

**Status and Life-history Aspects of *Villosa constricta*  
(Conrad 1838) (Notched Rainbow), in the Upper Neuse  
River Basin, North Carolina**

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**Abstract** - We report the findings of stream-survey data, a length-at-age study, and host-fish determination for *Villosa constricta* (notched rainbow). Visual surveys were done for freshwater mussels at 44 bridge crossings in the upper Neuse River basin in North Carolina. Three surveyors, each searching a 1-m wide lane, covered a 600-m long stream reach at each site. All mussels found were identified to species and measured, and females were checked for gravidity. Of the 24 sites where *V. constricta* occurred, the median number found was 3.5 (range = 1-54). We cut thin-sections of 71 individual shells collected from middens at 1 survey site and counted growth lines to determine mussel age. Shell ages ranged from 3 to 14 years. Lab trials determined that *Etheostoma flabellare* (fantail darter) served as a suitable host for this species.

### Introduction

*Villosa constricta* (Conrad) (notched rainbow; Bivalvia:Unionidae) ranges along the mid-Atlantic slope from the Catawba River basin in North Carolina north to the James River basin in Virginia (Johnson 1970). It primarily occurs in coarse sand in areas with some current, with a brooding season from August to June (Johnson 1970), and is listed as a species of special concern (Williams et al. 1993). Other crucial life-history information is relatively unknown. The type locality for this species is the North River in Rockbridge County, VA (Bogan 2002).

The national strategy for the conservation of freshwater mussels lists answering basic biology and ecology questions as a high priority to protect imperiled species (NNMCC 1998). In addition to determining the status of this species in the upper Neuse River basin, we sought to further study aspects of its basic life history. Watters et al. (1999) used lab trials to report several potential hosts for this species; however, a majority of those reported hosts do not co-occur with *Villosa constricta*, and transformation success was limited with not more than 5 juveniles produced from any single fish species. One of our objectives was to conduct host-fish trials to enhance our understanding of *V. constricta* life history and facilitate captive propagation. Also, discovery of muskrat middens containing a large number of shells of this species presented a unique opportunity to gain valuable life-history information on this species such as longevity and length-at-age data.

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## Methods

### Study area

The survey study area was defined as the Neuse River basin above Falls Lake (Fig. 1). The 1686-km<sup>2</sup> region covers portions of Orange, Durham, Person, Granville, and Wake counties in North Carolina. The main drainages in the area are the Eno, Little, and Flat River watersheds, but several other smaller watersheds feed directly into Falls Lake from Granville and Wake

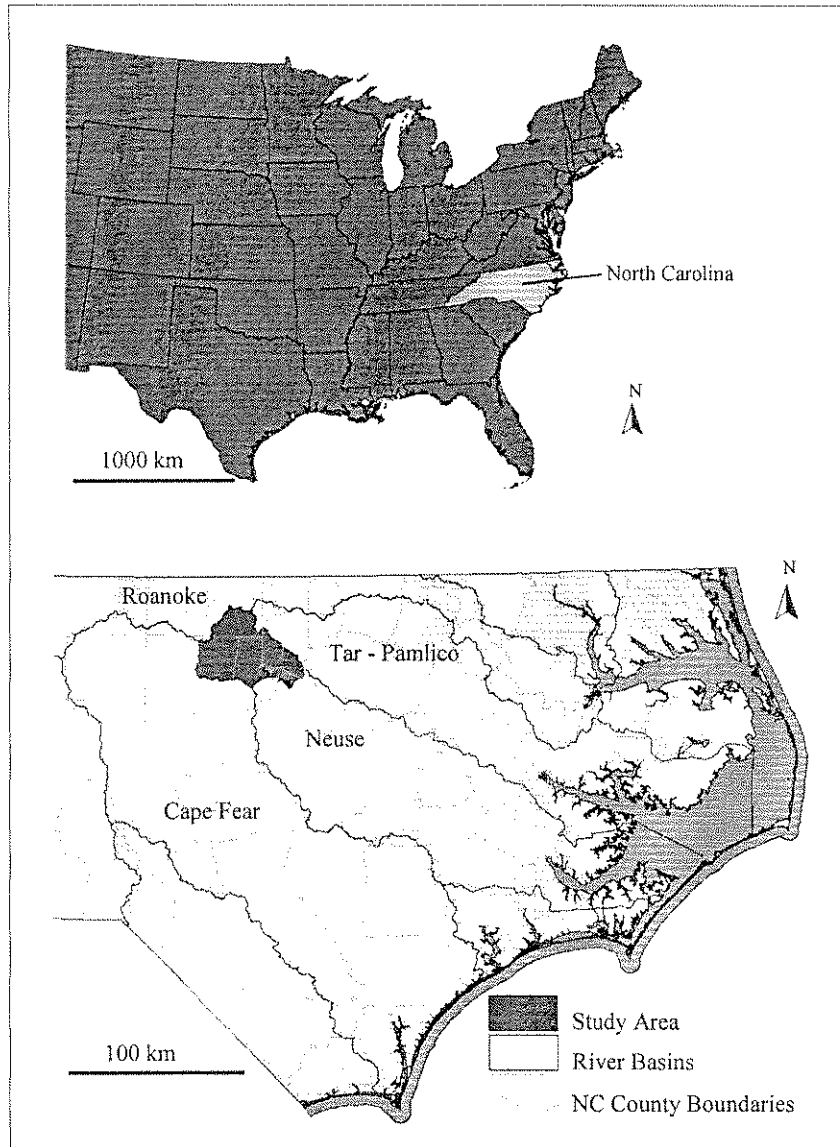


Figure 1. Upper Neuse River basin study area.

counties. The geology in this area results in a variety of stream types from rocky to sandy, and a diversity of stream-channel types are represented in this relatively small portion of the Piedmont. Durham, Hillsboro, Creedmoor, and Butner are the primary municipalities in the region, with Durham being the largest (population = 190,000). The dominant land uses within the sub-basin included forest (61%), urban (16%), and agriculture (18%). Various wetland types comprised 4% of the land cover (EPA 2000). With the exception of two sites along the mainstem Eno River where the setting was somewhat more urban, mussel-survey sites were restricted to rural areas where stream riparian zones are well buffered by forest and agricultural use was not intensive.

### Mussel survey

A total of 44 study sites were selected to cover the study area and surveyed from 24 April–21 August 2001 (Fig. 2). At each site, 3 surveyors each searched 1-m wide lanes (one next to each bank and one in the center of the stream) using view scopes and snorkeling to visually locate mussels. Surveyors covered the two 300-m stream reaches immediately upstream and downstream of the road crossing as well as the area under the crossing structure. To maximize consistency through time and between surveyors, only surface visual searches were done, and no excavation or rock flipping was used to locate mussels. Tactile searching was used as necessary when murky water, debris piles, or undercut banks made visual searches difficult;

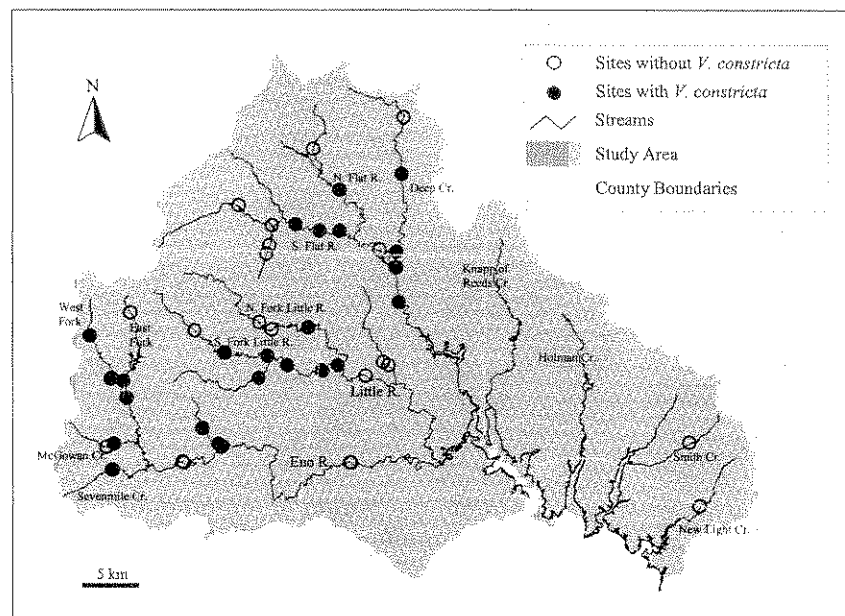


Figure 2. Map of 2001 study area and 44 survey sites in the upper Neuse River basin. Solid circles indicate sites containing *Villosa constricta*, and open circles represent sites where the species was not found.

however, only mussels felt on the sediment surface were taken. We collected all mussels found in the three lanes, and no mussels were included in the survey that fell outside of a surveyor's measured lane. All but 2 sites were completed within the same day. Those 2 sites were each completed in 2 consecutive days, and no substantial weather changes or rain occurred between days. We identified all mussels and recorded length, gender, and gravidity state for all sexually dimorphic species.

### Age and growth

A total of 71 (47 male, 24 female) *Villosa constricta* shells were collected for age and growth studies from middens at the West Fork Eno River at the most downstream crossing of SR 1004 in Orange County. We measured the length of each shell and assigned them unique identification numbers. Using a Buehler® low-speed, precision saw, a cut was made through both valves of each shell along the posterior ridge, creating four shell pieces for each individual. We then used an epoxy resin to adhere the cut side of the four resultant shell pieces to glass microscope slides. When the epoxy had cured, a final cut was made through the adhered shells to create thin-sections with a thickness of 500  $\mu\text{m}$  attached to the glass slides (Fig. 3). We then determined age by counting internal growth lines using a stereomicroscope (Neves and Moyer 1988). Final age for a mussel was determined by examining all four thin-sections. In most shells, the first year of growth had been eroded away, so one year was added to the age of those shells eroded in the area where the first year's growth line was typically located (Hanlon and Levine 2004). Lines not originating at the umbo or extending to the periostracum were assumed to be false annuli and not counted (Neves and Moyer 1988).

### Host-fish determination

Three fish-host trials were conducted in the laboratory (Zale and Neves 1982) to determine a suitable host for *Villosa constricta*. We collected 5 gravid females on 11 May 2004 from Deep Creek in Person County and 13 fish species by seine and backpack electrofisher from Richland Creek in Wake County, NC. On 12 May 2004, glochidia were extracted from mussels by flushing the marsupium with a water-filled syringe and tested for viability by exposure to a solution of NaCl. Glochidia were 100% viable according to this test. Fish were put into a tank containing 31 L of water at 20 °C, which was aerated vigorously. The glochidia were then dumped into the tank with the fish and stirred periodically to keep them in suspension. We checked the gills of some fish for infestation periodically throughout the exposure, and removed the fish from the glochidial suspension after 30 minutes. Fish were then maintained in 38-L aquaria at 20–22 °C and fed either commercial pellet food, frozen blood worms, or live earthworms. Each tank contained only a single species of fish. Tanks were then siphoned every 2–3 days through a 105-mm-mesh sieve, and the trial ended on 21 June 2004 (41 days post-infestation).

For the second trial, one gravid female *Villosa constricta* was collected in March 2005 from the Little River in Randolph County in the Yadkin-Pee Dee River basin and was maintained in a laboratory tank chilled to 6 °C until the trial began. Using a backpack electrofisher, a total of 16 species of fish was collected from Horse Creek in Wake County, NC (Neuse basin) in March 2004 to be used in the trial. Fish were maintained in the laboratory at 18 °C and fed regularly. On 19 April 2004, glochidia were extracted from the mussel by flushing the marsupium with a water-filled syringe. We anesthetized the fish with MS-222, and pipetted glochidia directly onto their gills. The fish were placed in a recovery tank and then moved back to their individual aquaria where they were fed daily. Individual aquaria contained only a single species. Tanks were siphoned every 2–3 days for 27 days post-infestation to check for live transformed juveniles.

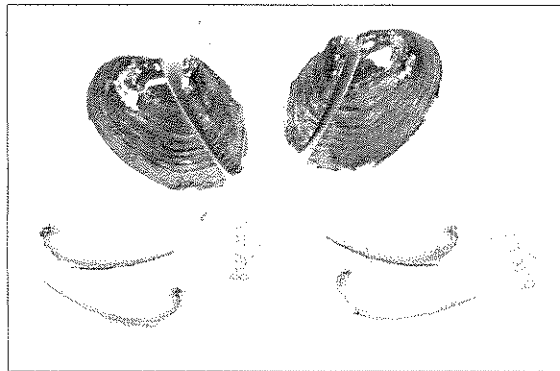
The third trial used 7 gravid *V. constricta* collected from the North Fork Little River in Orange County, NC in April 2005 and fish collected from Upper Barton Creek in Wake County, NC on 18 May 2005. Gravid mussels were maintained in a tank in the laboratory at 6 °C until the trial began. On 18 May 2005, glochidia were extracted from the females and directly pipetted onto one gill of 123 *Etheostoma flabellare* (Rafinesque) (fantail darters) and 6 *Percina roanoka* (Jordan and Jenkins in Jordan) (Roanoke darters) as described in the second trial. Fish were then maintained in 45-L tanks at 18 °C, fed regularly, and checked routinely for transformed juveniles until 43 days post-infestation.

## Results

### Mussel survey

We found *Villosa constricta* at 24 of the 44 sites surveyed (Fig. 2), and numbers found ranged from 1 to 54 (Table 1), totaling 190 individuals (143 males, 47 females) across all sites. The median number of individuals found at sites where the mussel occurred was 3.5 (quartiles = 1.75 and 8.25). This species tended to occur in coarse sand and sand/gravel mixtures in areas with some current. The surveyor in the middle of the stream found 114 individuals

Figure 3. Left and right valve of an individual *Villosa constricta* cut along the posterior ridge. The four thin-sections created from each individual were adhered to glass slides.



compared to only 41 along the left bank and 35 along the right bank. The sites where the greatest number of individuals were found (Deep Creek, and the North Fork Little River) were characterized by stable and consolidated sand, gravel, and cobble mixes across the entire channel. Gravid females were found on 4 May, 11 June, 12 July, and 20 August (Table 1). Mean length of all males ( $42.9 \pm 6.8$  mm) was significantly larger than that of females ( $35.5 \pm 5.8$  mm) (t-test,  $p < 0.001$ ).

### Age and growth

The collected shells ranged in age from 3–14 years (Fig. 4), with a median age of 5 (quartiles = 4 and 8). Shells that were three years of age clearly exhibited sexual dimorphism. Age in female shells collected was fairly evenly distributed from age 3 to age 10 with a median age of 7 (quartiles = 4.75 and 9.25); however, male shells were much more abundant at ages 3–5 with a median age of 5 (quartiles = 4 and 7) (Fig. 5). Males and females were closest in size at the youngest ages, but males grew at a faster rate than females. The largest and oldest shell found in the midden was a 14-year-old male measuring 54 mm in length that looked quite old and eroded.

Table 1. Number of *Villosa constricta* found, gravidity, and mean length at 24 sites where the species occurred in surveys of 44 sites in the upper Neuse River basin in North Carolina during 2001. M = # of males found, F = # of females found, and T = total # found.

Sub-basin	Stream	Road crossing	Date surveyed	M	F	T	% of females as gravid	Mean length (mm) (min, max)
Eno	Stroud's Cr.	SR 1002	18 May	2	0	2	-	43 (42, 44)
Eno	Stroud's Cr.	SR 1555	24 May	0	1	1	0	54 (54, 54)
Eno	McGowan Cr.	SR 1338	30 May	3	0	3	-	44 (43, 47)
Eno	Eno R.	SR 1336	27 June	3	2	5	0	39 (25, 49)
Eno	W. Fork Eno R.	SR 1004	3 July	1	0	1	-	61 (61, 61)
		(upper)						
Eno	E Fork Eno R.	SR 1332	6 July	1	0	1	-	48 (48, 48)
Eno	Sevenmile Cr.	SR 1120	12 August	1	1	2	0	55 (55, 55)
Eno	W. Fork Eno R.	SR 1004	20 August	5	1	6	100	38 (27, 53)
		(lower)						
Eno	Eno R.	SR 1561	21 August	2	0	2	-	54 (42, 65)
Flat	Deep Cr.	SR 1723	4 May	0	4	4	50	42 (39, 44)
Flat	Deep Cr.	SR 1734	11 June	38	16	54	18.8	38 (28, 52)
Flat	S. Flat R.	SR 1125	20 June	10	3	13	0	44 (33, 54)
Flat	Flat R.	SR 1614	28 June	1	2	3	0	39 (31, 47)
Flat	S. Flat R.	SR 1123	5 July	5	1	6	-	46 (38, 53)
Flat	S. Flat R.	US 501	18 July	12	4	16	0	38 (29, 46)
Flat	N. Flat R.	SR 1715	25 July	1	0	1	-	33 (33, 33)
Flat	Flat R.	SR 1771	9 August	1	0	1	-	41 (41, 41)
Little	Forrest Cr.	SR 1548	24 April	1	0	1	-	41 (41, 41)
Little	S. Fork Little R.	SR 1538	25 June	6	0	6	-	37 (31, 44)
Little	S. Fork Little R.	NC 157	26 June	10	1	11	0	54 (48, 60)
Little	N. Fork Little R.	SR 1538	12 July	19	9	28	12.5	42 (24, 55)
Little	S. Fork Little R.	NC 57	19 July	7	2	9	0	46 (41, 52)
Little	S. Fork Little R.	SR 1540	23 July	3	0	3	-	46 (38, 50)
Little	S. Fork Little R.	SR 1461	14 August	3	0	3	-	54 (48, 60)

**Host-fish determination**

Trial 1 yielded only two live individuals, which transformed on *Lepomis cyanellus* (Rafinesque) (green sunfish) (Table 2). This fish species was used again in Trial 2 along with other *Lepomis* spp., but that

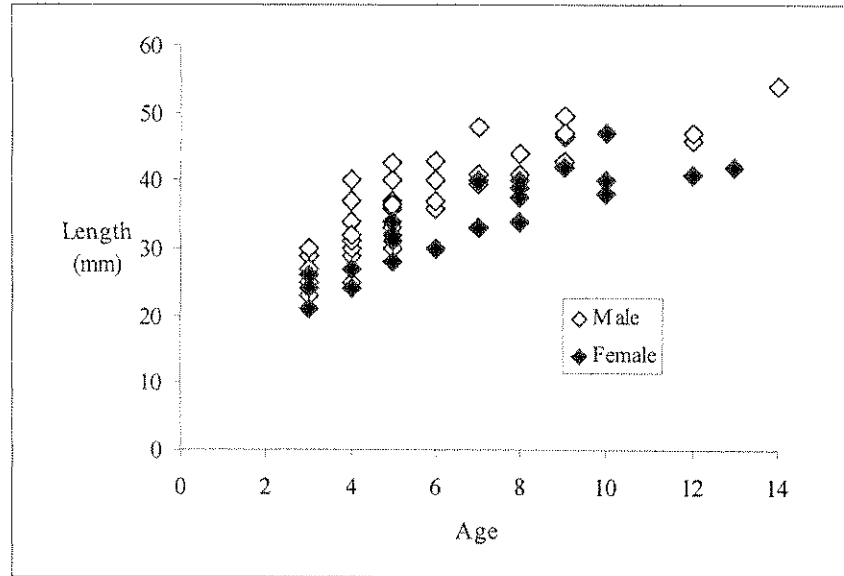


Figure 4. Length-at-age for male and female *Villosa constricta* in the West Fork of the Eno River in Orange County.

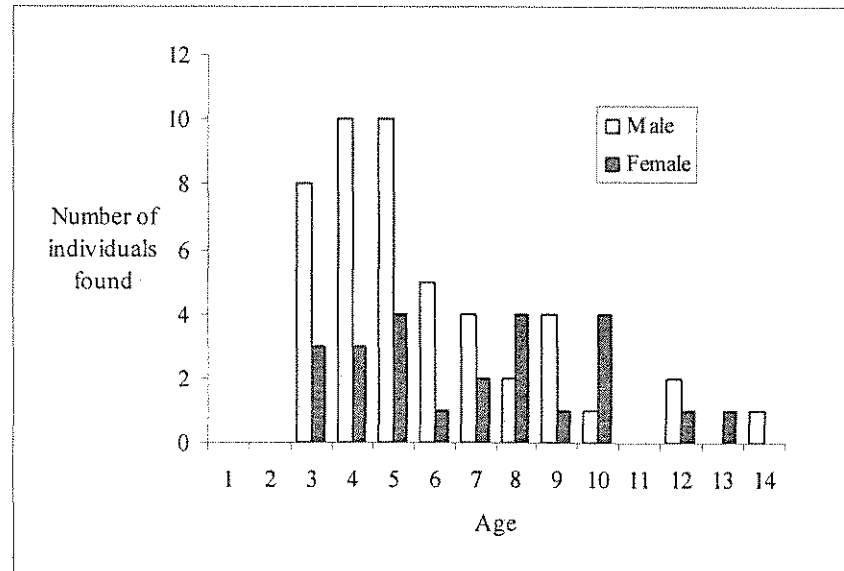


Figure 5. Age-frequency distribution of male and female *Villosa constricta* shells found in the West Fork of the Eno River in Orange County.

infestation produced juvenile mussels only from the fantail darters (Table 3). A total of 14 juveniles were produced by 5 *Etheostoma flabellare* with a transformation time of 22 ( $n = 13$ ) and 26 ( $n = 1$ ) days, so we decided to focus our efforts in Trial 3 on this darter species. By hand-infesting one gill each on 123 *E. flabellare*, we produced a total of 192 juvenile mussels with a mean transformation time of  $35.9 \pm 4.3$  days (SD) (Range = 21–44 days) at 18 °C. The only other species in that trial, *Percina roanoka*, did not produce any transformed mussels.

Table 2. Number of fish infested with *Villosa constricta* and the number of live juveniles produced during Trial 1.

Fish species (common name)	Fish species (scientific name)	# of fish exposed	# of days to complete metamorphosis	# of juvenile mussels recovered
Bluegill	<i>L. macrochirus</i>	6	n/a	0
Bluehead chub	<i>Nocomis leptocephalus</i>	6	n/a	0
Fantail darter	<i>E. flabellare</i>	5	n/a	0
Flat bullhead	<i>Ameiurus platycephalus</i>	2	n/a	0
Green sunfish	<i>Lepomis cyanellus</i>	8	22	2
Johnny darter	<i>Etheostoma nigrum</i>	5	n/a	0
Largemouth bass	<i>Micropterus salmoides</i>	3	n/a	0
Margined madtom	<i>Noturus insignis</i>	8	n/a	0
Northern hogsucker	<i>Hypentelium nigricans</i>	3	n/a	0
Redbreast sunfish	<i>L. auritus</i>	2	n/a	0
Swallowtail shiner	<i>Notropis procne</i>	6	n/a	0
White shiner	<i>Luxilus albeolus</i>	5	n/a	0

Table 3. Number of fish infested with *Villosa constricta* and the number live juveniles produced during Trial 2.

Fish species (common name)	Fish species (scientific name)	# of fish exposed	# of days to complete metamorphosis	# of juvenile mussels recovered
Bluegill	<i>Lepomis macrochirus</i>	2	n/a	0
Bluehead chub	<i>Nocomis leptocephalus</i>	3	n/a	0
Fantail darter	<i>Etheostoma flabellare</i>	4	22–26	14
Flat bullhead	<i>Ameiurus platycephalus</i>	1	n/a	0
Glassy darter	<i>Etheostoma vitreum</i>	1	n/a	0
Green sunfish	<i>Lepomis cyanellus</i>	2	n/a	0
Largemouth bass	<i>Micropterus salmoides</i>	4	n/a	0
Margined madtom	<i>Noturus insignis</i>	3	n/a	0
Northern hogsucker	<i>Hypentelium nigricans</i>	2	n/a	0
Notchlip redhorse	<i>Moxostoma collapsum</i>	4	n/a	0
Redbreast sunfish	<i>Lepomis auritus</i>	1	n/a	0
Roanoke darter	<i>Percina roanoka</i>	1	n/a	0
Satfin shiner	<i>Cyprinella analostana</i>	5	n/a	0
Swallowtail shiner	<i>Notropis procne</i>	1	n/a	0
Warmouth	<i>Lepomis gulosus</i>	1	n/a	0
White shiner	<i>Luxilus albeolus</i>	4	n/a	0



## Discussion

### Mussel survey

According to our surveys, this mussel was widespread across the study area and was the second-most abundant species next to *Elliptio complanata* (Lightfoot) in the Upper Neuse basin; however, rarely was it locally abundant. Despite surveying 600 m of stream length at each site, we found more than 10 individuals at only 5 sites and young individuals less than 40-mm long at only 12 sites. Of those 12 sites containing only larger *Villosa constricta*, only 1 of them had more than 3 individuals. Although growth rates may likely differ between sites, our age and growth data showed that the average males of this species are likely 7 years old before they reach 40 mm in length. The lack of individuals under 40 mm indicates that multiple year classes are missing in areas where the species is sparse (i.e., lack of recent recruitment).

In summary, there are few relatively healthy populations in the area that are consistently experiencing recruitment. Those sites with the healthiest populations were in highly stable channels with riffle-run complexes. By contrast, sites where the species was not found tended to be characterized by unstable shifting substrates. Mussels require stable substrates to persist in streams (Di Maio and Corkum 1995, Johnson and Brown 2000, Strayer 1999), but many of the streams in the upper Neuse basin are highly incised and lack the instream refugia that unionids require. Although much of the study area is currently forested and streams are generally well buffered, the habitat suffers from past land use and subsequent stream degradation (Trimble 1974). Many other mussel species in North Carolina are rarer than *Villosa constricta*, yet even this species of special concern, was rarely abundant at individual sites. Our survey findings serve to emphasize the importance of protecting unspoiled streams from further development to prevent the disappearance of rare species that depend on high quality habitats.

Overall, we only found 7 gravid females during the 2001 surveys. This was largely due to the fact that *Villosa constricta* was most abundant at sites surveyed at the end of the brooding season. The single individual we found gravid in July was fully gravid and likely just late in releasing its glochidia compared to the several other females found at the site. According to Johnson (1970), the brooding season begins in August and ends in June, so the individual found on 20 August was likely at the beginning of the next brooding period.

### Age and growth

Thin-sectioning is an effective means of estimating the age of freshwater mussels (Neves and Moyer 1988). In this study, female *Villosa constricta* appear closest in size to males at age 3 (the youngest shells found), but over time, males grow at a faster rate. This trend has been documented in other Lampsilines (Hanlon and Levine 2004, Rogers et al. 2001) and may be due to

females investing more energy into brooding rather than into shell growth. Overall, the male:female sex ratios were 2:1 in the middens. The abundance of males in shell middens was primarily due to the year classes aged 3 to 5, where males outnumbered females 3:1. Older mussels in middens had sex ratios closer to 1:1. Possible reasons why more young mussels were male include an actual higher number of males in certain year classes, differences in behavior at those ages resulting in increased predation of males, or delayed maturity of females causing their shells to appear more like that of a male. We clearly saw sexual dimorphism in age-3 shells, indicating that this species may be sexually mature at this age. *Toxolasma pullus* (Conrad) (Savannah lilliput), another Lampsiline from the North Carolina piedmont, was sexually dimorphic at age three (Hanlon and Levine 2004), and *Lampsilis cardium* (Rafinesque), *Lampsilis fasciola* (Rafinesque), and *Villosa iris* (I. Lea) have been shown to spawn and become gravid at age 3+ in captivity (James B. Layzer, Tennessee Technological University, Cookeville, TN, pers. comm.). However, perhaps some individuals of the species may mature at a slower rate while not exhibiting the female shell form until after age three. If so, this could cause misidentification of the sex of younger shells and an incorrectly skewed male:female ratio in these year classes.

The oldest shell in this study was estimated to be 14 years of age, and it appeared quite old and eroded. Of the 190 live *Villosa constricta* seen in our surveys of the upper Neuse basin, only 10 individuals (5.3%) were larger than this mussel. We suspect few mussels of this species live much longer than this. Although some freshwater mussel species may be extremely long-lived (Ziuganov et al. 2000), *V. constricta* appears to live less than two decades in this area. This appears to be the case with other small Lampsilines in coastal drainages in North Carolina (Hanlon and Levine 2004). This study demonstrates that useful life-history data important to species management can be garnered from shell middens.

#### Host-fish determination

Additional testing was needed to determine the host(s) this species actually uses for natural recruitment to occur. We used a batch-infestation method in the first trial in an attempt to expose each fish to the same concentration of glochidia; however, the larvae were likely too dilute in the exposure tank for sufficient infection of the fishes' gills. The lone producer of juvenile mussels (*Lepomis cyanellus*) in the first trial was retested by hand infestation and found not to support metamorphosis. Often when one species of *Lepomis* is found to serve as a host of a given mussel species, congeners will also serve as hosts for that species (C.B. Eads, unpubl. data; Fuller 1974; Jenkinson 1982). We used 16 individuals of the genus *Lepomis* in Trial 1 and 6 individuals in Trial 2, yet only 2 live juveniles were produced. Watters et al. (1999) used a total of 19 fish representing 4 species of this genus and also had poor transformation rates (total of 3 juvenile mussels). We conclude that *Lepomis* are poor hosts and would not recommend them for propagation of *Villosa constricta*.

Both the second and third trials indicated that *Etheostoma flabellare* is a suitable host for this species; however this species is rare in the Cape Fear River basin (Menhinick 1991) where *Villosa constricta* is abundant. So there is likely another natural host for this species. Since *Etheostoma nigrum* (Rafinesque) (johnny darter), was only used in our initial trial that was unsuccessful, we believe additional trials with this species and its close relative, *Etheostoma olmstedi* (Storer) (tessellated darter), should be conducted to determine viable hosts in the Cape Fear basin. *Etheostoma olmstedi* was one of the hosts reported by Watters et al. (1999) that does co-occur with *V. constricta*.

Our knowledge of the basic biology of freshwater mussels is still markedly deficient—particularly in the Atlantic Slope, where few life-history studies have been conducted. This study represents one step towards understanding these species, but additional work is needed to: 1) refine our understanding of freshwater mussel reproductive strategies; 2) develop a clear understanding of freshwater mussel diets, growth, and metabolism; 3) assess the effects of anthropogenic activity and environmental perturbation on population health, survival, and abundance; 4) clarify these species' role in sustaining the health of freshwater ecosystems; and 5) define the conservation measures that support the stewardship of freshwater mussel populations.

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