linnaeus, 1758) has been shown to vary up to several weeks from year to year (Ross, 1992; Hastie & Young, 2003). Lewis (1985) also observed a shift in the brooding period of *Anodonta grandis* (Say, 1829) between years.

While temperatures have often been cited as the driving force behind the timing of glochidial release (Chamberlain, 1934; Young & Williams, 1984; Holland-Bartels & Kammer, 1989; Kondo, 1993; Watters & O'Dee, 2000), we believe this population of *V. constricta* may have also been influenced by stream flows. Difference in temperature between years would not explain why glochidial release was two months earlier in 2006 than in 2005, because individuals were found gravid at much higher temperatures in 2005 compared to 2006. We believe the frequent storm events from mid-February through mid-April 2005 likely delayed glochidial release by one of two mechanisms or a combination thereof. *Villosa constricta* uses a small lure display to attract sight-feeding, insectivorous darters as their host (Eads et al., 2006). These host fish encounters, which are the primary trigger for glochidial release (Haag & Warren, 2000), likely decreased during this high flow period. Some darters and stream fish have been shown to decrease movement during high flows (Freeman, 2004) and decrease feeding during high turbidity (Bonner & Wilde, 2002). Alternatively, the higher flows may have triggered *V. constricta* to burrow, as was observed with the freshwater mussel *Margaritifera margaritifera* during its reproductive season (Schwalb & Pusch, 2007).

Our recognition of the beginning of the spawning and brooding season was marked by gravid females found on 26 August 2005. In 2006, no gravid females were detected on 11 August but gravid females were found in September. There was apparently no mass emergence to the substrate surface when we first detected spawning activity. On the contrary, August and September tended to yield the fewest epibenthic mussels compared with other months of the year. We believe a large portion of the population became gravid over an extended period of time between the September and December surveys in 2005. While this fits the general

description of a long-term brooder, we gained additional important information on its reproductive habits by using a mark-recapture strategy. Had we simply monitored the population monthly without marking individuals, we would have concluded this population was no different than other reports of bradytichitic species with a late summer or fall spawn. Instead, we detected *V. constricta* becoming gravid in March. Although likely a minority of the population, a proportion of the population spawned in winter. Of the 53 total individuals found gravid from September 2005 – April 2006, we documented 26 of those (49.1%) to be gravid by December. We found that three individuals became gravid after the December survey, but we cannot determine when the other gravid females initially found during February through April actually spawned.

Other studies that followed gonadal histology of Lampsilines over time have found very short and distinct spawning periods (Zale & Neves, 1982; Holland-Bartels & Kammer, 1989; Haggerty & Garner, 2000). While we did not sample gonadal tissue, based on vertical migration and brooding patterns, *V. constricta* seemed to have an extended spawning period at this site. In 2005 and 2006, it covered eight months of the year. Even if only a small portion of the population was spawning in winter, this represents behavioral diversity that should be considered in the management of this species. Additionally, it is reasonable to assume that this behavior is not limited to *V. constricta* but may also be found in other bradytichitic species. Perhaps this behavior is genetically controlled and should be considered in the context of collection of broodstock for a propagation program. Indeed, Jones and co-workers (2006) provided genetic management guidelines for mussel augmentation and recommended that broodstock be collected from different times of the year to account for differences in reproductive behavior.

Initially, it seems unlikely that a species that is generally a long-term brooder would produce more than a single brood in a year, but the overlapping spawning and glochidial release of *V. constricta* at this site could make this theoretically possible. Though only a small proportion of the population, we documented some individuals becoming gravid after others had already released glochidia. The short-term brooding *Margaritifera margaritifera* (Howard, 1915; Gordon & Smith, 1990) and *Glebulula rotundata* (Parker et al., 1979) have been reported to potentially produce two broods in a year. The same has been found for other short-term brooding *Elliptio* species (Price & Eads, 2011). We found that approximately half of the females spawned in two consecutive years, and one individual was observed to spawn in three consecutive years. A study of *M. margaritifera* found that approximately 60-65% of females did not reproduce in

the year following spawning activity (Bauer, 1987). In contrast, Haag & Staton (2003) found a high degree of participation in brooding and suggested that most females in a mussel population reproduce in most years.

We did not observe complete reproductive senescence in *V. constricta*; however, smaller females appeared slightly more likely to become gravid than larger ones. If we assume that size is a reasonable surrogate for age, it can be concluded that reproduction may slow, but not stop, as this species ages. Because vertical migration has been linked so closely with reproduction (Amyot & Downing, 1998; Watters et al., 2001), it may follow that the coinciding decrease in burrowing behavior and spawning behavior are related biological processes.

SUMMARY

The idea that mussels burrow into the substrate escaping detection by visual surveys is not a new one (Miller & Payne, 1988; Balfour & Smock, 1995; Smith et al., 2001); however, the cues that drive this movement and how that varies between species are poorly understood. Prior studies previously conducted with *Elliptio complanata* (Balfour & Smock, 1995; Amyot & Downing, 1997) and other species (Watters et al., 2001; Perles et al., 2003; Schwalb & Pusch, 2007), have documented varied vertical movement patterns in unionids. Our previous understanding of bradytictic and tachytictic brooders was also too generalized to fully describe the variability in the diverse freshwater mussel fauna of North America (Watters & O'Dee, 2000). Our research in New Hope Creek demonstrates that the vertical migration and reproductive patterns of *V. constricta* there are quite complex. We found that vertical movement varied by age, gender, season, and even within seasons. Reproductive activity occurred over several months and could not be linked to a specific temperature.

In addition to its traditional role in estimating population size the use of mark-recapture techniques can yield great insight into the biology and ecology of freshwater mussel species by tracking individual mussels (Villella et al., 2004). Our research describes the behavior of one species in one mussel bed in the Piedmont of North Carolina. Some of the behaviors described here may apply to other long-term brooding species across a wide geographical range, and some behaviors may have been specific to this species, the location of the study site or the time of our observations. In addition, it is unclear how repeated sampling and its frequency could potentially affect mussel behavior, vertical movement and the likelihood of encountering an individual on the surface of the substrate.

Environmental assessments are an essential

component of freshwater mussel conservation efforts. An erroneous estimate of mussel populations made during an environmental assessment could alter conclusions about a site's suitability for a road crossing or other development project. Survey design must be compatible with survey project objectives (Smith, 2006). Careful study of the environmental cues prompting species' emergence and surface activity is needed to ensure that study protocols match the life history of the target species being surveyed. An understanding of the timing of spawning and glochidial release, spawning interval, and other variables that reflect the biology of mussel species should be considered when designing mussel population studies. These studies were conducted over time at a single site. The same species in a different stream with different physical features, flow patterns, food resources, or land-use related inputs could display different movement patterns. The complexity of the behavior we observed in *V. constricta* and previously documented in other species emphasizes the importance of studying how individual species and populations respond to environmental factors before making conservation recommendations.

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TABLE1

Freshwater mussels species observed and marked during 14 surveys conducted in New Hope Creek, Cape Fear River Basin, NC.

Species	Number Found	Number of Males	Number of Females	% Male	% Female
<i>Elliptio complanata</i>	980	-	-	-	-
<i>Villosa constricta</i>	244	130	114	53.3	46.7
<i>Villosa delumbis</i>	62	36	26	58.1	41.9
<i>Lampsilis sp.</i>	59	52	7	88.1	11.9
<i>Villosa vaughaniana</i>	14	9	5	64	36
<i>Fusconaia masoni</i>	9	-	-	-	-
<i>Pyganodon cataracta</i>	8	-	-	-	-
<i>Strophitus undulatus</i>	4	-	-	-	-
<i>Alasmidonta varicosa</i>	1	-	-	-	-

SHORT-TERM EFFECTS OF SMALL DAM REMOVAL ON A FRESHWATER MUSSEL ASSEMBLAGE

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ABSTRACT

Dam removal is increasingly used to restore lotic habitat and biota, but its effects on freshwater mussels (family Unionidae) are not well known. We conducted a four-year study to assess short-term effects on mussels after removal of a small hydropower dam on the Deep River (Cape Fear River drainage), North Carolina, USA, in 2006. We conducted annual pre- and post-removal monitoring of mussel density, richness, and survival (post removal only) with transect surveys and quadrat excavation, and assessed changes in substrate composition at two impact sites (tailrace and impoundment) and two reference sites. Before-after-control-impact (BACI) analyses of variance did not detect a significant change in mussel density (total or individually for the three most abundant species), species richness, Eastern Elliptio (*Elliptio complanata*) mean length, or substrate composition in the tailrace or drained impoundment following dam removal. Apparent annual survival estimates of Eastern Elliptio at the tailrace site did not differ among sampling periods and were similar to control sites. We observed minimal mussel mortality from stranding in the dewatered reservoir. These results demonstrate that adverse short-term impacts of dam removal on downstream mussel assemblages can be minimized with appropriate planning, timing, and removal techniques, but additional monitoring is warranted to determine long-term effects on mussels within the restored river reach.

KEY WORDS Apparent survival, BACI, *Elliptio*, imperiled species, mussel density, quantitative sampling, restoration, Unionidae

INTRODUCTION

The diverse freshwater mussel fauna of the southeastern U.S. is highly imperiled, and loss of habitat and other effects of dams are among the most important factors in the decline of these animals (Richter et al., 1997; Strayer et al., 2004; Cope et al., 2008). The ecological costs of dams are well documented (Dynesius & Nilsson, 1994; Watters, 2000; Bednarek et al., 2001) and

removal is becoming a common river restoration tool (Bednarek et al., 2001; Poff & Hart, 2002), especially as the financial cost of maintaining these aging structures exceeds their benefits (Stanley & Doyle, 2003).

Much of the research guiding dam removal has been conducted on large, high dams, but the vast majority of future removal projects concern small or medium-

sized dams (Heinz Center, 2002). Much less research is available on the effects of these dams on physical and biological components of river ecosystems, and thus, dam removals commonly occur without sufficient information to predict their outcome. Habitat restoration through dam removal is an important conservation strategy in the long-term, but potential negative short-term effects of dam removal on mussels have been rarely investigated. For example, large quantities of sediment are often deposited and stored within an impoundment, and sediment mobilization during dam removal may impact or extirpate downstream mussel populations (Sethi et al., 2004). In some cases, tailraces of small dams or even the reservoirs themselves support important mussel assemblages (Nedeau et al., 2000; Singer & Gangloff, 2011), and these habitats are especially vulnerable to negative effects of dam removal.

Based on available information (e.g., Sethi et al., 2004) and input from state and federal agencies, removal of a small hydropower dam (Carbonton Dam) on the Deep River, North Carolina, was conducted follow-

ing procedures designed to minimize adverse effects on fish and mussels. These procedures included a gradual drawdown of the impoundment and dam removal during the fall-winter, a season regarded as less stressful to aquatic biota. We conducted a four-year study to examine the effectiveness of these measures on reducing negative effects on the mussel assemblage in the Deep River. We examined changes in mussel density, species richness, length, and survival (post removal only) and substrate characteristics at impacted and non-impacted sites prior to and after dam removal.

METHODS

Study Site

The Deep River is a fourth-order tributary of the upper Cape Fear River drainage in the Piedmont physiographic province in central North Carolina (Fig. 1). The drainage area upstream of Carbonton Dam is about 2,600 km² and the estimated average annual discharge at the dam site is 38 m³/s (based on the difference

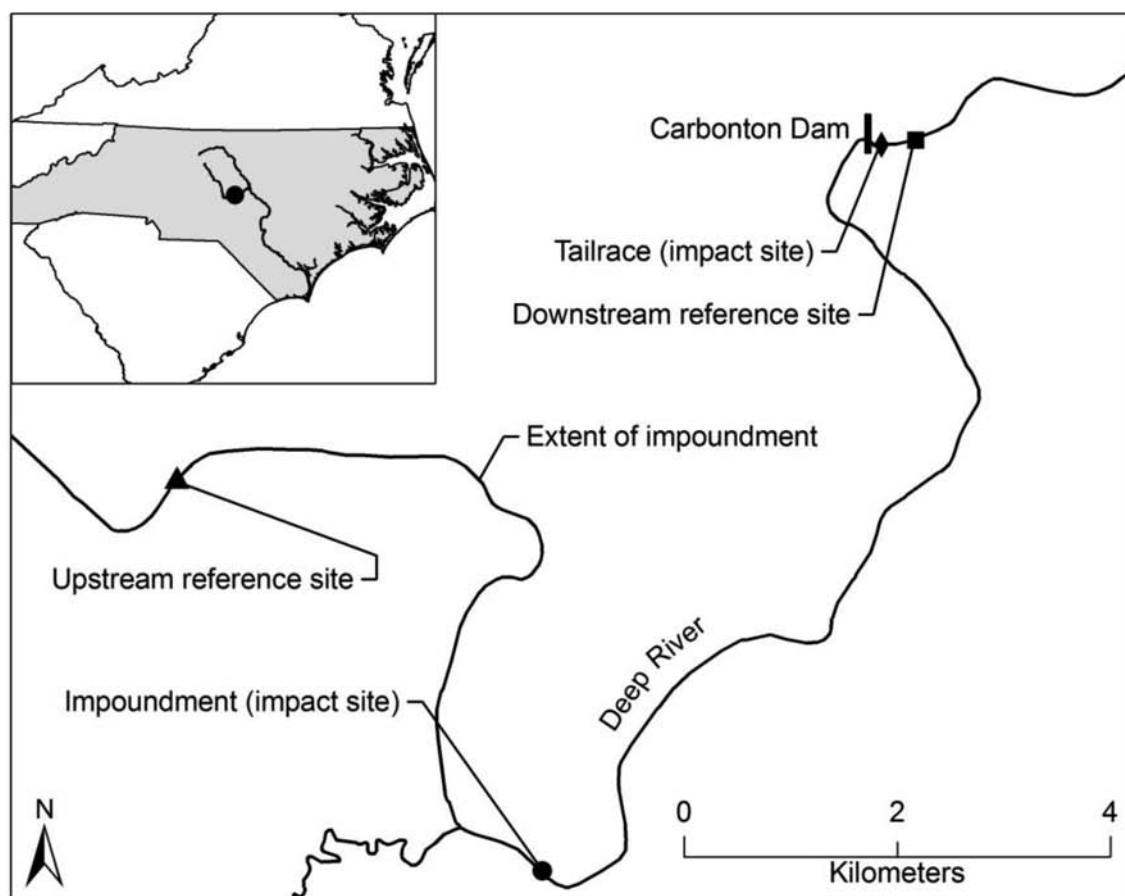


FIGURE 1

Location of Carbonton Dam in North Carolina (inset) and mussel sampling sites on the Deep River, North Carolina.

between discharge at USGS gaging stations 2102000 and 2101726). The watershed is primarily forested (63%), with smaller percentages of agricultural (12% pasture, 8% crops) and urban (11%) land use (NCDENR, 2005). Carbondon Dam was constructed in 1921 southwest of Sanford, North Carolina. The dam was 5 m high and 80 m wide with a hydropower generation capacity of one megawatt. The impoundment created by the dam was narrow and was contained within the banks of the Deep River (width = 45–80 m) and extended about 15 km upstream with a maximum depth of 8 m (Restoration Systems and Ecoscience Corporation, 2006). At least 14 other dams exist upstream on the Deep River, the closest being about 38 km upstream of Carbondon Dam (NCDENR, 2004).

Carbondon Dam was not navigable (i.e., without a lock) and lacked any engineering for fish passage. Water quality degradation, including low dissolved oxygen and excessive algal production, also had been recorded within the impoundment (NCDENR, 2005) and presumably contributed further to fragmentation of riverine habitat. State and federal environmental agencies prioritized the dam for removal to restore connectivity between populations of several state threatened and endangered mussel species (Table 1) and a federally endangered fish species (Cape Fear Shiner, *Notropis mekistocholas*) that occurred in this segment of the river. The dam was operated in a run-of-the-river flow regime (minimal water storage capacity) until June 2004. In 2005, a private environmental restoration company purchased the dam for removal to provide stream mitigation. Sufficient lead time between the purchase of the dam and the target removal date allowed state and federal resource agencies and the company to develop and recommend procedures that could minimize the impact of dam removal on aquatic life. These procedures included a gradual drawdown of the impoundment (over a two- to three-week period) and removal in the fall-winter, a time regarded as less stressful to aquatic biota in the region because large precipitation events are less frequent, dissolved oxygen concentrations are highest, and water temperatures are cool. These procedures also were attempts to minimize erosion and transport of sediment and large woody debris stored in the impoundment (see Bednarek et al., 2001). Drawdown of the impoundment, using existing powerhouse gates, began on October 15, 2005, and proceeded for approximately three weeks, and removal was completed in February 2006 (Restoration Systems and Ecoscience Corporation, 2006).

Sampling design

We conducted annual pre- and post-dam removal mussel surveys at two impacted sites and two reference sites in June from 2005 to 2008 (1 pre-removal and 3

post-removal samples; Fig. 1). However, we were unable to conduct a pre-dam removal mussel survey at the impoundment site (see below) prior to the drawdown and dam removal because of excessive depth. Criteria used to select sites included the presence of mussels (based on preliminary mussel surveys) and accessibility. One impacted site was located within the impounded reach, 10 river km (rkm) upstream of the dam, and the other was in the dam tailrace, 70 m downstream of the dam. One reference site was located 18 rkm upstream of the dam, beyond the influence of the impoundment, and the other was 400 m downstream of the dam. In larger rivers with high dams, 400 m may not be sufficient distance to qualify as a reference site (e.g., Vaughn & Taylor, 1999). Because of the small size of the Deep River and other channel morphological features, this distance appeared adequate to isolate the site from short-term effects of dam removal, and the high substrate stability we observed at the site during the study (see Results) supported the use of this site as a reference. We attempted to standardize sampling area within the boundaries of mussel aggregations. Sampling areas were 48 m long (upstream to downstream) by 5 m wide (bank toward channel) at all sites except for the tailrace site, which was 48 m long by 17 m wide; a wider sampling area was used at the tailrace site because mussels were broadly distributed across the wider river channel.

We used a combination of visual-tactile and quadrat excavation sampling to maximize the accuracy of mussel species richness and density estimates. Visual-tactile sampling is preferred for estimating species richness because it allows rapid coverage of large areas and collection of large numbers of individuals, but it can underestimate density by failing to detect burrowed and small individuals (Smith et al., 2000; Strayer & Smith, 2003). In contrast, quadrat excavation provides less biased density estimates, but because it is slower and more laborious, it is less effective for estimating richness.

At each site, we established 12, 3-m wide transects spaced 4 m apart (on center) positioned perpendicular to the shoreline. Sample areas were relocated in subsequent years by GPS coordinates and permanent markers on the river bank. Nine of the transects were randomly selected for sampling by visual-tactile methods, and the three remaining transects were sampled with quadrats. During sampling, a white metal chain was placed along the center line of the transect to indicate its location, and the transect length was measured to allow determination of transect area (length $\times$ 3 m). For visual-tactile surveys, three experienced personnel simultaneously snorkeled along each transect, each searching about 1 m of the width of the transect. Snorkelers searched for mussels visually and by feeling through the substrate (a heterogeneous mixture of silt, sand,

and gravel), and then placed all mussels into individually labeled dive bags. The annual mean sampling time among transects (all three snorkelers combined) ranged from 23–113 min among sites and years (overall mean = 58 min, coefficient of variation among sample dates at a site averaged 19.8%). After completing each transect, all mussels were identified to species, measured (total length, mm), and batch marked with a Dremel® tool by etching the periostracum of each valve of the mussel with a mark unique to the survey year (i.e., 1–4). After processing, each mussel was placed into the substrate within the transect from which it came. In addition to annual visual-tactile sampling, we conducted weekly visual surveys (1–2 h each sampling event) of the newly exposed river banks at the impoundment site during much of the drawdown process (October 2005 – February 2006) to document the extent of mussel stranding and mortality.

For the three quadrat-sampled transects, 10 randomly selected points were located within each transect according to coordinates based on the distance from shore and the width of the transect (total of 30 quadrats per site). At each sampling point, a 0.25-m² metal quadrat frame was placed on the substrate surface, and all sediment within the quadrat was excavated down to aggregated substrate or to 10 cm and placed in a 20-L container and processed on shore. Mussels from quadrat samples were processed as described for those from visual-tactile samples.

We developed site-specific calibration factors to account for bias in density estimates from visual-tactile sampling relative to quadrat sampling, and to standardize density estimates from these two methods. Calibration factors were computed and used to adjust density estimates after Peterson & Paukert (2009) as follows. First, the quadrat density estimate was divided by the visual density estimate (using combined data from all mussel species) for each year, and then an arithmetic mean among years was calculated. This mean calibration factor was then multiplied by each visual density estimate to yield an adjusted visual density estimate that represented a complete census mussel estimate comparable to quadrat sampling. The adjusted density estimates from visual data were used in all analyses. We considered stream sites as the experimental unit rather than transects (*sensu* Hurlbert, 1984). Estimated site density was expressed as the mean density among all 12 transects; thus $N=1$ density estimate per site per year.

We analyzed substrate removed from quadrats during mussel sampling to examine potential changes in substrate composition associated with dam removal. We fractioned substrate samples into particle size cat-

egories of cobble/boulder (>64.0 mm diameter), gravel (64.0 – 2.0 mm), and sand/silt/clay (<2.0 mm) (Bovee & Milhous, 1978) by passing the sediment through a set of nested sequential sieves. We then determined the relative percentage of each of these three particle size categories by measuring the total wet weight (nearest kg) of each fraction with an analog hanging balance (Viking Pelouze® Model 7810, Pelouze Scale Company, Evanston, IL).

Data analysis

A before-after-control-impact (BACI) design was used to assess temporal differences in mussel density and length, species richness, and substrate composition among sites. Species richness was defined as the total number of species observed using both visual and quadrat methods at a given site and year. We followed an analysis of variance (ANOVA) procedure for asymmetric BACI described by Underwood (1991, 1994) and Smith (2002) with a significance probability of 5% ($\alpha = 0.05$). The response variables included in the BACI analysis were density (all mussel species, and separately for Eastern Elliptio (*Elliptio complanata*), Eastern Creekshell (*Villosa delumbis*), and Eastern Pondhorn (*Unio merus carolinianus*)), Eastern Elliptio mean length, total mussel species richness, and percentage of each substrate category (boulder/cobble, gravel, and sand/silt/clay). The general model for this analysis was

$$y = \mu + BA + T(BA) + CI + L(CI) + (BA \times CI) + \text{error},$$

where y is the measured response variable (i.e., mussel density, species richness, mean mussel length, substrate composition), μ is the grand mean of the measured response variable, BA is the mean effect of the before or after period (i.e., 2005 before, 2006–2008 after), $T(BA)$ is the effect of sampling date (i.e., year) within the before or after period, CI is the mean effect of the control (i.e., upstream or downstream reference sites) or impact treatment (i.e., tailrace or impoundment sites), $L(CI)$ is the effect of location (i.e., site) within the control or impact treatment, and $(BA \times CI)$ is the effect of the before or after period in the control or impact treatment (i.e., the BACI effect). The response variables conformed to the normality assumption for ANOVA (Shapiro-Wilk W test, $P>0.05$; Zar, 1996).

We conducted two separate BACI analyses; one in which the tailrace (impact) response variables were compared to those of the two reference sites (controls), and another in which the impoundment (impact) variables were compared to those of the reference sites. Because we were unable to conduct a pre-dam removal mussel survey in the impoundment, we used data from

the first post-removal sample (taken 4 months after removal in 2006) to represent pre-removal (2005) conditions so as to conform to the balance needed for the BACI data analysis. We assumed that the mussel assemblage in the impoundment before dam removal was similar to that seen 4 months after removal because minimal bank erosion was observed, major changes in sediment composition in the former impoundment were not observed during our study, and we found few dead mussels that were stranded by receding water (see Results). In addition to BACI analyses including mean mussel length, we also compared length distributions of Eastern Elliptio sampled at the upstream reference, tailrace, and downstream reference sites between years before (2005) and after dam removal (2006–2008; all methods combined) with pairwise Kolmogorov-Smirnov two-sample tests.

Apparent survival of the Eastern Elliptio at each site was estimated with the Cormack-Jolly-Seber model (with model averaging) in the software program MARK (Lebreton et al., 1992; White & Burnham, 1999), using recapture rates of marked individuals (visual and quadrat data combined) in successive annual samples. Apparent survival was defined as the probability that an individual mussel was alive and available for recapture (White & Burnham, 1999). The Cormack-Jolly-Seber model is an open population model, which we considered most applicable because it allowed for immigration or recruitment and emigration or death occurring between sampling periods. Capture probability was estimated to describe the chance of capturing an individual that is present during the study period. We did not estimate survival or capture probability for any other species because of their rarity and low sample sizes (see Results).

RESULTS

Trends in mussel and habitat parameters

Among all sites and years, a total of 11 mussel species were collected, including one state endangered, three state threatened, and one significantly rare species (Table 1). Cumulative richness among all years was highest at the tailrace site (10 species), and lowest at the impoundment site (5). Estimates of species richness over time were variable and showed no clear pattern at most sites except for the impoundment site where richness appeared to decline gradually after dam removal (Fig. 2). Eastern Elliptio was the numerically dominant species at all sites and accounted for 88–95% of the mussels. Eastern Pondhorn, Eastern Creekshell, and Triangle Floater (*Alasmidonta undulata*) were also found at all sites but in much lower densities (Table 1). Most species were represented by four or fewer indi-

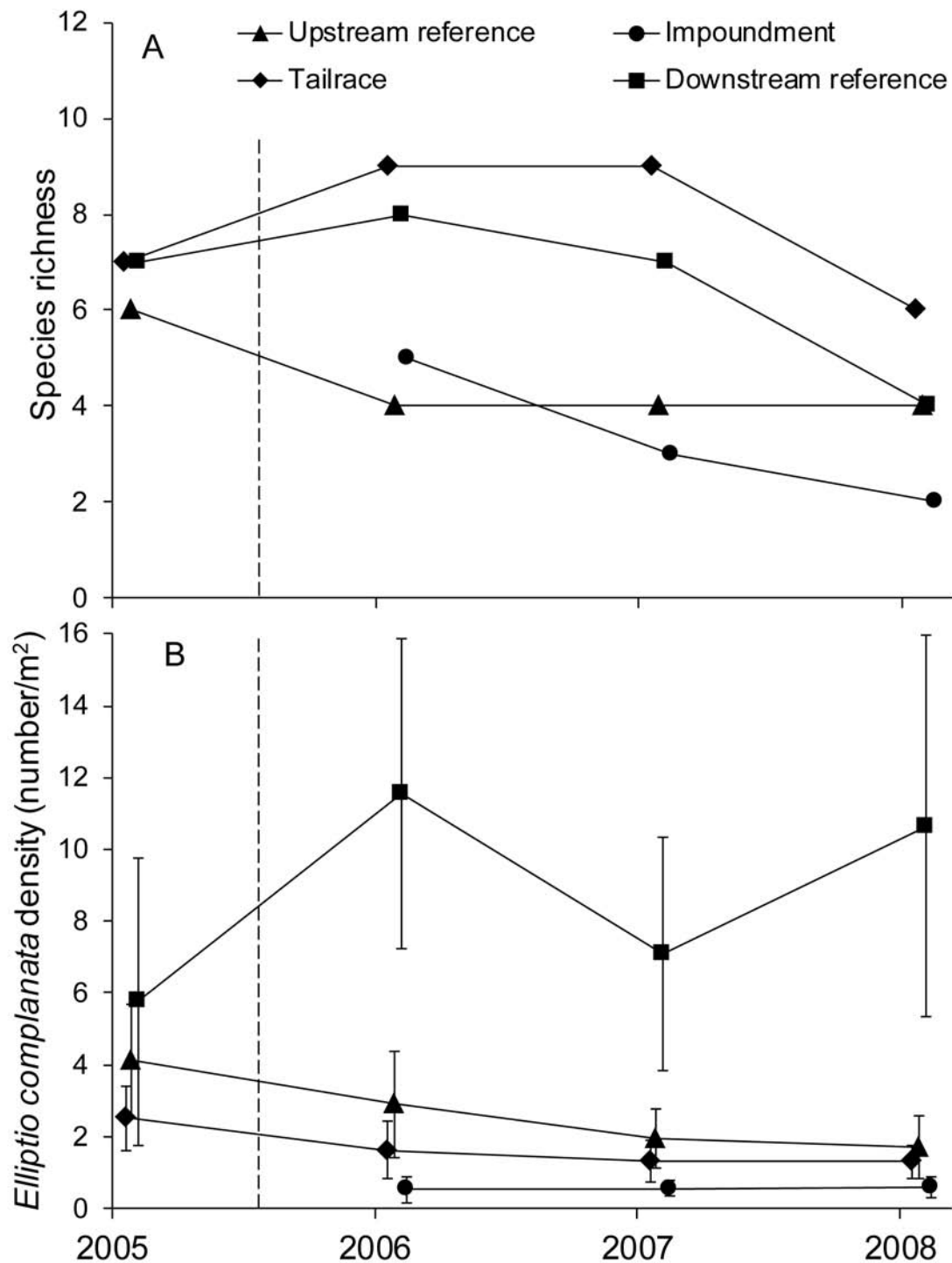
viduals at each site. Density of Eastern Elliptio was highest but most variable, both within and among years, at the downstream reference site (5.8 to 11.6 mussels/m² among years; Fig. 2). Density of Eastern Elliptio was lowest at the previously impounded site (0.52 to 0.57 mussels/m²; Fig. 2).

We observed only minimal short-term changes in substrate composition at the two impact sites among years (Fig. 3). The percentage of fine sediment (i.e., sand/silt/clay) at the tailrace site appeared to increase slightly from 38.3% pre-dam removal to 49.4% the first year after removal, but it then declined to 24.7% by the third year after removal; however, standard error for estimates of fine sediments overlapped among most years. Similarly, the percentage of fine sediment at the impoundment site appeared to increase from 30.1% immediately after removal (2006) to 49.6% in 2007, but it then decreased to 36.4% by 2008. The substrate composition at the two reference sites remained stable among years (Fig. 3A, B).

The mean length of Eastern Elliptio varied widely among sites, but not among years within sites. Mean length of Eastern Elliptio over the four years ranged from 51.8–53.9 mm (52.6 mm overall mean) at the upstream reference site, 53.1–54.7 mm (53.7 mm overall mean) at the impoundment site, 73.4–77.1 mm (75.4 mm overall mean) at the tailrace site, and 69.6–73.0 mm (71.1 mm overall mean) at the downstream reference site. Length distributions differed significantly among years (before and after dam removal) at the tailrace and downstream reference sites, but not at the upstream reference site (Table 2). The differences at the tailrace site were the result of variable shifts in length frequency about the mode, but at the downstream reference site, the differences reflected a change in the mode and skewness, indicating a reduction in modal size and increased numbers of smaller individuals.

BACI effects

At the tailrace site, no significant dam removal effects were detected by the BACI analysis for any mussel or substrate response variable. We found significant control/impact effects in the mean length of Eastern Elliptio ($P < 0.0001$) and proportions of boulder/cobble ($P = 0.003$) and sand/silt/clay ($P = 0.002$), indicating consistent differences between the control and impact sites that did not change after removal of the dam (see Fig. 3). We also detected significant location effects (nested within control/impact, $P < 0.05$) in all mussel variables (except for total species richness) and in the proportions of boulder/cobble ($P = 0.004$) and sand/silt/clay ($P = 0.007$), reflecting differences between the two control sites.

**FIGURE 2**

(A) Total species richness and (B) mean (among sampling transects) Eastern Elliptio (*Elliptio complanata*) density at each site on the Deep River, North Carolina, from 2005-2008. Error bars are 95% confidence intervals; the vertical dashed line represents the date of removal of the Carbonton Dam. Only means were included in the BACI analyses.

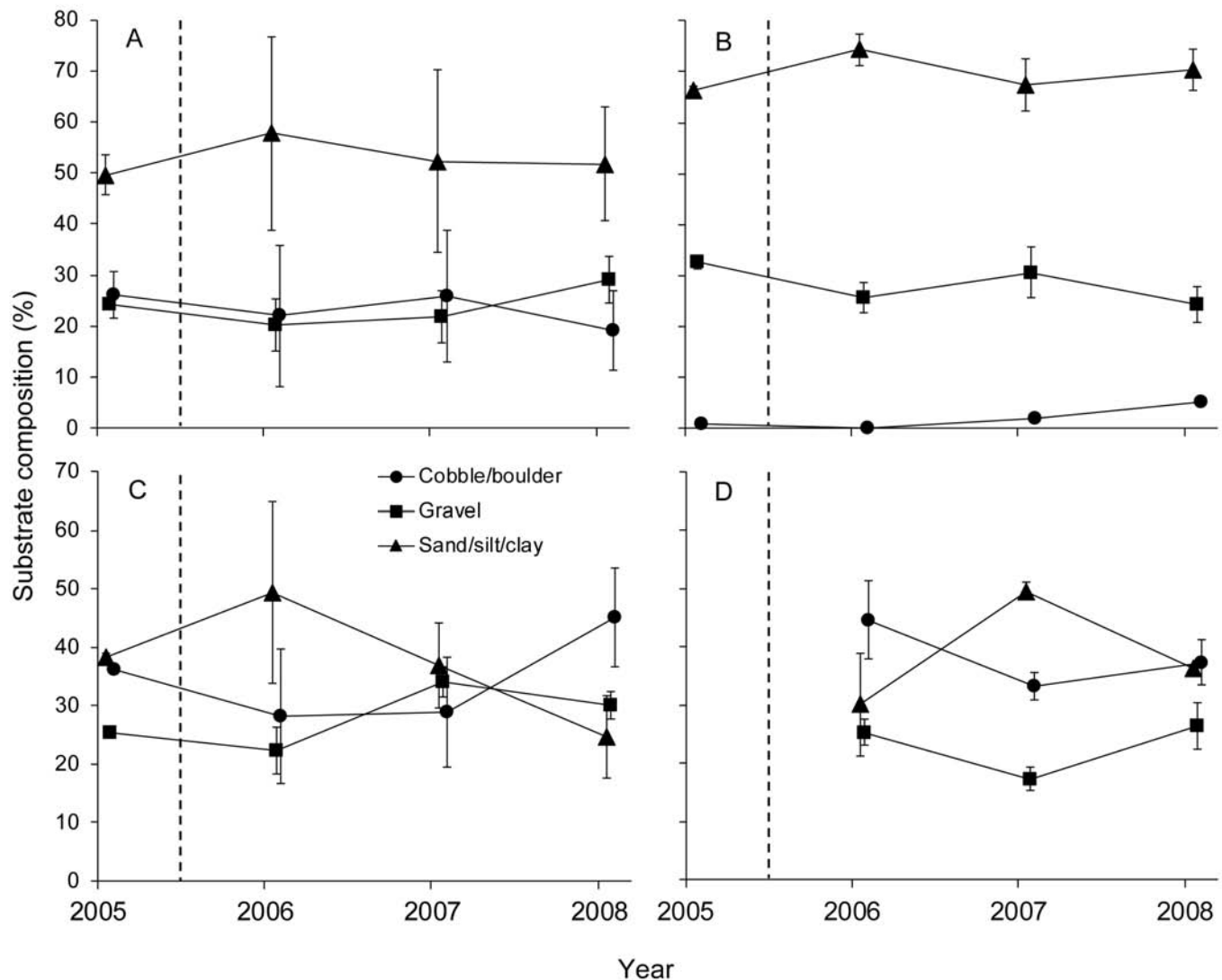


FIGURE 3

Mean (among sampling transects) percentage ($\pm$ SE) of each sediment particle size class at the (A) upstream reference, (B) downstream reference, (C) tailrace, and (D) impoundment sites on the Deep River, North Carolina. The vertical dashed line represents the date of removal of the Carbonton Dam. Only means were included in the BACI analyses.

At the impoundment site, no significant dam removal effects were detected by the BACI analysis for any mussel or substrate response variable. Significant control/impact effects ($P < 0.05$) were evident for all mussel variables (except for total species richness) and in proportions of boulder/cobble ($P = 0.0003$) and sand/silt/clay ($P = 0.003$) indicating differences between the control and impact sites that did not change after removal of the dam (see Figs. 2 and 3). We detected significant location effects (nested within control/impact, $P < 0.05$) in all mussel variables (except for total species richness) and in boulder/cobble ($P = 0.001$) and sand/silt/clay ($P = 0.02$), indicating differences between the two control sites. During the drawdown process, only a few individu-

als (< 10 total) of Eastern Elliptio and Paper Pondshell, *Utterbackia imbecillis*, were observed stranded on the newly exposed banks at the impoundment site.

Mussel survival

The mean recapture rate of Eastern Elliptio ranged from 12.8% ($SD = 2.3$) at the tailrace site to 24.5% ($SD = 11.6$) at the impoundment site. Cormack-Jolly-Seber capture probabilities among years ranged 17-30% at the tailrace site, 34-52% at the downstream reference site, 19-34% at the upstream reference site, and 30-34% at the impoundment site. Annual apparent survival of Eastern Elliptio at the tailrace site was similar over time, including the interval that spanned dam removal

and all subsequent intervals. Apparent survival at the tailrace site was similar to control sites and confidence intervals for all of these estimates overlapped broadly (Table 3). Survival estimates at the impoundment site for all intervals and for all sites from 2007-2008 were not informative due to the wide confidence intervals, which resulted from the model sensitivity to the low number of years sampled.

DISCUSSION

We found little evidence of short-term effects of dam removal on the mussel assemblage in the Deep River after removal of Carbonton Dam. There were no detectable differences that were attributable to dam removal in abundance of all species, and individually for the three most abundant species, nor in Eastern Elliptio mean length or length distribution. Our estimates of Eastern Elliptio survival were lower than previously reported survival rates for this species in a free-flowing stream (Villella et al., 2004), but survival in our study did not differ between impact and control sites. Species richness was variable among years at all sites due to sampling error, but it remained highest at the tailrace site throughout the study.

Sediment erosion, transport, and deposition in downstream reaches are among the most important negative physical effects of dam removal (Heinz Center, 2002), and release of fine sediment stored in the impoundment was a potential risk in the removal of Carbonton Dam. At the tailrace and impoundment sites, fine sediment appeared to increase slightly the year after removal, as expected, but by the end of the study (3 years after removal) it had declined to levels comparable to, or lower than, those observed before dam removal. However, BACI analysis showed no significant effects of dam removal on substrate composition. Several features of Carbonton Dam or the removal process appear to have minimized negative effects of downstream sediment transport. The reservoir apparently stored a relatively low volume of sediment because of its run-of-river flow regime and because numerous upstream impoundments capture sediment prior to its reaching the dam. Coarser materials, mostly sand and gravel, as well as woody debris, had accumulated immediately upstream of the dam, but these materials were graded with heavy equipment after drawdown to form a bench along one side of the river so that the material would erode more slowly (Restoration Systems and Ecoscience Corporation, 2006). The majority of the flow at the tailrace site during the drawdown and removal process was directed to the side of the river opposite from the diverse mussel bed that we monitored during this study, and this likely buffered the mussel bed from sediment deposition as

well as scour. Also contributing to the lack of short-term adverse effects on mussels was a lack of organic and inorganic contaminant accumulation in the sediments stored in the impoundment (USFWS, 2005; Hewitt et al., 2006).

Stranding of mussels in dewatered impoundments can result in high mortality, in some cases affecting imperiled species (Nedea et al., 2000). The reservoir behind Carbonton Dam was confined within the banks of the river, which prevented vast areas of the impoundment bottom from being exposed during drawdown. Therefore, stranding and aerial exposure of large numbers of mussels during impoundment draining was minimized — a result we verified through multiple qualitative observations throughout the drawdown process. Nevertheless, we observed a decline from 5 to 2 species in the dewatered impoundment over time; one of those undetected species (Eastern Floater, *Pyganodon cataracta*) is adapted to lentic environments. Changes in mussel assemblages in dewatered reservoirs after dam removal may be unavoidable as habitats revert from lentic to lotic characteristics, but we may expect additional stream species to colonize these restored habitats in the future. Because most imperiled species are dependent on lotic habitats, increases in habitat availability for these species can offset negative effects to previous reservoir assemblages.

To our knowledge, the only other study that addressed the effects of dam removal on unionoid mussels is Sethi et al. (2004), who reported substantial mussel mortality in both the former impoundment and tailrace reach after removal of Rockdale Dam on Koshkonong Creek, Wisconsin. Rockdale Dam appeared to have stored much larger amounts of sediment than Carbonton Dam, and it was dewatered rapidly (36 h), exposing large areas of substrate in the former reservoir and resulting in stranding and mortality of mussels. Moreover, downstream habitats were inundated with sediment as the newly forming river channel mobilized material in the former Rockdale impoundment. Three years after dam removal, mussel density downstream of Rockdale Dam had declined by about 32%.

Sethi et al. (2004) recommended that negative effects of dam removals could be minimized by a slow drawdown period (i.e., months to years) that would allow mussels to migrate with decreasing water levels and allow stabilization of reservoir sediments. Even though the impoundment above Carbonton Dam had a limited littoral zone and apparently held less sediment, the slower dewatering process (3 weeks) likely minimized mussel stranding in the former reservoir and sedimentation or scouring of downstream habitats. However, the effectiveness of a gradual drawdown on reducing mussel stranding and mortality may vary among species according to

differences in mobility and burrowing behavior (Gough et al., 2012). Timing of dam removal also may influence the potential for negative effects. Rockdale Dam was dewatered in early September (Sethi et al. 2004), when water temperatures presumably remained high. Removal of Carbonton Dam in the fall-winter may have further reduced mussel mortality by minimizing heat and oxygen stress. Because of variation in factors such as dam configuration and river and impoundment morphology, the optimal timing and methods for reservoir dewatering and dam removal will be case-specific. Potential toxicity of sediments stored in the impoundment also is a critical factor in assessing potential negative effects of dam removal (USBR, 2006; Cope et al., 2008). Consideration of these variables during the planning and execution of future dam removals is necessary to ensure the best possible outcome for aquatic biota.

Positive effects of existing dams also should be evaluated prior to removal. Despite their many negative aspects, in some cases small dams may enhance downstream habitats by trapping toxicants or sediments or by increasing downstream oxygen and food concentration (Gangloff et al., 2011; Singer & Gangloff, 2011), and these reaches often support dense mussel assemblages or rare species, as we observed in the Deep River. In dam removal planning, consideration of potential short-term negative effects of dam removal on localized populations (e.g., in tailrace or impoundment reaches) should to be weighed against long-term negative effects of population fragmentation at the watershed scale. Even though we observed no short-term negative effects of dam removal in our study, we also detected no evidence of recolonization of the former impoundment or increased density downstream in this time frame. Because of the slow growth and low recruitment rates of many mussel species, such responses may not be evident for several years or even decades. Long-term monitoring of effects of dam removal on mussel populations has not occurred, but this is vital to assess the full benefits or risks of this conservation strategy.

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TABLE 1

Mean density (number/m²) across years (and range) and cumulative species richness among all years at four study sites on the Deep River, North Carolina, from 2005-2008 and respective state conservation status in North Carolina (T = threatened, E = endangered, SR= significantly rare).

Species	Conservation status	Study site			
		Upstream reference	Impoundment	Tailrace	Downstream reference
<i>Alasmodonta undulata</i> Triangle Floater	T	0.003 (0-0.013)	0.003 (0-0.006)	0.013 (0.003-0.033)	0.017 (0.008-0.042)
<i>Elliptio complanata</i> Eastern Elliptio		2.67 (1.71-4.12)	0.541 (0.517-0.574)	1.69 (1.30-2.50)	8.77 (5.76-11.56)
<i>Elliptio icterina</i> Variable Spike		0.017 (0-0.054)			
<i>Elliptio roanokensis</i> Roanoke Slabshell	T	0.008 (0-0.033)		0.028 (0.015-0.043)	0.036 (0-0.067)
<i>Elliptio</i> sp. lance group		0.074 (0.013-0.174)		0.014 (0-0.036)	0.004 (0-0.017)
<i>Lampsilis cariosa</i> Yellow Lampmussel	E	0.024 (0-0.040)		0.009 (0-0.033)	
<i>Pyganodon cataracta</i> Eastern Floater			0.020 (0-0.040)	0.014 (0-0.037)	0.017 (0-0.025)
<i>Strophitus undulatus</i> Creeper	T			0.002 (0-0.003)	0.015 (0-0.025)
<i>Unio merus carolinianus</i> Eastern Pondhorn		0.010 (0-0.027)	0.017 (0.007-0.028)	0.012 (0.003-0.033)	0.496 (0.372-0.584)
<i>Utterbackia imbecillis</i> Paper Pondshell				0.001 (0-0.003)	
<i>Villosa delumbis</i> Eastern Creekshell	SR	0.015 (0-0.033)	0.005 (0-0.007)	0.123 (0.094-0.143)	0.356 (0.190-0.510)
Total richness		8	5	10	8

TABLE 2

Pairwise comparisons of Eastern Elliptio (*Elliptio complanata*) length distributions before (2005) and after (2006–2008) dam removal at three study sites on the Deep River, North Carolina (impoundment not included due to lack of pre-removal data). Statistics are the Kolmogorov-Smirnov maximum difference in cumulative frequencies (D) and associated probability (P) of the paired distributions coming from the same population.

Years compared	Study site					
	Upstream reference		Tailrace		Downstream reference	
	D	P	D	P	D	P
2005, 2006	0.1132	0.0960	0.0903	0.0281	0.1429	<0.0001
2005, 2007	0.1375	0.0773	0.0973	0.0362	0.1038	0.0012
2005, 2008	0.0965	0.4928	0.0746	0.1976	0.1665	<0.0001

TABLE 3

Estimated apparent survival and the 95% confidence interval (Cormack-Jolly-Seber model) of Eastern Elliptio (*Elliptio complanata*) between sampling dates from 2005–2008 at four study sites on the Deep River, North Carolina.

Study site	2005–2006	2006–2007	2007–2008
Upstream reference	0.77 (0.51–0.91)	0.80 (0.46–0.95)	0.68 (0–1)
Impoundment		0.95 (0–1)	0.61 (0–1)
Tailrace	0.69 (0.58–0.78)	0.72 (0.54–0.85)	0.64 (0–1)
Downstream reference	0.68 (0.61–0.73)	0.67 (0.61–0.73)	0.64 (0–1)

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OUR PURPOSE

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

- 1) Advocate conservation of freshwater molluscan resources;
- 2) Serve as a conduit for information about freshwater mollusks;
- 3) Promote science-based management of freshwater mollusks;
- 4) Promote and facilitate education and awareness about freshwater mollusks and their function in freshwater ecosystems;
- 5) Assist with the facilitation of the National Strategy for the Conservation of Native Freshwater Mussels (Journal of Shellfish Research, 1999, Volume 17, Number 5), and a similar strategy under development for freshwater gastropods.

OUR HISTORY

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

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