Hydrobiologia (2009) 636:191–201
DOI 10.1007/s10750-009-9948-9

PRIMARY RESEARCH PAPER

Influence of urban tributaries on freshwater mussel populations in a biologically diverse piedmont (USA) stream

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Received: 17 February 2009 / Revised: 9 August 2009 / Accepted: 11 September 2009 / Published online: 19 October 2009

Abstract The Southeastern USA is currently experiencing a period of rapid growth of human populations that is likely having profound effects on the region's unique aquatic biota. Using both survey data and experimental protocols, we assessed the influences of water and habitat quality on freshwater mussel populations in a small Piedmont stream. Chewacla Creek is a high-quality stream located near the rapidly growing towns of Auburn and Opelika in east-central Alabama. From 1999 to 2007 we monitored freshwater mussel populations and measured substrate and water chemistry variables in Chewacla Creek. Surveys revealed that mussel abundance decreased substantially concomitant with degraded habitat and water quality downstream of three highly urbanized tributaries. We conducted sentinel trials using adult mussels (*Villosa lienosa*) in Chewacla Creek and a tributary (Parkerson Mill Creek) that receives wastewater discharge. Sentinel mussels were placed in cages at three locations downstream of the effluent discharge point and at one upstream site (control). Sentinel mussels exposed to wastewater discharge exhibited lower survival rates compared to control animals. Together, survey and experimental data suggest that degraded tributary subcatchments may fragment mussel populations in high-quality streams. Moreover, our data indicate that protection of sensitive aquatic taxa necessitates effective management of water quality across large spatial scales.

Keywords Urbanization · Unionidae · Alabama · Water quality · Habitat quality

Handling editor: R. Bailey

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Introduction

North America's freshwater ecosystems and their biota in formerly rural areas are under increasing pressure from rapidly expanding human populations (Benke, 1990; Groffman et al., 2003). Despite the passage of several high profile federal statutes intended to stem the loss of rare species and their habitats, many taxa and their necessary habitats continue to decline (Neves et al., 1997; Warren et al., 1997; Ricciardi & Rasmussen, 1999). Freshwater mussels (Bivalvia: Unioniformes) were historically widespread in North American streams and rivers but are now among the most globally imperiled freshwater organisms (Williams et al., 1993; Bogan, 1996; Ricciardi & Rasmussen, 1999; Lydeard et al., 2004; Strayer et al., 2004; Williams et al., 2008). Of the

~250 species of Unioniformes historically found in the Southeastern U.S., 75% are thought to be extinct or at risk of extinction (Williams et al., 1993; Neves et al., 1997).

The Unioniformes have unique life history characteristics compared to other freshwater invertebrates. Many freshwater mussel species are long-lived (up to ~100 years) and the larvae of most species are obligate fish parasites. Recruitment rates may fluctuate greatly from year-to-year. As adults, mussels are relatively sedentary filter- and deposit-feeders (reviewed in Vaughn & Hakenkamp, 2001) but are occasionally displaced by large, streambed-altering floods (Vannote & Minshall, 1982; DiMaio & Corkum, 1995; Tucker, 1996; Hastie et al., 2001). Unionids often aggregate in stable regions of the streambed or in microhabitats that experience less intensive flooding or scouring (Strayer, 1999; Johnson & Brown, 2000; Howard & Cuffey, 2003; Gangloff & Feminella, 2007a). Incised channels with unstable sand or unconsolidated gravel substrates typically support few mussels (Hartfield, 1993; Brown & Curole, 1997; Brim-Box et al., 2002; Gillies et al., 2003).

Catchments that drain urbanized landscapes frequently experience radical physical and chemical alterations. Both the removal of streamside vegetation and increased impervious surface typically result in increased surface runoff and hydrologic variability (Trimble et al., 1987; Potter, 1991). These changes, in turn, lead to increased rates of erosion, increased loads of suspended and deposited fine sediments (Glenn, 1911; Ellis, 1936; Magilligan & Stamp, 1997), and elevated nutrient concentrations (Paul & Meyer, 2001; Groffman et al., 2003).

Physicochemical changes associated with urbanization have profound implications for aquatic biota and ecosystem function. For example, fish assemblages in small streams in the Southeastern U.S. vary with level of urbanization and are likely a consequence of stream thermal regime and substrate composition. Rural or forested catchment fish assemblages are dominated by cyprinids (minnows) and other lithophilic (gravel or cobble associate) spawners while urbanized catchments are dominated by centrarchids (sunfish) and other more tolerant fish taxa (Roy et al., 2003; Helms et al., 2005). Similarly, in larger streams of the Southeastern U.S., mussel extirpation rates are greatest in areas with high levels of impervious surfaces in developing suburbs (Gillies et al., 2003).

Changes to stream physicochemical variables are likely the principal factors responsible for the extinction and extirpation of many southeastern mollusks (Neves et al., 1997; Brim-Box & Williams, 2000; Gillies et al., 2003; Lydeard et al., 2004). Although several recent studies have examined associations between non-point-source pollution and changes in freshwater mollusk assemblages (Arbuckle & Downing, 2002; Gillies et al., 2003) relatively few have quantified in situ effects of point-source impacts like municipal wastewater discharges or attempted to understand how point-source and non-point source impacts act in synergy. Horne & McIntosh (1979) conducted among the first studies of wastewater effluent effects on freshwater mussels in a Texas river. They found that although physicochemical conditions were not acutely toxic to mussels, large sections of the study river were devoid of mussels downstream of the effluent discharge. Goudreau et al. (1993) examined effects of wastewater discharge on mollusk assemblages in the Clinch River in Virginia, USA. They reported that unionids were often absent from large (~4 km) reaches below wastewater treatment facilities. Based on laboratory toxicity assays, Goudreau et al. (1993) hypothesized that glochidial sensitivity to monochloroamine and unionized ammonia may be responsible for mussel extirpations downstream of wastewater outflows.

However, when wastewater is discharged into already impaired urban streams it becomes difficult to separate effects of water quality from those of physical habitat degradation linked to catchment land use (Paul & Meyer, 2001; Groffman et al., 2003). Here, we examine the impacts of the quality of water and habitat on a freshwater mussel assemblage in a 4th-order, high-quality stream flowing through a rapidly developing catchment. We employ both correlative and experimental methodologies to assess the relative impacts of wastewater discharge and habitat degradation on survival rates of freshwater mussels.

Methods

Study site

Chewacla Creek is a major tributary of Uphapee Creek and the Tallapoosa River. Uphapee Creek drains 862 km^2 of the Piedmont and Gulf Coastal

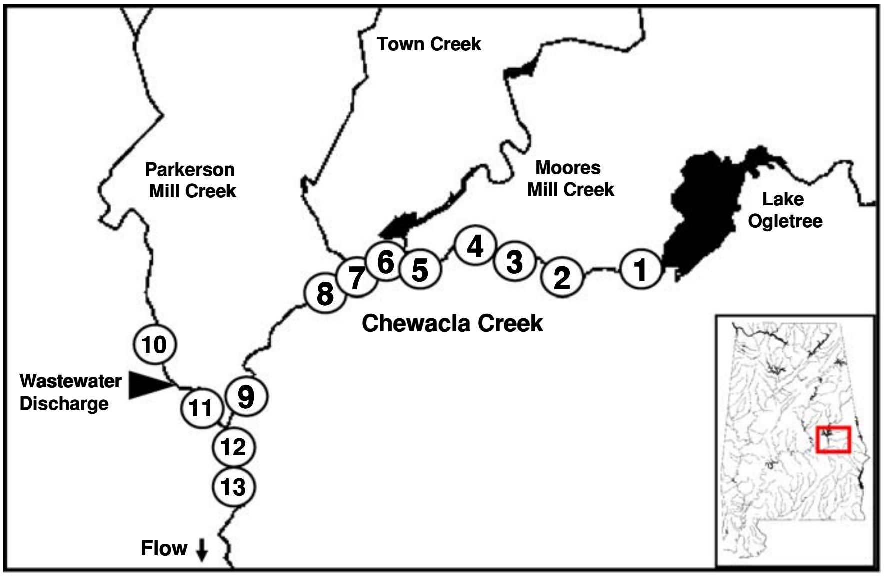

Fig. 1 Map of study site in East-central Alabama showing approximate locations of study sites downstream of Lake Ogletree. Site 14 is not depicted in this map but is located in Chewacla Creek ~20 km downstream of Site 13

Plain Physiographic Provinces (Fig. 1; Jenkinson, 1973). The headwaters of Chewacla Creek are near the southern limits of Opelika, Alabama and the stream flows west until it is impounded by the dam that forms Lake Ogletree. Lake Ogletree is a small, shallow impoundment in the upper reaches of Chewacla Creek that supplies Auburn with drinking water (Fig. 1). After leaving Lake Ogletree, Chewacla Creek flows southwest along the southern border of Auburn, Alabama, then south to its confluence with Opintlocco Creek. The two streams form Uphapee Creek.

During the last 15 years, the rapidly growing cities of Opelika and Auburn have greatly influenced land use patterns in the Chewacla Creek watershed. The communities combined have ~70,000 residents and both rely heavily on three small (4th–5th Order) streams for drinking water, waste removal, and recreation (Chewacla Creek, Saugahatchee Creek (a Tallapoosa River tributary), and Little Uchee Creek (an adjoining Chattahoochee River tributary). Currently, the lower section of Chewacla Creek (extending from the Lee County Road 010 bridge to the Opintlocco/Uphapee confluence) is classified as a high-quality fish and wildlife waterway by the state of Alabama (http://www.adem.state.al.us/WaterDivision/WQuality/WQMainInfo.htm). However, both Parkerson Mill and Moores Mill creeks are listed by the Alabama Department of Environmental Management (ADEM) as impaired waterways and both streams were included on Alabama's 2008 303d streams list (http://www.adem.state.al.us/WaterDivision/WQuality/WQMainInfo.htm).

The city of Auburn's south side wastewater plant is permitted to discharge a peak flow of 9 million gallons per day (mgd, ~0.3 m/s) into Chewacla Creek from its secondary treatment facility. Mean discharge levels over the past 3 years ranged from 3.8 to 4.5 mgd. Treated waters are chlorinated prior to their release into Parkerson Mill Creek. Effluent standards for chlorine are 0.01 mg/l in Alabama and the facility has been cited for exceeding these limits (0.02 mg/l) on two occasions in the past 3 years (http://www.epa-echo.gov/cgi-bin/ideaotis.cgi). We are unaware of any other recent infractions for water quality violations involving this facility.

Land use in much of the Uphapee sub-basin remains mixed agriculture/silviculture. Heaviest residential and urban development occurs in the headwaters and along the northern edge of the sub-basin (Fig. 1). The Uphapee sub-basin also includes the entire Tuskegee National Forest, the smallest National Forest Unit in the U.S., a ~45 km^2 parcel of recovering agricultural fields and pine plantations straddling the Piedmont-to-Coastal Plain transition zone (i.e., fall line; McGregor, 1993). Above the fall line, Uphapee sub-basin streams are high-gradient, low-conductivity streams with gravel/bedrock dominated substrate (Hurd, 1971; Jenkinson, 1973). Below the fall line, Uphapee basin streams are more typical of Coastal Plain systems and have higher conductivities, lower gradients, and the dominant substrates are sand, small gravel, or claystone.

Water and habitat quality measurements

We monitored water quality annually from 2000 to 2007 and monthly during cage trials. Additionally, we attempted to collect water samples during both high and low flow periods to estimate the extremes of water conditions during the trial. Water grab samples were made in mid-channel, placed on ice, and returned to the lab as soon as possible. We measured nitrate-nitrogen (NO_3-N), inorganic phosphorus (as both total phosphate, TP, and soluble reactive phosphate, SRP), and total ammonia nitrogen (TAN) concentrations using standard methods (APHA, 1998; Table 1).

All analyses were run either immediately or according to recommended holding times, most on the day of collection. We ran standards with all analyses and maintained a log of previous runs as part of our quality assurance protocol and for comparison with previous analyses of Chewacla Creek and adjacent watersheds. Percent recoveries for all water quality samples ranged from 93 to 95%. Two blanks (field and equipment) were analyzed per sampling date and duplicates were run on one sample (all three parameters) per sampling date to ensure assay precision (APHA, 1998).

Substrate measurements were made at nine sites in Chewacla Creek from ~2 km downstream of Lake Ogletree (site 2) to 600 m downstream of the Parkerson Mill confluence (site 13). We used a modification of the standard particle size measuring protocol first described by Wolman (1954). At each site, we measured 20 particles at randomly selected locations along 15 transects located at 10 m intervals (total site length = 150 m, particle $n = 300$ per site). All particles >2 mm diameter were measured but sand (2–0.2 mm diameter), silt (<0.02 mm diameter), wood, organic material (algae mats, macrophytes, leaf pack), and bedrock were noted categorically. We calculated the mean percentage of particles classified as sand and silt in each transect and site.

Table 1 List of water chemistry parameters measured for each grab sample collected from Chewacla and Parkerson Mill creeks, Lee County, Alabama from 2003 to 2006

Variable	Method
Total ammonia nitrogen (TAN)	Phenate
Nitrate nitrogen (NO_3-N)	Diazotizing
Nitrite nitrogen (NO_2-N)	Cadmium reduction
Total nitrogen	Persulfate digestion, UV spectrophotometry
Total phosphorus	Persulfate digestion, ascorbic acid
Soluble reactive phosphorus (SRP)	Ascorbic acid

Methodologies described in American Public Health Association (1998)

Mussel surveys

In order to assess mussel abundance and assemblage structure, we conducted timed searches and quadrat excavation episodically between 1999 and 2006 (Table 2). We searched reaches using a visual-tactile protocol at a rate of ~1 h per 50 longitudinal m of stream channel. Mussel abundance was estimated as number of mussels captured per hour and reported as catch per unit effort (CPUE). In addition to timed searches, we searched for and collected dead shells during visits to monitor and clean cages (see below). We used results from both recent qualitative and semi-quantitative surveys to create species lists for the study reaches (Table 2; Gangloff & Feminella, 2007b). We used historical records (e.g., Hurd, 1971; Jenkinson, 1973) and examined specimens in the Auburn University Museum to assess mussel assemblage changes over the last 35 years.

Sentinel mussel trials

Mussels (*Villosa lienosa*, $n = 36$ per trial) were obtained from a reach of Chewacla Creek, upstream of Lake Ogletree and in a relatively rural area southeast of Auburn and Opelika (Fig. 1). Previous surveys indicated that *V. lienosa* was relatively common (~50–250 per 50 m of stream reach, Gangloff & Feminella, 2007b) in Chewacla Creek upstream of Lake Ogletree. *Villosa lienosa* is found in the study reach (but at lower densities than in reaches above Lake Ogletree) and was historically found in all three tributaries. Mussels were labeled with Bee Tags® (The Bee Works, Orillia, Canada) and placed in hardware cloth cages. We recorded sex and measured maximum length (range 36.5–63.4 mm) of all mussels prior to beginning of the trials. Mussels were assigned to cages

Table 2 List of sites referenced in this study and site parameters including site description, link magnitude (LM) the number of upstream first order tributaries, years during which mussel surveys were conducted and the total number of mussels detected by surveys, mussel catch per unit effort (CPUE, number of mussels detected per hour), and the number of mussel species found alive during each year's surveys

Site description	LM	Mussel survey	Number Mussels	Mussel CPUE	Live Taxa
1. Chewacla Creek 200–350 m downstream Lake Ogletree	55	2003	64	16.0	5
		2004	21	5.3	4
		2005	10	2.5	4
2. Chewacla Creek ~2 km downstream Lake Ogletree	55	2000	93	18.6	7
		2001	12	2.4	5
		2002	19	3.8	6
		2006	25	8.3	7
3. Chewacla Creek ~3 km downstream Lake Ogletree (Pretty Hole)	56	2002–2006	0	0	0
4. Chewacla Creek ~3.5 km downstream Lake Ogletree (Sink Hole)	57	2002–2006	0	0	0
5. Chewacla Cr. 50–200 m upstream Moores Mill Creek	58	2005	0	0	0
6. Chewacla Creek 0–150 m downstream of Moores Mill Creek	73	2000–2002	0	0	0
7. Chewacla Creek 0–150 m upstream Town Creek	73	2000–2002	0	0	0
8. Chewacla Creek 0–150 m downstream Town Creek.	82	2005	0	0	0
9. Chewacla Creek 50–200 m upstream Parkerson Mill Creek	85	2005	12	3.0	3
		2007	42	2.33	5
10. Parkerson Mill Creek at Lee County Road 10, upstream Auburn wastewater discharge	14	2001	0	0	0
11. Parkerson Mill Creek nr Chewacla confluence, downstream Auburn wastewater discharge	14	2004–2006	0	0	0
12. Chewacla Creek 0–150 m downstream Parkerson Mill Creek	103	2005	1	0.17	1
		2006	1	0.17	1
13. Chewacla Creek 450–600 m downstream Parkerson Mill Creek	105	2005	0	0.0	0
		2006	2	0.33	2
14. Chewacla Creek at U.S. Hwy 80, Macon Co., AL	183	2007	115	6.39	7

and groups such that the sexes and size class were evenly distributed.

We constructed mussel cages using two layers of hardware cloth that allowed for water circulation and prevented the escape of animals. The hardware cloth cages measured 190 × 90 × 160 cm and were held together with non-toxic hot glue and plastic cable ties. Cages were filled with ~6 cm of gravel and sand (collected in situ) and anchored into the streambed using 30 cm lengths of rebar.

We conducted sentinel mussel trials during two periods (from 29 August 2005 to 5 January 2006 and from 10 February to 10 June 2006). Each trial consisted of four treatment groups that corresponded to four locations in Parkerson Mill and Chewacla Creeks. Each treatment group consisted of three cages, each with three mussels. The control group was located in Chewacla Creek approximately 100 m upstream of the confluence of Parkerson Mill Creek (site 9; Fig. 1). Cages were also placed in Chewacla Creek approximately 50 and 600 m downstream of the Parkerson Mill Creek confluence (sites 12 and 13, respectively, and in Parkerson Mill Creek ~50 m upstream of its confluence with Chewacla Creek (site 11; Fig. 1). Site 11 is just downstream from the discharge for the city of Auburn's wastewater treatment plant.

Cages were monitored weekly or every other week to ensure they remained stable in the streambed and to measure mortality rates. During cage inspections, we removed accumulated debris, clipped 1 or 2 cable ties, and then removed the mussels while leaving the cage embedded in the streambed. Occasionally, cages were buried by sedimentation, or needed sediment removed to allow mussels adequate space. During cage monitoring, we examined mussels and assessed body condition by attempting to pry open shells using fingernails; healthy mussels are very difficult to open in this manner (MMG personal observation). We examined soft tissues for any sign of deterioration and noted the condition of marsupial gills. After a brief examination, all mussels were returned to the cage. All mussels that were alive at the completion of the trial were killed and accessioned into the Auburn University Museum.

Statistical analyses

Within cage trials, individual sentinel mussels were considered replicates in survival calculations ($n = 9$ per site). Survivorship at a given point in time was calculated as the proportion of mussels still alive at each trial site. Trial data were analyzed separately. We used χ^2 analyses to test for differences in survival between the cage treatments in each trial.

Water chemistry data from the first four sites downstream of Lake Ogletree were pooled because we had only a few water samples at any individual site and these sites were all upstream of any point-source impacts to stream water quality. All nutrient concentrations were found to be normally distributed (Kolmogorov–Smirnov test $P < 0.05$) so we used parametric ANOVAs to examine differences across sites and LSD post-hoc tests to assess between-site differences.

We transformed proportional substrate data using the function: Arcsine (Square root N) but were not able to achieve normality. To account for non-normal data, we used a non-parametric Kruskal–Wallis test to examine substrate differences across sites.

Results

Physicochemical variables

Nutrient concentrations generally increased along a downstream gradient from Lake Ogletree, although increases for some were not statistically significant. Concentrations of all nutrients were relatively low in Chewacla Creek immediately downstream of Lake Ogletree and immediately upstream of Parkerson Mill Creek and elevated just below urbanized tributaries (Fig. 2). Nutrient concentrations peaked in lower Parkerson Mill Creek (downstream of the treated wastewater discharge) and in Chewacla Creek immediately downstream of the Parkerson Mill Creek confluence (sites 12 and 13; Fig. 2). ANOVA revealed significant differences in both NO_3-N and PO_4-P concentrations across sites ($F > 4.35$, $P < 0.001$, Fig. 2). TAN was not significantly different across sites but concentrations were always greatest in the lower Parkerson Mill Creek site. Post-hoc tests found

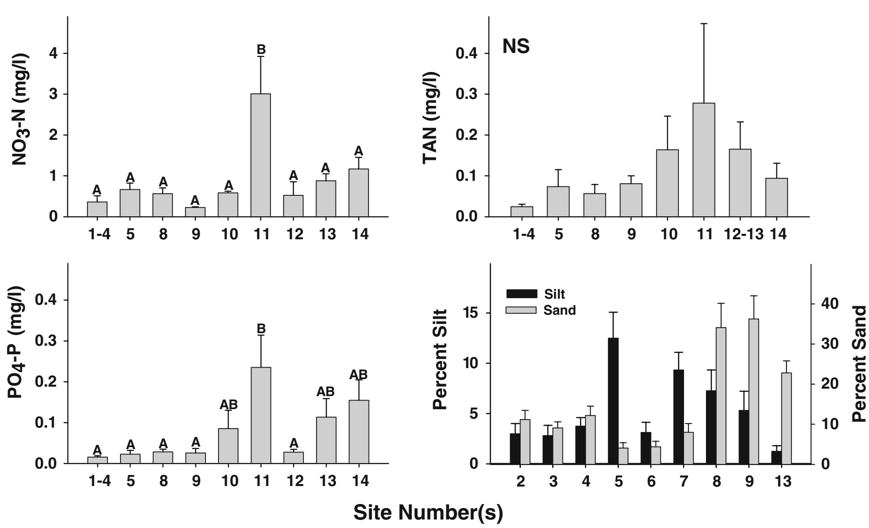

Fig. 2 Mean (±SE) of physicochemical parameters at study sites. Water quality data not available for sites 6 and 7. Substrate data not available for sites 1, 10, 11, 12, and 14. Substrate data were non-normal and Kruskal–Wallis test does not allow testing for differences between sites

that NO_3-N was only significantly different at the lower Parkerson Mill Creek site. NO_3-N concentrations at all other sites were not significantly different from one another (Fig. 2). PO_4-P concentrations were significantly greater at both the lower Parkerson Mill Creek site and in Chewacla Creek immediately downstream from the Parkerson Mill Creek confluence (Fig. 2).

Substrate composition also changed dramatically up- and downstream of the tributaries and Kruskal–Wallis tests indicated significant differences in substrate sizes across sites. Upstream of the urbanized tributaries, sand and silt compose a relatively low proportion of the substrate in Chewacla Creek (Fig. 2). However, as we moved downstream, the proportion of sand and silt tended to increase. We observed greater percentages of sand and silt in Chewacla Creek downstream from Town and Parkerson Mill creeks. However, we did not observe increased sand and silt composition in Chewacla Creek downstream from Moores Mill Creek. Moores Mill Creek is impounded just upstream of its confluence with Chewacla Creek and this likely impedes downstream sediment movements into Chewacla Creek (Fig. 2).

Mussel surveys

Over the 8-year-period, mussel surveys yielded nine live unionid taxa (and three additional taxa as weathered or fresh-dead shell material) between Parkerson Mill Creek and Lake Ogletree (Table 2). Mussel abundance and richness were relatively high in Chewacla Creek at sites 1 and 2 but declined at downstream reaches. Mussel assemblages appeared to recover downstream of Town Creek (site 9) but then abundances declined again immediately downstream of the confluence of Parkerson Mill Creek (sites 12 and 13; Table 2).

Cage trials

Average within-site survivorship of sentinel mussels over the two trial periods ranged from 100% at the control site in Chewacla Creek to 22% in Parkerson Mill Creek at site 11, just below the effluent discharge (Fig. 3). In both trials, within-trial survivorship was lowest for the Parkerson Mill Creek cage site but was matched by the downstream site (site 13) during the second trial (Fig. 3). Between-site survivorship was significantly influenced by site in both trials (autumn

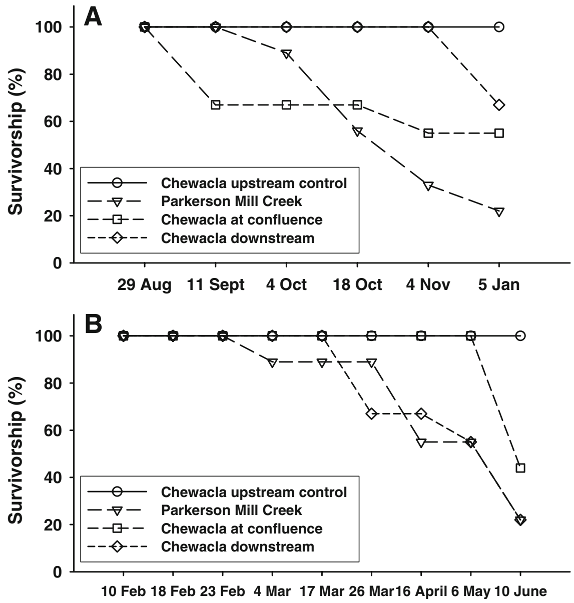

Fig. 3 Percent survivorship of sentinel mussels at four cage locations in Chewacla and Parkerson Mill Creek from 29 August 2005 to 5 January 2006 (**A**) and from 10 February to 10 June 2006 (**B**). Nine sentinel mussels were used at each cage location

$\chi^2 = 11.69$, $P = 0.009$; spring $\chi^2 = 14.6$, $P = 0.002$; Table 3). Direct comparisons between sites indicated that there were significant differences between the control site (site 9) and all sites during both the autumn and spring. However, few significant differences in survival were observed between the Parkerson Mill Creek site and the confluence and downstream cage sites (Table 3).

Mussels in Parkerson Mill Creek were easier to pry open and exhibited reduced adductor strength compared to the sentinel mussels at all other cage sites. This suggests that the animals in Parkerson Mill Creek were in poorer condition. Female mussels were generally easier to pry open than males and most were gravid during the trials.

Discussion

In Chewacla creek, mussel abundance, assemblage diversity, and survival varied with proximity to urban catchments. Our data indicate that urban-impacted catchments may have profound impacts on native freshwater mussels. The field surveys indicate that locations immediately downstream of urban-impacted

Table 3 Comparison of χ^2 analyses for survivorship of sentinel mussels from cage trials in Chewacla and Parkerson Mill creeks

Trial	Cage comparison	χ^2	*P* value
Fall 2006	1 vs. 2	3.60	0.058
	1 vs. 3	2.10	0.147
	1 vs. 4	11.46	0.001
	2 vs. 3	0.23	0.629
	2 vs. 4	3.60	0.058
	3 vs. 4	5.14	0.023
Spring 2006	1 vs. 2	0.00	1.00
	1 vs. 3	1.00	0.317
	1 vs. 4	11.46	0.001
	2 vs. 3	1.00	0.317
	2 vs. 4	11.46	0.001
	3 vs. 4	6.92	0.009

Overall Fall 2005 trial $\chi^2 = 11.69$, $P < 0.009$; Spring 2006 trial $\chi^2 = 14.6$, $P < 0.002$. Cage designations are as follows: 1. Parkerson Mill Creek, 2. Chewacla Creek 600 m downstream of Parkerson Mill Creek, 3. Chewacla Creek 50 m downstream of Parkerson Mill Creek, 4. Chewacla Creek 100 m upstream of Parkerson Mill Creek confluence

catchments support lower densities and lower biodiversity of mussels compared to the control sites. Moreover, immediately downstream of each confluence with an urban-impacted catchment Chewacla creek exhibited poor habitat (high sedimentation) and poor water quality. Our sentinel mussel experiment demonstrated that mussels placed within and immediately downstream of an urban-impacted catchment were less likely to survive compared to those placed upstream of the urban catchment.

High mortality rates could have been the result of poor water quality, extreme sedimentation, a combination of the two, or a factor not measured in this assessment. Because both water and habitat quality are likely poor in all three urban-impacted catchments, it may not be possible to disentangle the relative importance of water quality and substrate degradation on mussel survival. Downstream of each urbanized tributary, the water and habitat quality of Chewacla Creek recover gradually but mussel abundance generally remains low (CPUE < 5 mussels hr) for nearly 2 km. Habitat quality immediately downstream of Town and Parkerson Mill creeks is impaired by sedimentation and the effect is likely cumulative so impacts downstream of Parkerson Mill Creek are likely more substantial than those below other tributaries.

The sentinel trial data suggest that mussel survival can be reduced in urbanized streams and in mainstem reaches immediately downstream of their confluence. Although it is not possible in this case to determine the cause of mussel mortality, two lines of evidence implicate sedimentation rather than chronic low-level nutrient exposure punctuated by episodic acute exposures to higher concentrations during run-off events. First, most mortality did not occur until after 6 weeks (Fig. 3). If mortality were a consequence of acute water quality degradation, we would have expected higher mortality rates and earlier death of sentinel mussels. The TAN levels we measured were an order of magnitude less than levels reported to be acutely toxic to mussels although they were similar to chronic exposure toxicity levels (Augspurger et al., 2003). Additionally, adult freshwater mussel LC50s (i.e., the concentration at which 50% of a test population are dead after a given exposure period) for TAN are typically greater than LC50s for juvenile mussels or glochidia larvae, suggesting that adult mussels are less sensitive to TAN than earlier life stages (Augspurger et al., 2003). Thus, it seems unlikely that caged mussel mortality was solely attributable to TAN toxicity. Second, large quantities of shifting sand often buried cages and made them difficult to relocate. Mussel mortality tended to coincide with bed movements associated with high flow events. However, cages were usually recovered (i.e., un-buried) within 2–3 days of peak flows so it is unlikely that cage-derived inhibition of mussel movement led directly to mortality. This suggests that sediment mobilization events are stressful to mussels. However, we do not yet understand why sediment movements are detrimental to mussels.

Sentinel mussel mortality may also be driven by chronic exposure to other, un-measured toxicants. For example, we did not measure the water quality parameter for which this City of Auburn facility has been most frequently cited, chlorine (http://www.adem.state.al.us/WaterDivision/WQuality/WQMainInfo.htm). This is notable because chlorine reacts with ammonia to produce monochloroamine and unionized ammonia (Brungs, 1973). Both monochloroamine and unionized ammonia are acutely toxic to freshwater mollusks (Goudreau et al., 1993). Although survivorship was not significantly different between mussels in the Parkerson Mill and downstream Chewacla Creek cages, our qualitative assessments of mussel health (as assessed by adductor strength and marsupial condition) suggested that Parkerson Mill Creek mussels were not as healthy as those in Chewacla Creek upstream of the confluence. Thus, we cannot discount the possibility that unmeasured compounds in Parkerson Mill Creek may have contributed to sentinel mussel mortality.

inally, we expected that mortality rates of our sentinel mussel trials were conservative with regards to other unionids because *V. lienosa* is still relatively abundant in many Southeastern streams, including throughout much of the Chewacla Creek study reach, and is thus likely relatively tolerant to water quality degradation and other disturbances (Parmalee & Bogan, 1998; Brim-Box & Williams, 2000; Gangloff & Feminella, 2007b). Perhaps a more sensitive species would have experienced greater mortality as a consequence of water quality or high rates of sedimentation.

To date, relatively few toxicological studies have been conducted using adult mussels because such studies reveal that adults are typically less sensitive to water quality degradation than juvenile or larval stages (reviewed in Ingersoll et al., 2007). Thus, although adult mussels may not experience high mortality rates as a consequence of the water quality of Parkerson Mill creek, it is possible that recruitment of impaired-stream populations is limited by high rates of larval or juvenile mortality. We did not attempt to quantify larval mortality in this study and collecting suitable numbers of juveniles from wild populations in Chewacla Creek would have been excessively time-consuming. Future studies should use cultivated juveniles or larvae from mussel propagation programs to minimize impacts to natural populations.

Chewacla Creek supports some of the highest remaining mussel biodiversity in the Piedmont ecophysiolographic province in the Southeastern U.S. (Gangloff & Feminella, 2007b). Moreover, this region is currently experiencing rapid increases in human population size and economic development. Many other southeastern Piedmont watersheds have experienced much greater rates of urbanization and have lost a significant portion of their native biodiversity (Gillies et al., 2003). Our field surveys and sentinel mussel trials strongly suggest that urbanized tributaries may have significant negative consequences for populations of endangered freshwater mussels. These impacts appear to persist downstream for a considerable distance in Chewacla Creek (M. Gangloff, pers. obs.). Ensuring the survival of highly fragmented mussel populations in urban or developing watersheds like the Uphapee subbasin will require significant reductions of both point and non-point source nutrient loading. Reductions in sediment inputs and stabilization of both riparian and instream habitat within tributary sub-catchments are critical to limiting migration of fines into mainstem reaches and will likely reduce non-point nutrient loading of tributaries. Meaningful conservation of imperiled aquatic biota in Piedmont streams will likely require substantial changes to ongoing development practices in these sensitive watersheds.

Acknowledgments We thank the Friends of the Chewacla and Uphapee Creek Watershed for providing financial support for water samples. Roger Birkhead, Steven Butler, Robert Hamm, Emily Hartfield, Brian Helms, and Hilary Register assisted with field work. Michael Ward graciously allowed us to access Chewacla and Parkerson Mill creeks through his property.

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