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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; Watters, 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; Lefevere & 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 ± 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.

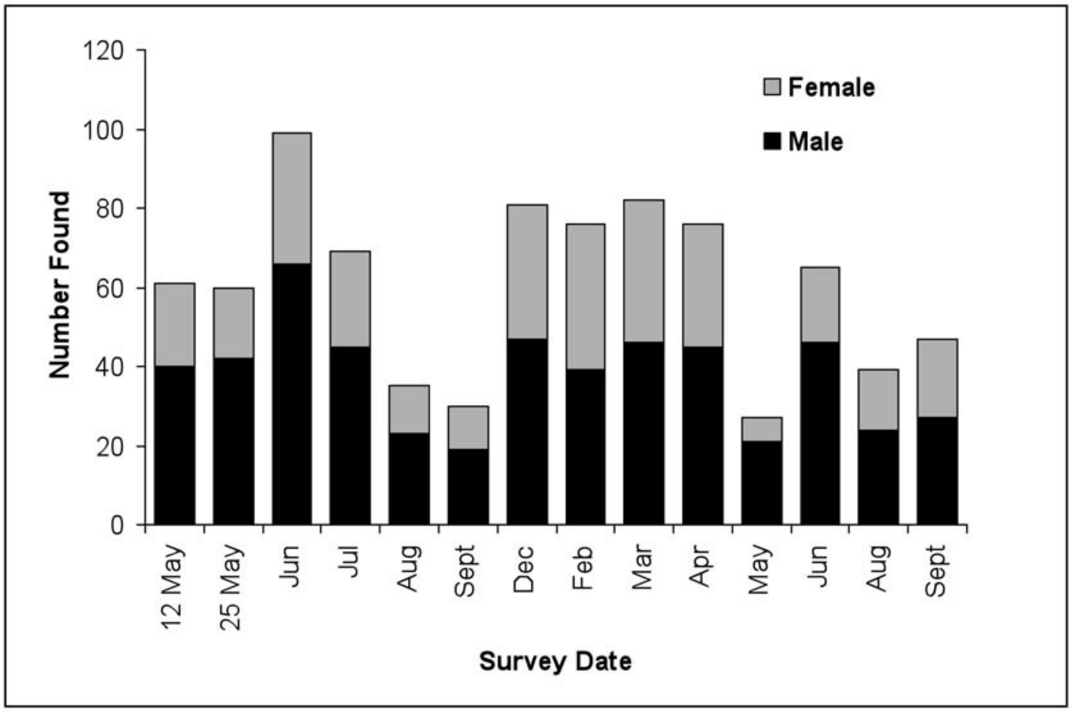

FIGURE 1

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

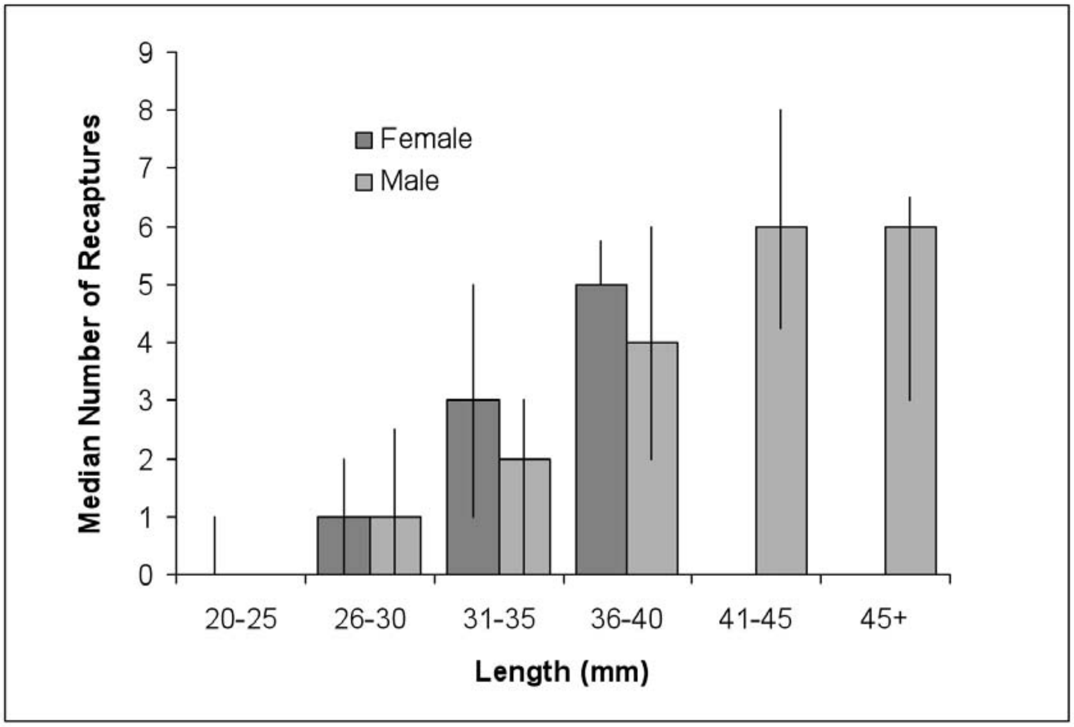

FIGURE 2

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

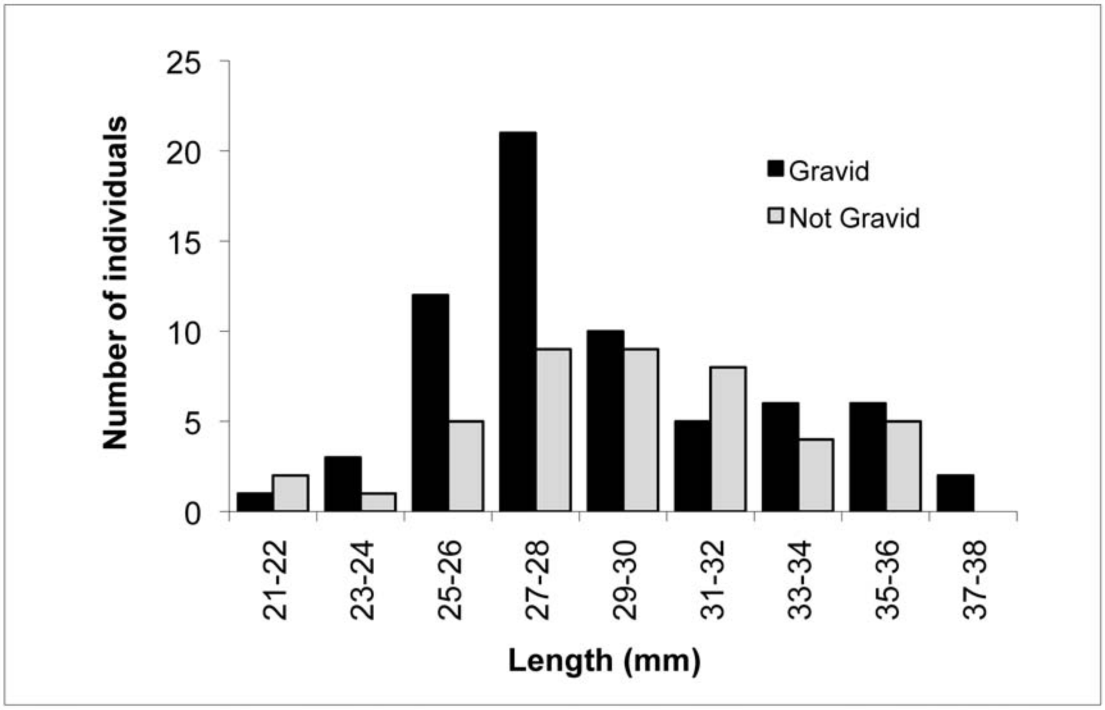

FIGURE 3

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

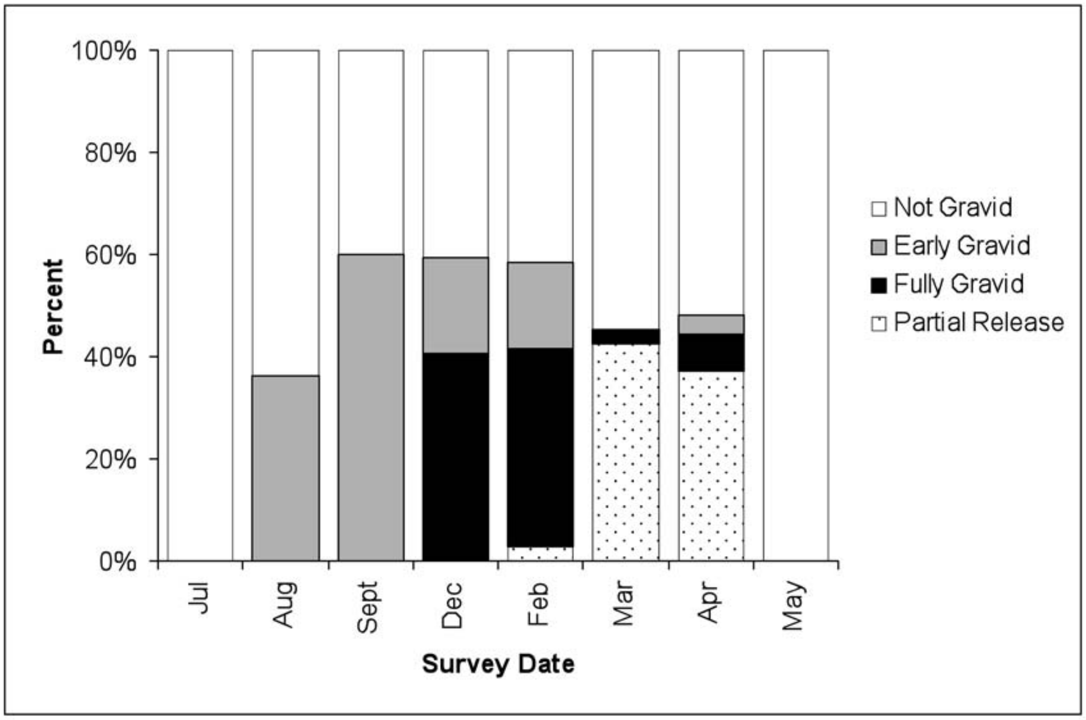

FIGURE 4

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,

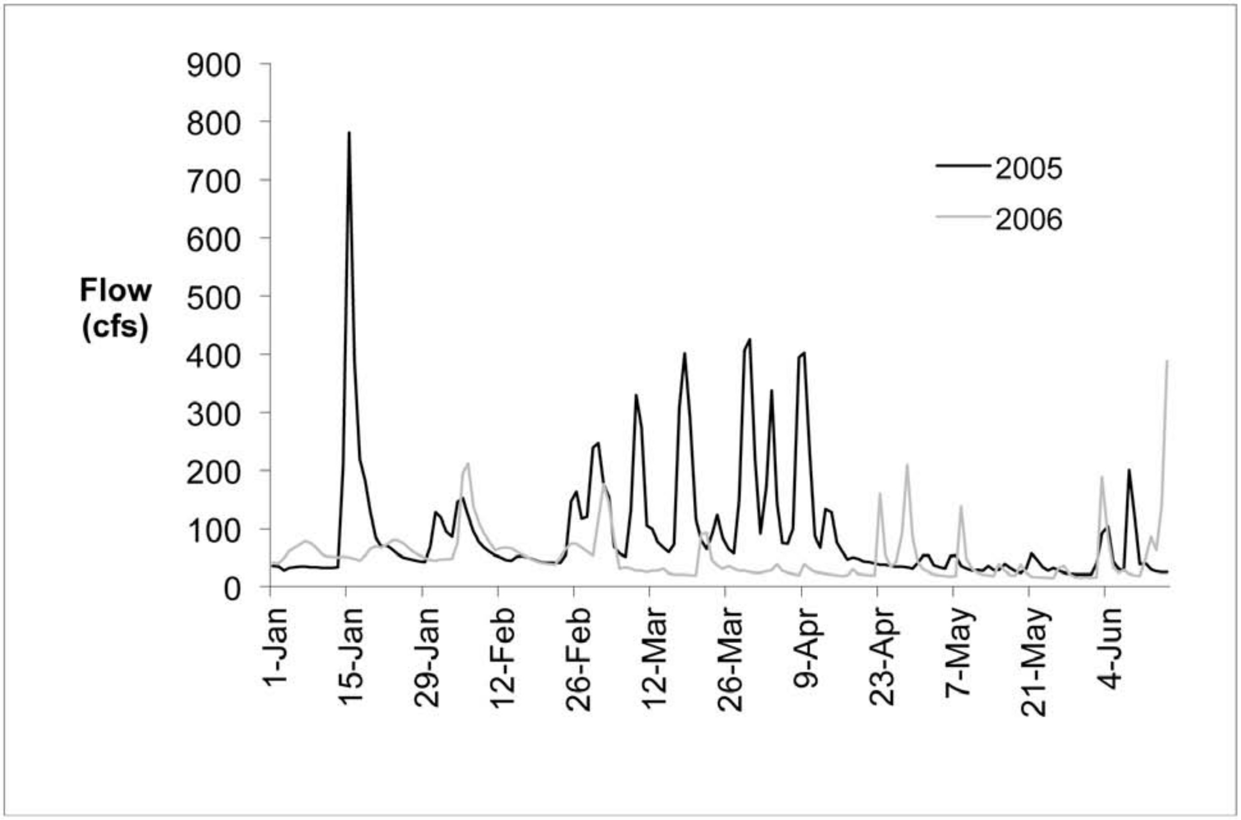

FIGURE 5

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 coworkers (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 (Villella 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 japanensis* (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 bradytichtic 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 bradytichtic 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 *Glebula 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 bradytichtic and tachytichtic 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
Elliptio complanata	980	-	-	-	-
Villosa constricta	244	130	114	53.3	46.7
Villosa delumbis	62	36	26	58.1	41.9
Lampsilis sp.	59	52	7	88.1	11.9
Villosa vaughaniana	14	9	5	64	36
Fusconaia masoni	9	-	-	-	-
Pyganodon cataracta	8	-	-	-	-
Strophitus undulatus	4	-	-	-	-
Alasmidonta varicosa	1	-	-	-	-