

**Initial Implementation of a Long-term Freshwater Mussel Monitoring
Program for the Chattooga River**

Francis Marion-Sumter National Forest, South Carolina, 2012



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Introduction

The Chattooga River watershed drains approximately 180,000 acres with 122,000 acres under Federal Management in the Francis Marion-Sumter National Forest (FMSNF) in South Carolina, Nantahala National Forest in North Carolina, and Chattahoochee-Oconee National Forest in Georgia (Figure 1). In 1974 Congress designated 57 miles of the Chattooga River as Wild and Scenic River. The FMSNF administers river uses associated with the Chattooga Wild and Scenic River Corridor (Thomas 2004). The watershed is popular for a number of uses, including fishing, hiking, and horseback riding and contains sections of world-class whitewater rafting and kayaking. The Chattooga River also supports populations of several freshwater mussel species, including a Region 8 Forest Sensitive Species, *Alasmidonta varicosa* (brook floater).

In 2004 - 2005 the FMSNF partnered with the Southern Research Station, Center for Aquatic Technology Transfer (CATT) to develop a long-term monitoring proposal (Roghair et al. 2005) with goals of detecting population trends in *Alasmidonta varicosa* and the *Elliptio* species complex, and documenting distribution of exotic *Corbicula fluminea* (Asian clam) in the Chattooga River upstream of Tugaloo Lake. Members of the *Elliptio* species complex present in the Chattooga River include *Elliptio angustata* (Carolina lance) and *Elliptio producta* (Atlantic spike). Live specimens of the *Elliptio* complex are exceedingly difficult to distinguish under field conditions, so we combine all *Elliptio* species (hereafter *Elliptio spp.*) for reporting purposes. *Corbicula fluminea* are an introduced species originating in Asia, and are generally abundant when present.

We completed the first samples in support of the FMSNF Chattooga mussel monitoring program in October, 2012. Our objectives were to 1) compare present distribution of mussel species to historic distributions, 2) complete baseline population estimates, 3) document the location of sample sites, and 4) provide suggestions for the improvement of the monitoring program.

Methods

Sample Area & Site Selection

The sample area on the Chattooga River extends from the confluence with Camp Creek (site 1) upstream 44.2 km to the confluence with Lick Log Creek (site 14) (Figure 1, Table 1, Appendix A). Within this area, 14 sites were selected for reconnaissance in 2004. We eliminated two of the sites in 2012 (sites 2 and 9) due to lack of shoals (i.e. wadeable riffle and run stream habitat with sand and/or gravel substrate) or poor access (Table 1). The 12 remaining monitoring sites are located in wadeable stream reaches near points of good land access. To facilitate precise location of the sites during future

sampling, we thoroughly documented site locations with written descriptions, measurements, digital photographs, and GPS points (Table 2, Appendices A and B).

Sample Design

We deployed 6 – 8 biologists and technicians to each sample site. Mussel density was estimated at each site using systematic sampling with three random starts (Strayer and Smith 2003). Each random start represented a single sample consisting of a systematic transect array of 0.25 m² quadrats. Total wadeable area was measured for each site and sample locations were determined onsite with a portable computer running an Excel form. Total sampling effort was site-dependent but the number of quadrats was set to sample 2.5% of total wadeable area at each site.

Individual quadrats were sampled by searching the substrate visually, using a viewing bucket or snorkel gear, before and after light disturbance of the substrate. Light disturbance increased the chances of finding mussels buried just below the surface or covered with a thin layer of sediment. All live mussels were identified, measured, and returned to the substrate. Relic shells were collected and returned to the lab. For detailed methods, see Appendix C.

Results

We found live or relic *Alasmidonta varicosa* at 8 sites, *Elliptio spp.* at all 12 sites, and the introduced *Corbicula fluminea* at only the two most downstream sites (Table 1). Previous inventories found *Alasmidonta varicosa* only as far upstream as site 9, whereas we found live *Alasmidonta varicosa* 9.6 km further upstream at site 11 (Table 3). Like previous inventories, we found *Elliptio spp.* at sites throughout the sample area (Table 4).

Population estimates for the 12 sites ranged from 0 to 436 individuals (0.00 to 0.62 per m²) for *Alasmidonta varicosa* and 33 to 689 individuals (0.04 to 0.84 per m²) for *Elliptio spp.* (Figures 2 and 3, Table 5). Confidence intervals at site 6 are notably large because the majority of the site's mussels were observed within a single quadrat (Figure 3; Appendix C, Figure C2). Estimates for both *Alasmidonta varicosa* and *Elliptio spp.* varied throughout the study area (Figures 2 and 3). *Alasmidonta varicosa* were most abundant at sites 6 and 7, and were not detected upstream of site 11 (Figures 2 and 3). *Elliptio spp.* were present at all sites, but were most abundant from site 11 downstream, though there was considerable variation (Figures 2 and 3).

Total shell length of live *Alasmidonta varicosa* ranged from 38 mm to 73 mm and *Elliptio spp.* ranged from 18 mm to 82 mm (Tables 6 and 7). All relic shell lengths were within the range of the measured live mussels. The most prevalent sampled shell size range for both species is 41-50 mm (Figure 4).

Discussion

The Chattooga River mussel monitoring program is designed to assess changes in the population of the sample area as a whole (i.e. all 12 sites combined). Although numbers of mussels present at any individual sample site may decline, numbers at other sites may increase or be unchanged; the goal of distributed sampling is to assess the overall state of the resource throughout the entire sample area. The basic analytical approach is to use a paired t-test to assess differences in mussel density at time-*a* and time-*b*, using as pairs, observations at each site at the two times (Strayer and Smith 2003). It may also be possible to examine each site individually, depending on how many mussels are encountered. However, in interpreting site-specific changes, it will always be difficult to tease apart natural changes from non-natural ones and the real strength of this approach is to make an assessment of the sample area as a whole. The optimal sampling interval to capture long-term trends is 5-6 year intervals (Wendell Haag, pers. comm.). If successive monitoring continues (i.e. at years 5, 10, 15, etc.) then use of time-series analysis should be considered to capture any gradual, long-term population decline or increase.

We focused our search efforts on areas previously described as the preferred habitat of the target mussel species: relatively shallow (< 1 m deep) riffles and runs with sand and/or gravel substrate (i.e. shoals) (Adkins 1995). We examined additional habitats at two sites, with mixed success. At site 7, a brief (<2 min.) qualitative search using snorkeling gear found 12 *Alasmidonta varicosa* in swift, chest deep, mid channel water. *Alasmidonta varicosa* were also present in high numbers within the sampled shoal area at site 7 (Table 1). At site 2, we could not locate a suitable shoal area. The site was comprised of deep pool, riffle, and run habitat with boulders. We qualitatively searched as much habitat as possible and observed only *Corbicula fluminea*. Given these results, we believe focusing on shoal habitat is effective for quantifying mussel populations in the Chattooga River.

Because some mussels may be buried deeply in the substrate, our sampling methods (visual inspection with light disturbance) are assumed to miss some unknown fraction of mussels present at any given site. Further, very small mussels are often missed by visual inspection of the substrate, so this method will also likely underestimate abundance of juveniles. The only juveniles we detected were two 18 – 19 mm *Elliptio spp.* at site 11. Complete excavation of quadrats and processing substrate across a series of graded sieves is necessary to eliminate these two sources of bias. However, excavation of substrate is very time-consuming and is not feasible for many routine monitoring programs. With regard to the stated goals of the FMSNF, sampling a larger number of sites using a method with lower precision and known sources of bias is preferable to obtaining more precise estimates at fewer sites (Wendell Haag, pers. comm.).

The bell-shaped length-frequency distributions (Figure 4) are indicative of healthy populations. Maximum shell length is about 70 mm for *Alasmidonta varicosa*, 140 mm for *Elliptio angustata*, and 120

mm for *Elliptio producta* (Bogen and Alderman 2008). We found shell lengths ranging from 38 – 73 mm for *Alasmidonta varicosa* and 18 – 82 mm for *Elliptio spp.*, which are similar to the ranges found by Alderman (2004) of 22 -71 mm and 19 – 85 mm respectively. If complete quadrat excavation had been performed the length distributions would likely show more smaller individuals (Hornbach and Deneka 1996). Populations with recruitment have plenty of mid-sized individuals, which is what we found for *Alasmidonta varicosa* and *Elliptio spp.* Furthermore, if the length distributions do not change over time, it is indicative that recruitment is occurring. Future monitoring should continue to measure shell length in order to watch for a shift toward larger individuals, which would indicate a decline in recruitment. Complete excavation of a limited number of quadrats at each shoal could give a more complete picture of recruitment and may allow us to develop a correction factor for inferences about the true size structure of the population (Hornbach and Deneka 1996, Strayer and Smith 2003). Future work should carefully weigh the benefits of complete excavation against the increase in time required at each site.

We detected at least 1 mussel species at each of the 12 sample sites. It was especially encouraging to expand the known range of *Alasmidonta varicosa* within the Chattooga River by nearly 10 km. Population estimates dropped off sharply for *Elliptio spp.* and completely for *Alasmidonta varicosa* upstream of site 11 despite no noticeable change in stream habitat. Water quality, water temperature, flow conditions, or a lack of necessary host fish species for reproduction could be affecting population persistence or abundance upstream of site 11, however investigating reasons for the decrease in mussel abundance is beyond the scope of the monitoring program.

Total sampling time at an individual site for an experienced crew of 6 averaged 1 hour, which was less than originally anticipated. Given our efficiency it may be tempting to increase the number of random starts and the percent shoal-area sampled, with the assumption that it would tighten confidence intervals. However, this is not needed for two reasons: 1) for scarce species like *Alasmidonta varicosa* it is difficult to sufficiently sample to have enough power to detect small changes in abundance at a single site, and 2) when comparing the population at time-*a* versus *b* using a paired t-test, the confidence intervals at each site do not come into play; rather it is the difference between the estimates of the mean across all sites between years that allow for inference regarding population changes (Wendell Haag, pers. comm.).

Data Availability

The 2012 mussel inventory data reside in a MS Access database, which is managed by the CATT and a copy has been provided to Jeanne Riley, FMSNF Fish Biologist. We will support the migration of this data into the USFS database tool, Natural Resource Information System Aquatic Surveys (NRIS AqS), as needed. In the interim, we are working with the FMSNF to develop custom queries and reports for the MS Access database.

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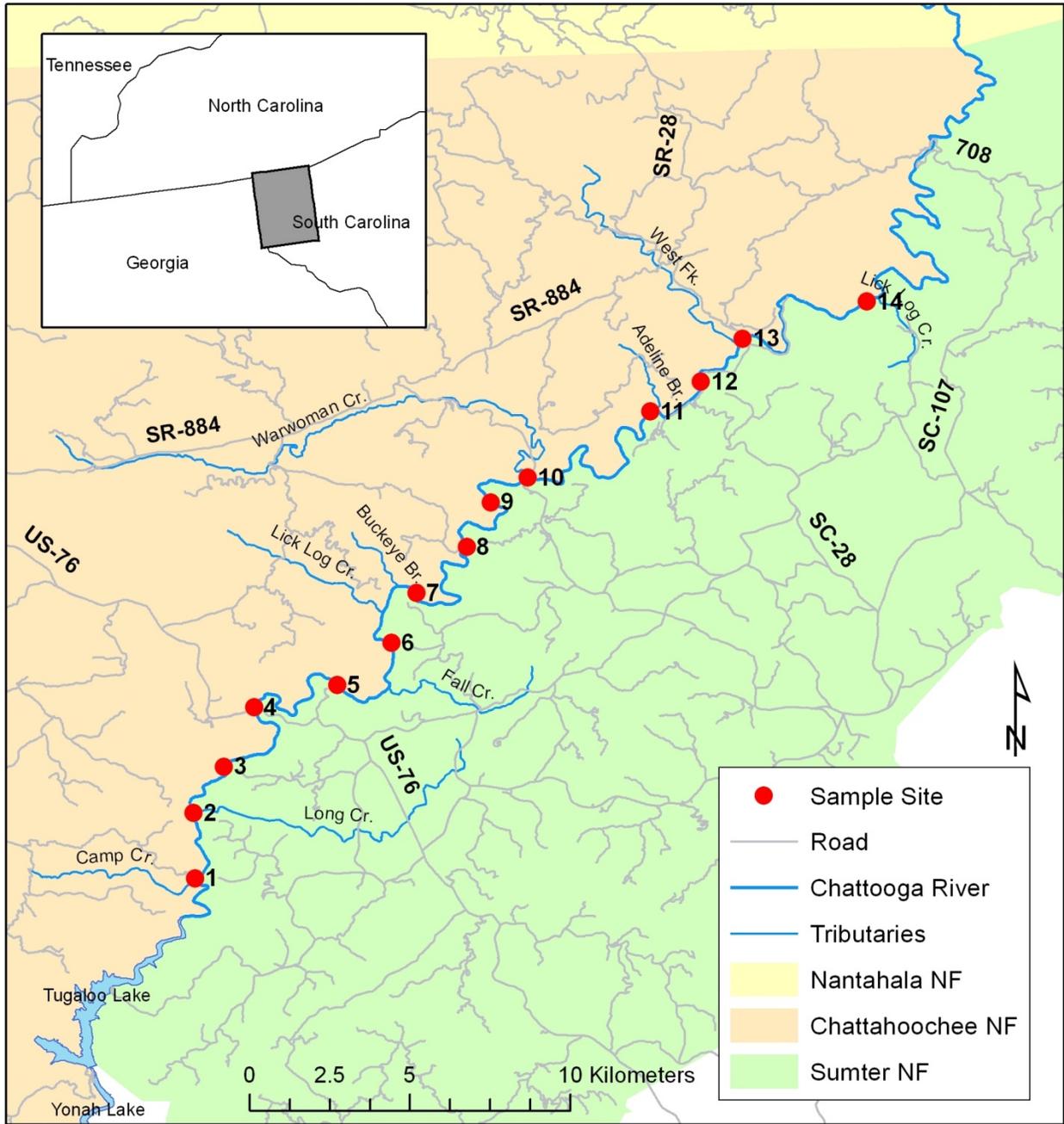


Figure 1. Locations of sites inventoried for mussels on the Chattooga River; October 2012.

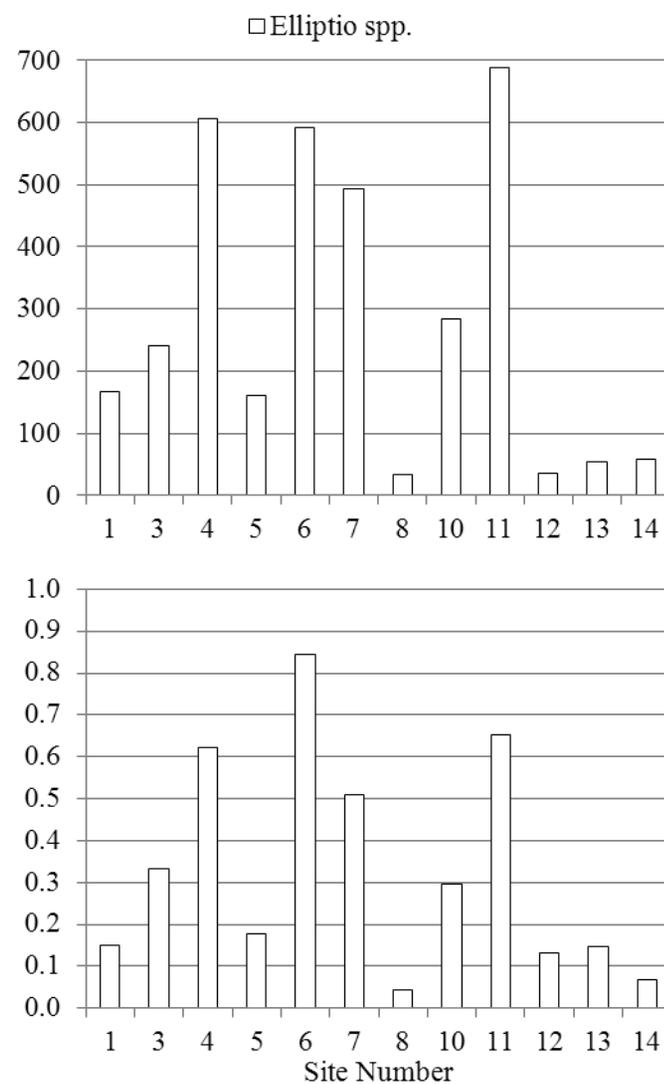
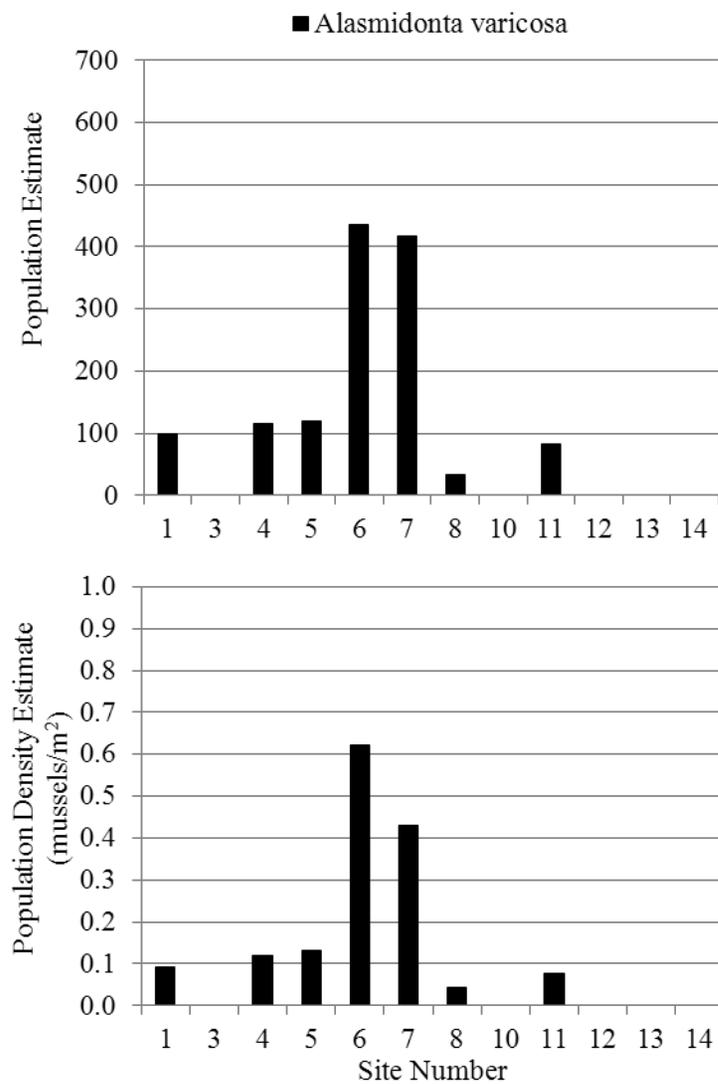


Figure 2. Population estimates (upper graphs) and population density estimates (mussels/m²; lower graphs) for *Alasmidonta varicosa* and *Elliptio* spp. at each sample site.

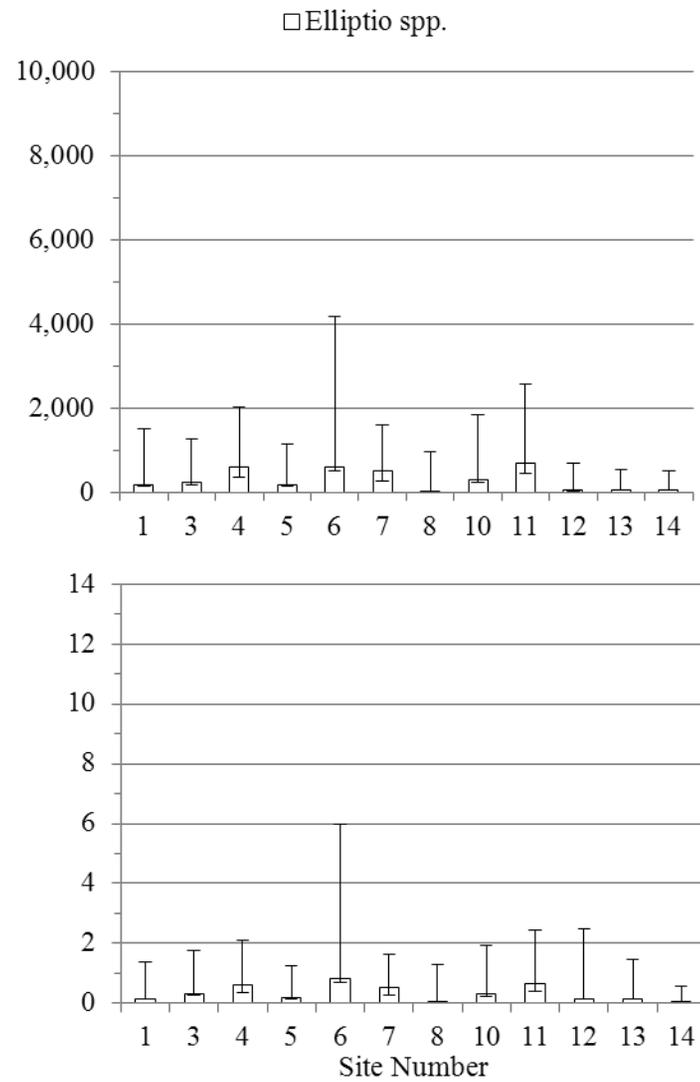
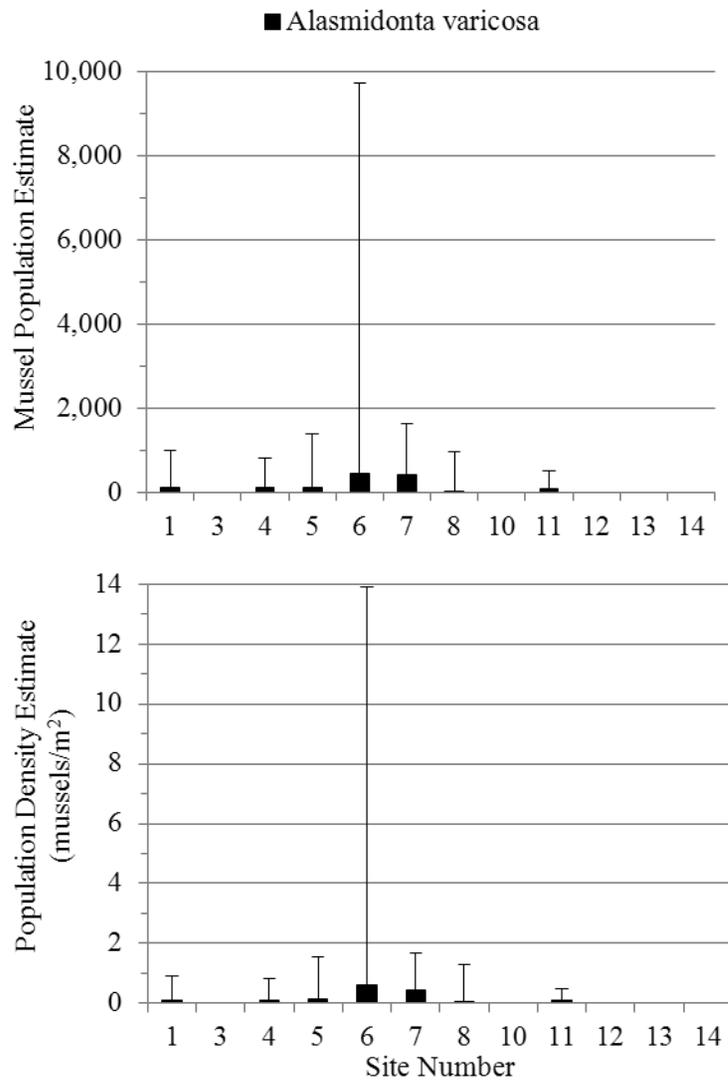


Figure 3. Population estimates (upper graphs) and population density estimates (mussels/m²; lower graphs) with confidence intervals for *Alasmidonta varicosa* and *Elliptio* spp. at each sample site.

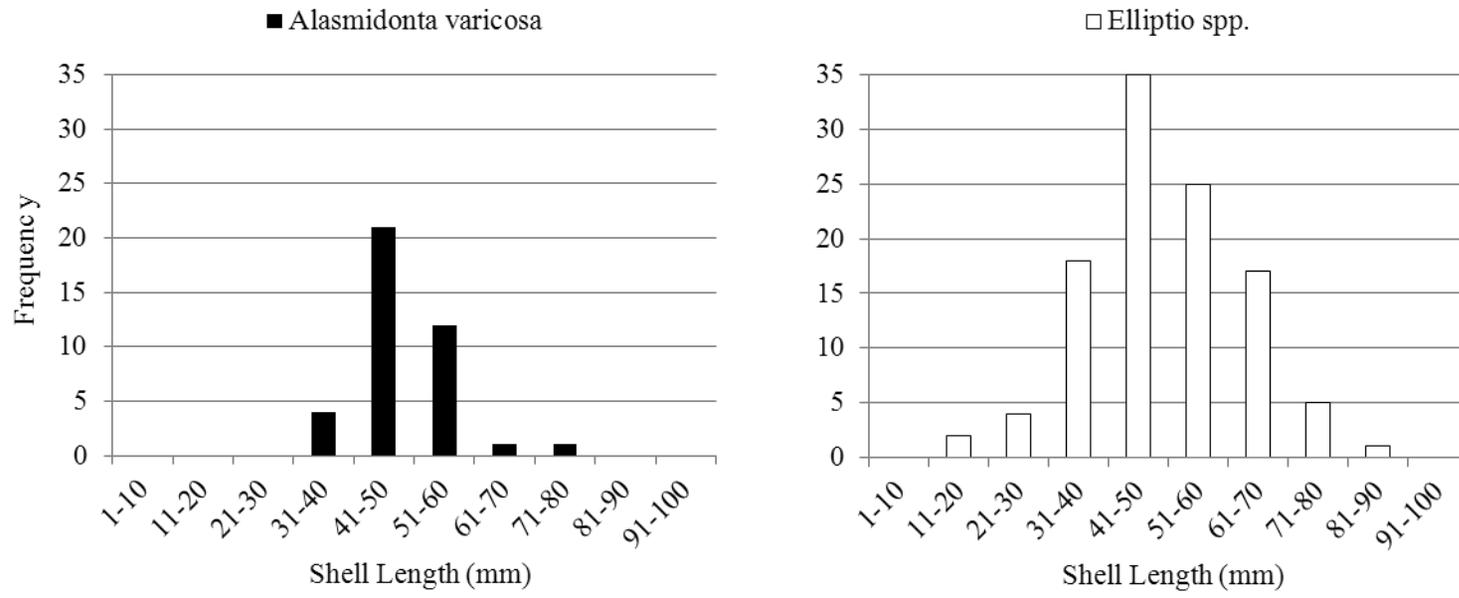


Figure 4. Total shell lengths (mm) for live *Alasmidonta varicosa* and *Elliptio* spp. combined for all sample sites.

Table 1. Mussel inventory results summary. River kilometer 0.0 at Camp Creek is located approximately 2 km upriver of where the Chattooga River flows into Tugaloo Lake (Figure 1). Counts of live *Alasmidonta varicosa* and *Elliptio spp.* are shown along with presence (P) or absence (A) of relics in parentheses.

| | Site # | Site Name | Date | River Km | Shoal Size (m) | # Transects | # Quads | Water Temp (C) | <i>Alasmidonta varicosa</i> | <i>Elliptio spp.</i> | <i>Corbicula fluminea</i> |
|------------|--------|-------------------------|------------|----------|----------------|-------------|---------|----------------|-----------------------------|----------------------|---------------------------|
| Upstream | 14 | Lick Log Creek | 10/13/2012 | 44.2 | 43x20 | 6 | 120 | 12 | 0 (A) | 2 (P) | Absent |
| | 13 | Hwy 28 Bridge | 10/10/2012 | 38.5 | 61x06 | 9 | 57 | 11 | 0 (A) | 2 (P) | Absent |
| | 12 | Hwy 28 Wildlife Parking | 10/13/2012 | 36.4 | 27x10 | 3 | 31 | 14 | 0 (A) | 1 (P) | Absent |
| | 11 | Adeline Branch | 10/10/2012 | 34.3 | 62x17 | 9 | 153 | 11 | 3 (A)* | 25 (P) | Absent |
| | 10 | Earls Ford/Warwoman Cr | 10/11/2012 | 26.8 | 80x12 | 9 | 108 | 10 | 0 (A) | 8 (P) | Absent |
| | 9 | Islands (no access) | 10/13/2012 | 24.7 | -- | -- | -- | -- | -- | -- | -- |
| | 8 | Sandy Ford | 10/11/2012 | 21.8 | 75x10 | 9 | 90 | 12 | 1 (A) | 1 (P) | Absent |
| | 7 | Buckeye Branch | 10/11/2012 | 17.5 | 57x17 | 6 | 102 | 12 | 11 (A) | 13 (P) | Absent |
| Downstream | 6 | Fall Creek | 10/11/2012 | 14.6 | 70x10 | 9 | 90 | 14 | 14 (A) | 19 (P) | Absent |
| | 5 | Thriffts Ferry | 10/12/2012 | 11.5 | 90x10 | 9 | 90 | 12 | 3 (A) | 4 (P) | Absent |
| | 4 | Hwy 76 Bridge | 10/12/2012 | 7.4 | 65x15 | 9 | 129 | 12 | 4 (A) | 21 (P) | Absent |
| | 3 | Woodall Shoals | 10/12/2012 | 4.4 | 90x08 | 9 | 72 | 13 | 0 (P) | 6 (P) | Absent |
| | 2 | Long Creek (no shoals) | 10/13/2012 | 2.3 | -- | -- | -- | -- | -- | -- | Present |
| | 1 | Camp Creek | 10/12/2012 | 0.0 | 50x22 | 6 | 132 | 14 | 3 (A) | 5 (P) | Present |

*In previous inventories *Alasmidonta varicosa* were not found this far upriver.

Table 2. Sample site coordinates for site access parking, shoal downstream sample start, and shoal upstream sample end locations.

| | Site # | Site Name | Coordinates (UTM NAD83) | | |
|-----------------|-------------------|-------------------------|-------------------------|----------------------|---------------------|
| | | | Parking | Downstream Start | Upstream End |
| <i>Upstream</i> | 14 | Lick Log Creek | 17 S 306136 3866808 | 17 S 305044 3867259 | 17 S 305059 3867289 |
| | 13 | Hwy 28 Bridge | 17 S 301879 3866269 | 17 S 301838 3866170 | 17 S 301874 3866236 |
| | 12 | Hwy 28 Wildlife Parking | 17 S 300789 3864898 | 17 S 300736 3864853 | 17 S 300733 3864876 |
| | 11 | Adeline Branch | 17 S 299530 3863416 | 17 S 299424 3863947 | 17 S 299441 3864007 |
| | 10 | Earls Ford/Warwoman Cr | 17 S 296230 3861609 | 17 S 296224 3861958 | 17 S 296294 3861958 |
| | 9 | Islands (no access) | 17 S 296367 3861509 | 17 S 295272 3861207* | -- |
| | 8 | Sandy Ford | 17 S 295025 3859832 | 17 S 294616 3859829 | 17 S 294590 3859896 |
| | 7 | Buckeye Branch | 17 S 293044 3858172 | 17 S 293298 3858426 | 17 S 293304 3858361 |
| | 6 | Fall Creek | 17 S 293110 3856773 | 17 S 292622 3856878 | 17 S 292565 3856914 |
| | <i>Downstream</i> | 5 | Thrifts Ferry | 17 S 290953 3855535 | 17 S 291201 3855600 |
| 4 | | Hwy 76 Bridge | 17 S 289161 3854958 | 17 S 289043 3854949 | 17 S 289041 3855013 |
| 3 | | Woodall Shoals | 17 S 288482 3853081 | 17 S 288231 3853118 | 17 S 288265 3853219 |
| 2 | | Long Creek (no shoals) | 17 S 288173 3851446 | 17 S 287412 3851697* | -- |
| 1 | | Camp Creek | 17 S 287159 3849751 | 17 S 287413 3849658 | 17 S 287434 3849710 |

* Sample site coordinate from CATT visit in 2004 (Roghair et al. 2005)

Table 3. Presence or absence of live *Alasimdonga varicosa* at Chattooga river sample sites from multiple mussel inventories (Adkins 1995, Alderman 2004, Alderman 2008, and Roghair et al. 2005).

✓ species observed, ✗ species not observed, -- site not sampled.

| | | Alasimdonga varicosa | | | | |
|------------|-----------------------------|----------------------|----------|----------|------|------|
| | | Adkins | Alderman | Alderman | CATT | CATT |
| Site # | Site Name | 1995 | 2004 | 2008 | 2004 | 2012 |
| Upstream | 14 Lick Log Creek | ✗ | -- | -- | -- | ✗ |
| | 13 Highway 28 Bridge | ✗ | ✗ | ✗ | ✗ | ✗ |
| | 12 Highway 28 Wildlife Area | -- | -- | -- | ✗ | ✗ |
| | 11 Adeline Branch | -- | -- | -- | -- | ✓ |
| | 10 Earls Ford/Warwoman Cr. | ✗ | ✗ | -- | ✗ | ✗ |
| | 9 Islands | -- | ✓ | -- | -- | -- |
| | 8 Sandy Ford | ✓ | ✓ | ✓ | -- | ✓ |
| | 7 Buckeye Branch | -- | ✓ | -- | -- | ✓ |
| | 6 Fall Creek | -- | ✓ | -- | ✓ | ✓ |
| | 5 Thrifts Ferry | -- | -- | -- | -- | ✓ |
| Downstream | 4 Highway 76 Bridge | ✓ | ✓ | ✓ | ✓ | ✓ |
| | 3 Woodall Shoals | ✓ | ✓ | ✓ | ✓ | ✗ |
| | 2 Long Creek | -- | -- | -- | -- | -- |
| | 1 Camp Creek | ✓ | ✓ | -- | -- | ✓ |

Table 4. Presence or absence of live *Elliptio spp.* at Chattooga river sample sites from multiple mussel inventories (Adkins 1995, Alderman 2004, Alderman 2008, and Roghair et al. 2005).

✓ species observed, ✗ species not observed, -- site not sampled.

| | | Elliptio spp. | | | | |
|------------|-----------------------------|---------------|----------|----------|------|------|
| | | Adkins | Alderman | Alderman | CATT | CATT |
| Site # | Site Name | 1995 | 2004 | 2008 | 2004 | 2012 |
| Upstream | 14 Lick Log Creek | ✓ | -- | -- | -- | ✓ |
| | 13 Highway 28 Bridge | ✓ | ✓ | ✓ | ✓ | ✓ |
| | 12 Highway 28 Wildlife Area | -- | -- | -- | ✓ | ✓ |
| | 11 Adeline Branch | -- | -- | -- | -- | ✓ |
| | 10 Earls Ford/Warwoman Cr. | ✓ | ✓ | -- | ✓ | ✓ |
| | 9 Islands | -- | ✓ | -- | -- | -- |
| | 8 Sandy Ford | ✓ | ✓ | ✓ | -- | ✓ |
| | 7 Buckeye Branch | -- | ✓ | -- | -- | ✓ |
| | 6 Fall Creek | -- | ✓ | -- | ✓ | ✓ |
| | 5 Thrifts Ferry | -- | -- | -- | -- | ✓ |
| Downstream | 4 Highway 76 Bridge | ✓ | ✓ | ✓ | ✓ | ✓ |
| | 3 Woodall Shoals | ✓ | ✗ | ✓ | ✓ | ✓ |
| | 2 Long Creek | -- | -- | -- | -- | -- |
| | 1 Camp Creek | ✓ | ✓ | -- | -- | ✓ |

Table 6. Total shell lengths (mm) of live *Alasmidonta varicosa* inventoried at the each sample site.

| | Site # | Site Name | <i>Alasmidonta varicosa</i> Shell Length (mm) |
|------------|--------|-------------------------|---|
| Upstream | 14 | Lick Log Creek | none |
| | 13 | Hwy 28 Bridge | none |
| | 12 | Hwy 28 Wildlife Parking | none |
| | 11 | Adeline Branch | 45 46 73 |
| | 10 | Earls Ford/Warwoman Cr | none |
| | 9 | Islands (no access) | -- |
| | 8 | Sandy Ford | 49 |
| Downstream | 7 | Buckeye Branch | 39 42 45 48 51 51 51 52 52 52 53 |
| | 6 | Fall Creek | 38 39 42 45 45 47 47 48 51 51 53 54 55 63 |
| | 5 | Thriffts Ferry | 43 46 49 |
| | 4 | Hwy 76 Bridge | 41 44 45 46 |
| | 3 | Woodall Shoals | none* |
| | 2 | Long Creek (no shoals) | -- |
| | 1 | Camp Creek | 39 44 49 |

**Alasmidonta varicosa* relic found.

Table 7. Total shell lengths (mm) of live *Elliptio* spp. inventoried at each sample site.

| | Site # | Site Name | Elliptio spp. Shell Length (mm) |
|----------|------------|-------------------------|--|
| Upstream | 14 | Lick Log Creek | 36 69 |
| | 13 | Hwy 28 Bridge | 54 59 |
| | 12 | Hwy 28 Wildlife Parking | 39 |
| | 11 | Adeline Branch | 18 19 30 34 37 39 40 41 41 43 46 48 48 49 53 55 57 57 61 62 65 65 66 73 78 |
| | 10 | Earls Ford/Warwoman Cr | 39 41 43 44 46 61 61 65 |
| | 9 | Islands (no access) | -- |
| | 8 | Sandy Ford | 46 |
| | 7 | Buckeye Branch | 36 43 48 49 49 50 50 50 51 51 56 60 82 |
| | 6 | Fall Creek | 29 34 37 43 45 49 49 51 51 52 56 58 59 59 59 61 62 64 73 |
| | Downstream | 5 | Thrifts Ferry |
| 4 | | Hwy 76 Bridge | 32 39 39 40 42 42 43 45 45 45 48 49 52 52 55 56 58 60 62 63 63 |
| 3 | | Woodall Shoals | 29 36 42 62 72 73 |
| 2 | | Long Creek (no shoals) | -- |
| 1 | | Camp Creek | 26 44 48 58 67 |

Appendix A – Mussel Inventory Site Maps

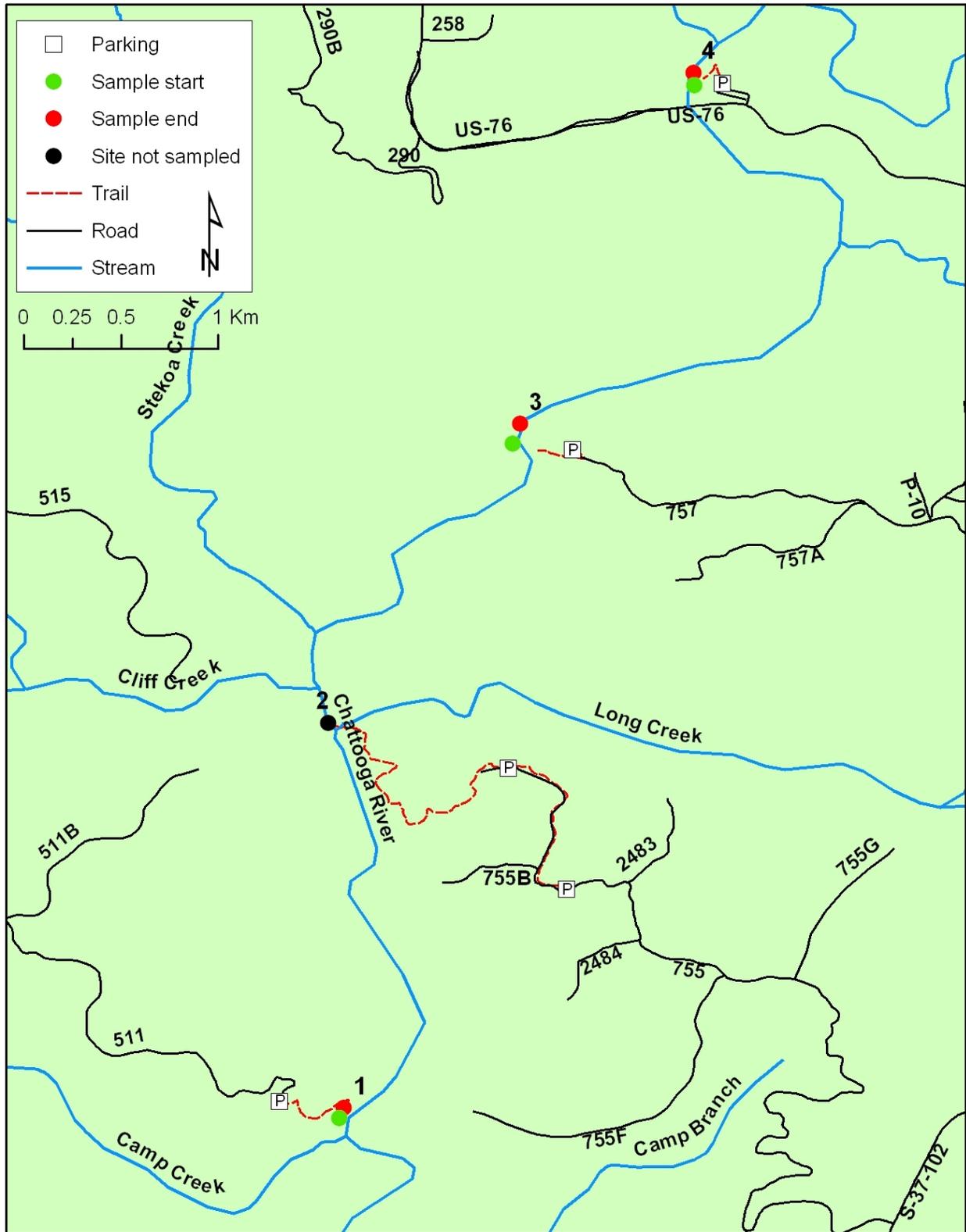


Figure A1. Location of sites Camp Creek (1), Long Creek (2), Woodall Shoals (3), and Highway 76 Bridge (4) on the Chattooga River.

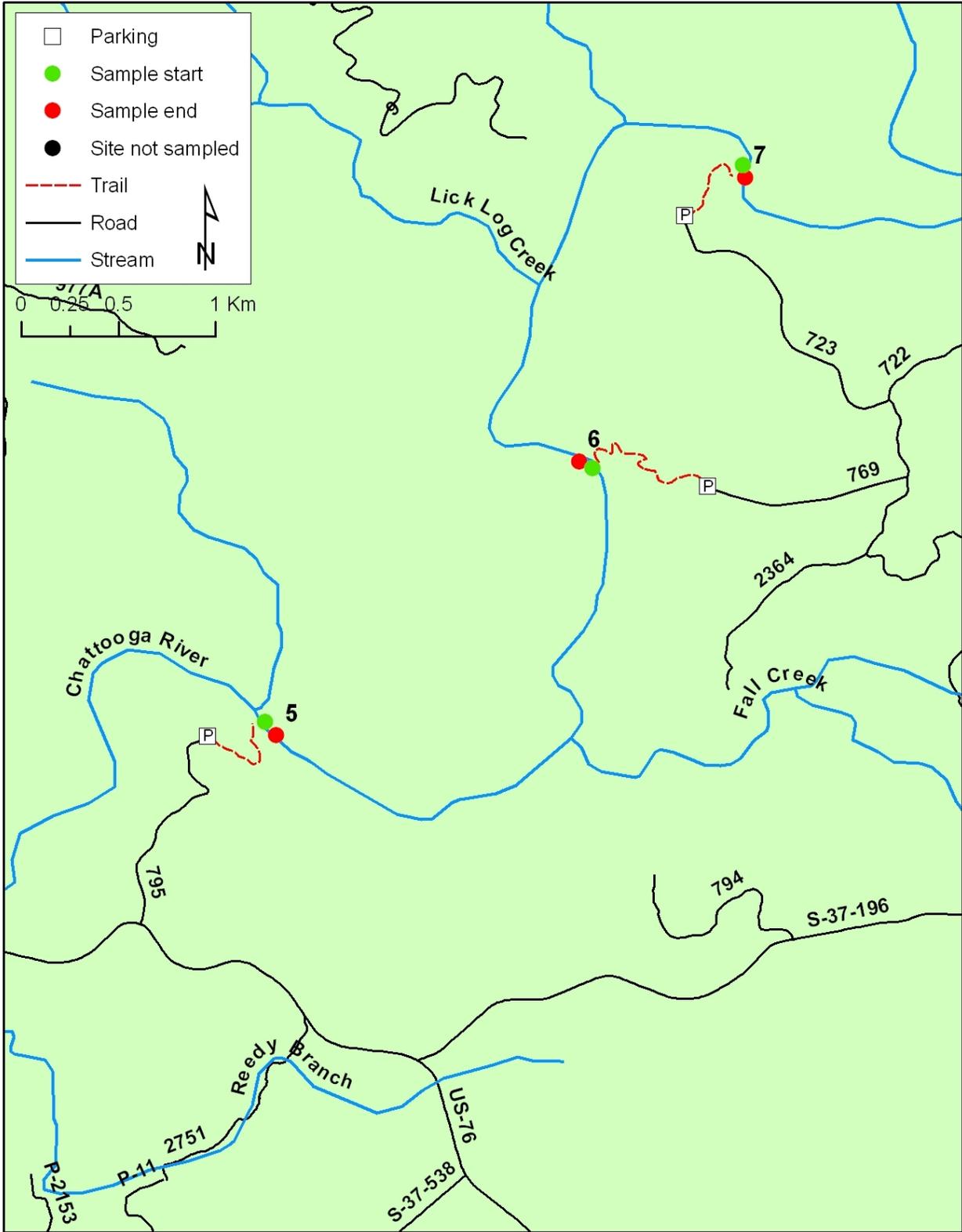


Figure A2. Location of sites Thrifts Ferry (5), Fall Creek (6), and Buckeye Branch (7) on the Chattooga River.

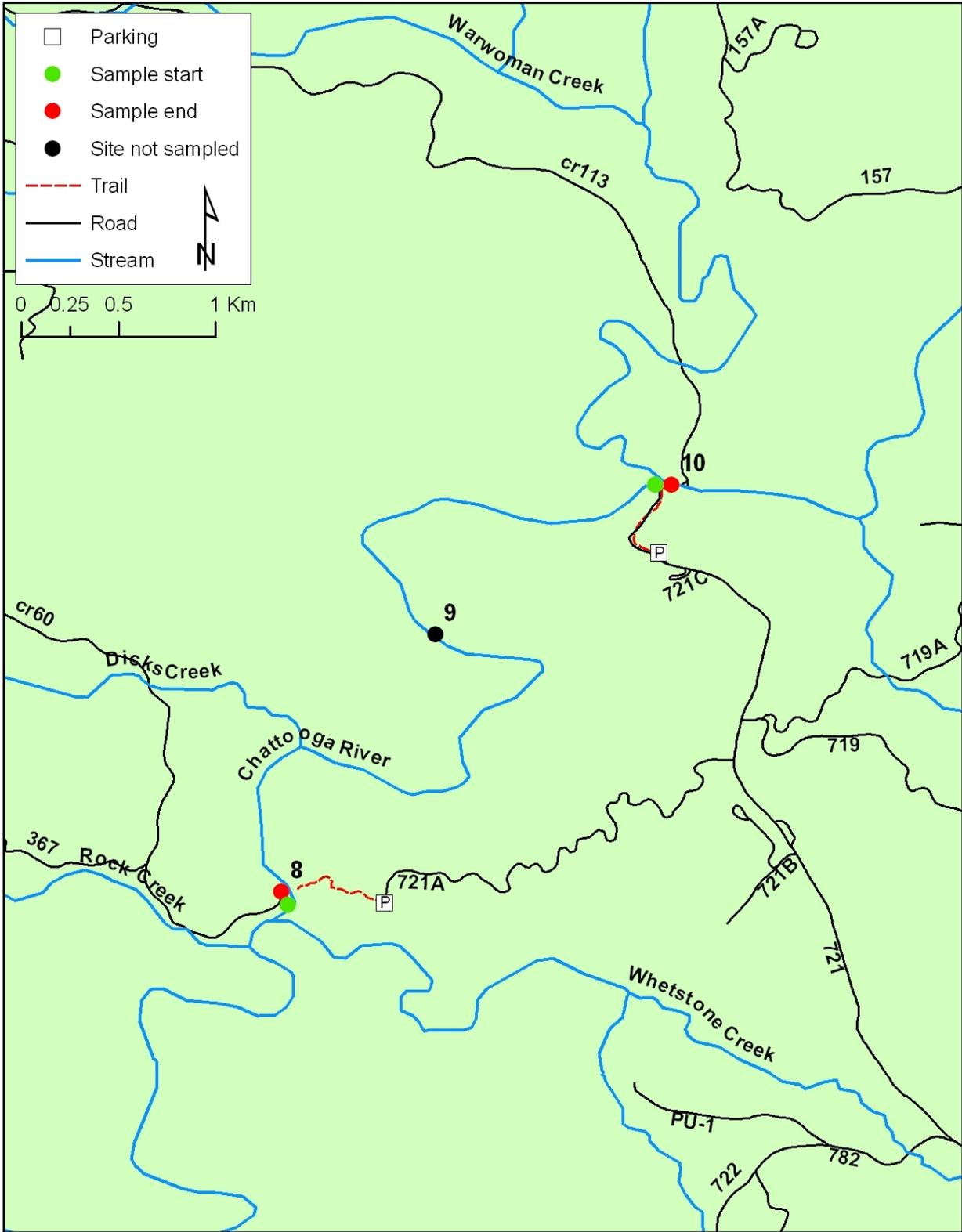


Figure A3. Location of sites Sandy Ford (8), Islands (9), and Earls Ford/Warwoman Creek (10) on the Chattooga River.

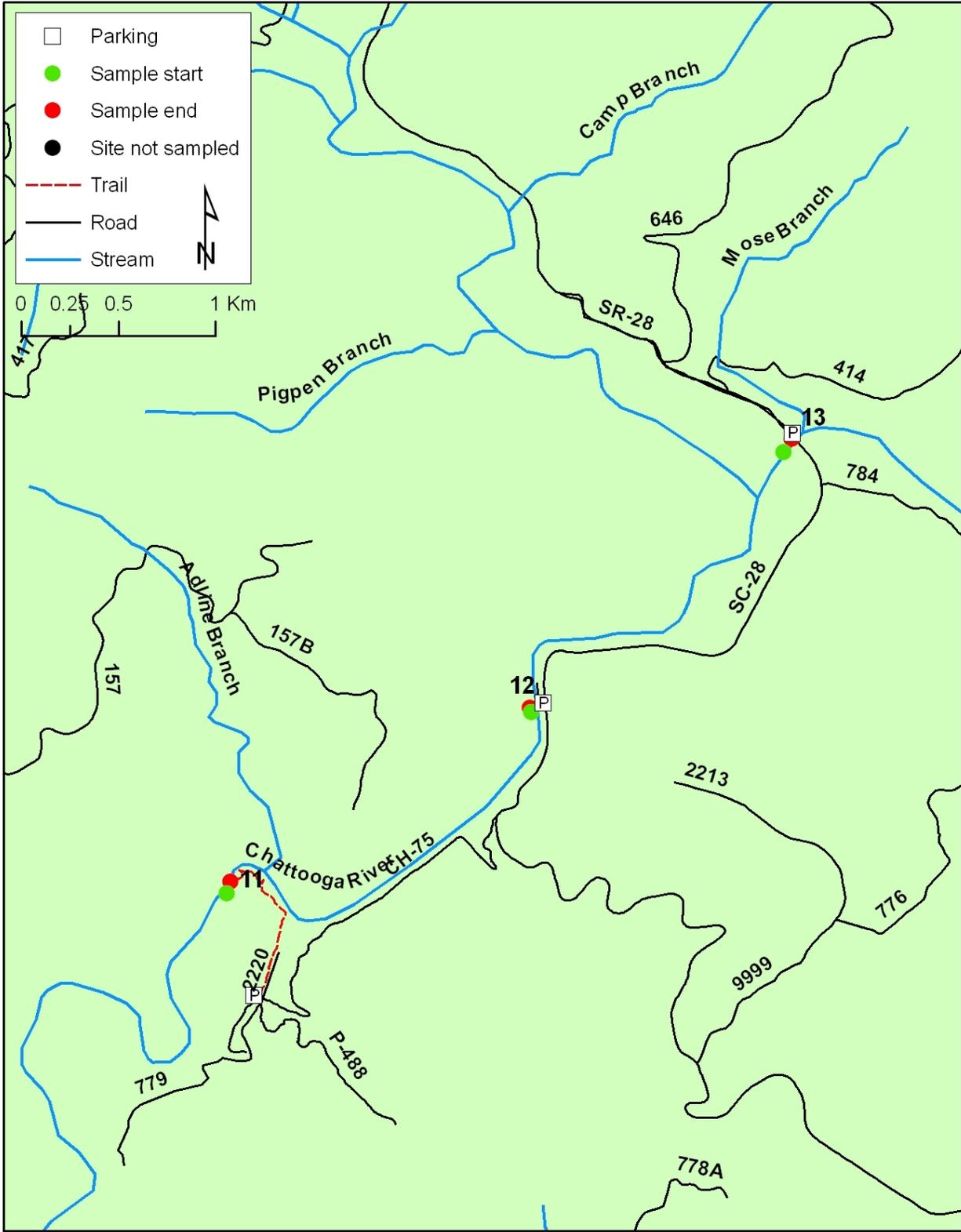


Figure A4. Location of sites Adeline Branch (11), Highway 28 Wildlife Parking (12), and Highway 28 Bridge (13) on the Chattooga River.

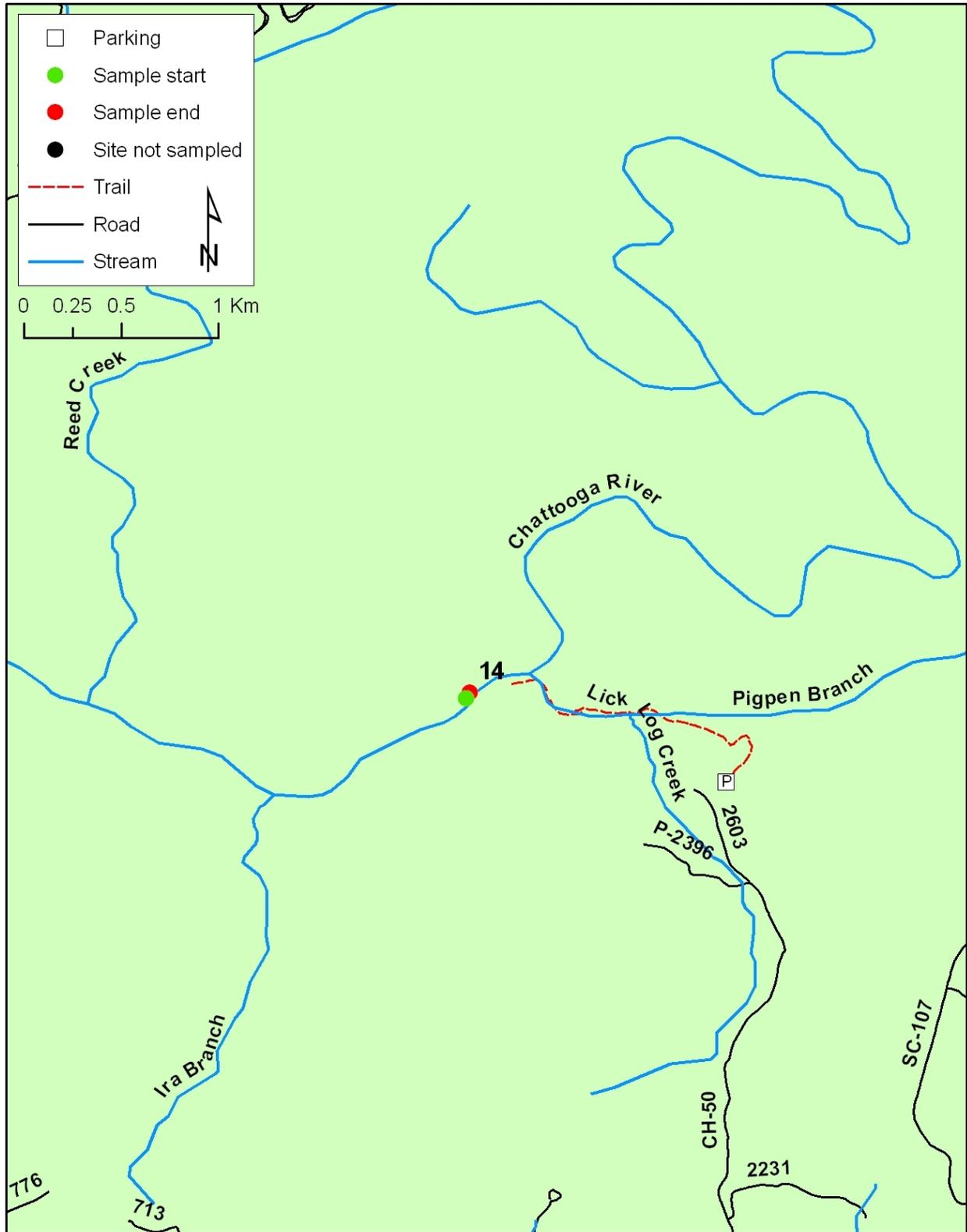


Figure A5. Location of site Lick Log Creek (14) on the Chattooga River.

Appendix B – Mussel Inventory Site Details

Site: 1, Camp Creek

Access: From Clayton, GA take 441 / 23 south, turn left on Camp Creek Rd, turn left on FS511. At end of road there is parking; follow trail to river; 7 min walk.

Length x Width: 50 x 22 m

Side of river: GA

Species: *Elliptio* spp., *Alasmidonta varicosa*, *Corbicula fluminea*

Downstream start: Start upstream of Camp Creek approximately 100 m. Start at bottom of run before water depth is too deep and transitions into pool. There is a tree that leans out over the river about 5 m upstream from the start.

Upstream end: End at the downstream end of beach before water depth is too deep.



Looking upstream



Looking downstream



Looking upstream

Site: 2, Long Creek

Access: Follow FS-755 to end. There is a large turn around area with plenty of parking; park here if it has rained recently. If roads are dry, can try driving to very end of 755, which descends off to the right of the parking circle. At the end of this old section of 755 there is small turn around and a trail. Follow trail until it nears Long Creek, where the trail then takes a left downhill on a very steep footpath. Hike is approx. 40 min.

Length x Width: None; there are no shoals to sample at this site

Side of river: SC

Species: Only *Corbicula fluminea* were observed during informal spot checks in 2012

Downstream start: NA

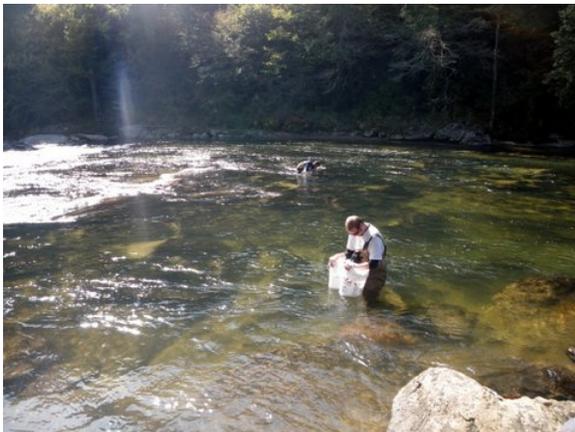
Upstream end: NA



Looking downstream



Looking upstream



Site: 3, Woodall Shoals

Access: From Hwy 76 take 538, then FS-757 to parking area at end. Follow trail to the left of the kiosk to river; 5 min walk. Climb over bedrock upstream of trail to access shoal area.

Length x Width: 90 x 8 m

Side of river: SC

Species: *Elliptio* spp., *Alasmidonta varicosa*

Downstream start: Start on upstream edge of very large bedrock outcrop.

Upstream end: Stop just around bend before riffle.



Looking downstream



Looking upstream



Looking downstream



Looking upstream

Site: 4, Hwy 76 Bridge

Access: On Hwy 76, near bridge over river, park at river access picnic and parking area with facilities on SC side. Follow paved trail behind facilities building to sand beach; 5 min walk.

Length x Width: 65 x 15 m

Side of river: SC

Species: *Elliptio* spp., *Alasmidonta varicosa*

Downstream start: Start at riffle crest that is downstream of sand beach.

Upstream end: End at pine tree that is close to the water's edge at the upstream end of the sand beach.



Looking downstream



Looking upstream



Looking upstream

Site: 5, Thrifts Ferry

Access: From Hwy 76 take FS-795 to parking turn around. Follow trail to the right of kiosk to boat landing; 6 min walk. Best place to wade across river to GA side is at the campsite about 100 m upstream of the boat landing.

Length x Width: 90 x 10 m

Side of river: GA

Species: *Elliptio* spp., *Alasmidonta varicosa*

Downstream start: Start upstream of boat landing and riffle; start at the small tributary flowing in on the left.

Upstream end: End 90 m upstream of the tributary where water depth starts to increase.



Tributary in on left



Looking upstream



Looking across river from river crossing location



Site: 6, Fall Creek

Access: From Hwy 76, turn left on 196 (Chattooga Ridge Rd), left on Falls Creek Rd, left on FS-722, left on FS-769. Park in loop turn around parking area. Follow trail to river; 10 min walk.
NOTE: water at this site is swift with some deep spots, recommend wetsuits.

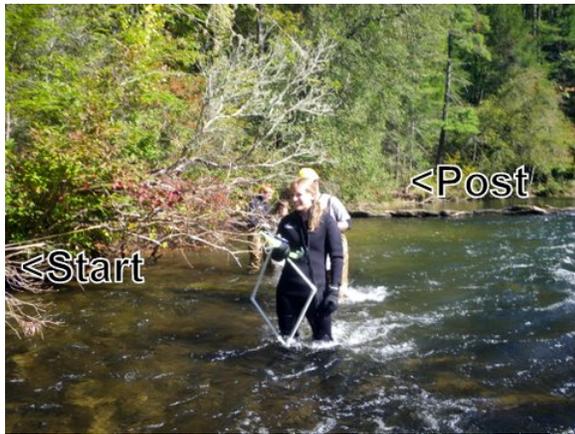
Length x Width: 70 x 10 m

Side of river: SC

Species: *Elliptio* spp., *Alasmidonta varicosa*

Downstream start: Just downstream of where the trail ends at the river, there is a wood post at the water's edge. From this post, start approx. 45 m upstream, above deep section above bedrock outcrop.

Upstream end: Stop just downstream of the big hemlock.



Looking downstream



Looking upstream

Site: 7, Buckeye Branch

Access: From Hwy 76, turn left on 196 (Chattooga Ridge Rd), left on Falls Creek Rd, left on FS-722, left on FS-723. Park in loop turn around parking area. Follow very steep trail to river; 10 min walk. NOTE: water at this site is swift (sample area is a run) and a little deep; recommend wetsuits.

Length x Width: 57 x 17 m

Side of river: GA

Species: *Elliptio* spp., *Alasmidonta varicosa*

Downstream start: Start upstream of bedrock and drop off into deep water.

Upstream end: Stop at downstream side of small island / gravel bar.



Looking upstream; end at small island



Looking across river from end of trail



Site: 8, Sandy Ford

Access: From Hwy 76, turn left on 196 (Chattooga Ridge Rd), left on 193 (Earls Ford Rd), turns to gravel (Rd 721), continue straight (will pass horse camp on left) turn left on 721A. Park in loop turn around parking area. Follow trail to river; 5 min walk. Wade across river to sand beach on GA side. NOTE: This site could also be accessed from the GA side.

Length x Width: 75 x 10 m

Side of river: GA

Species: *Elliptio* spp., *Alasmidonta varicosa*

Downstream start: Start at top of riffle before bend in river.

Upstream end: Stop at midpoint of sand beach.



Looking downstream; start at riffle crest



Looking upstream



Looking upstream



Looking downstream

| | |
|-------------------|---|
| Site: | 9, Islands |
| Access: | No access; old road bed used to access site in 2005 is now completely reforested. |
| Length x Width: | NA |
| Side of river: | NA |
| Species: | NA |
| Downstream start: | NA |
| Upstream end: | NA |

Site: 10, Earls Ford / Warwoman Creek

Access: From Hwy 76, turn left on 196 (Chattooga Ridge Rd), at 4-way stop, turn left on 193 (Earls Ford Rd), follow to end, park in parking area. Follow trail to river; 5 min walk.

Length x Width: 80 x 12 m

Side of river: SC

Species: *Elliptio* spp.

Downstream start: Start at bedrock outcrop downstream of sand beach.

Upstream end: Stop at dead tree stump that sticks out of the bank and is downstream of the island.



Looking downstream



Looking downstream



Looking upstream



Site: 11, Adeline Branch

Access: From Hwy 28 take FS-779. Follow 779, it will ford a small stream, when it goes uphill, at the crest there will be an intersection with another road to the left that continues uphill. Park in the small pull-off area on the right. Follow the trail that is on the right just before the intersection. Trail will come to a horse camp, go through horse camp and continue on trail around bend in river (trail becomes less defined here); 10 min walk.

Length x Width: 62 x 17 m

Side of river: SC

Species: *Elliptio* spp., *Alasmidonta varicosa*

Downstream start: Start 62 m downstream of bedrock on along right bank.

Upstream end: Stop at bedrock along right bank and dead/down pine tree in river to left of bedrock.



Looking downstream



Looking upstream



Looking upstream

Site: 12, Hwy 28 Wildlife Viewing Parking

Access: Park in paved river access parking lot with facilities just off Hwy 28 just down the road from the Hwy 28 bridge.

Length x Width: 27 x 10 m

Side of river: GA

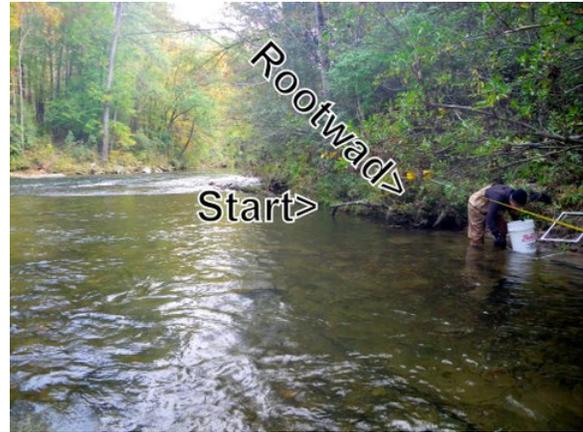
Species: *Elliptio* spp.

Downstream start: Go downstream of boat landing and parking area. Cross river and locate run below pool and above riffle. At riffle crest on left bank start at rootwad.

Upstream end: End just upstream of crest of run into pool. There is some large wood along the left bank at this location.



Looking across river



Looking downstream



Looking downstream



Looking upstream

Site: 13, Hwy 28 Bridge

Access: Park at parking area on GA side of river just before bridge. On opposite side of road from parking area, hike ditch alongside the road to the river; 1 min walk.

Length x Width: 60 x 6 m

Side of river: SC

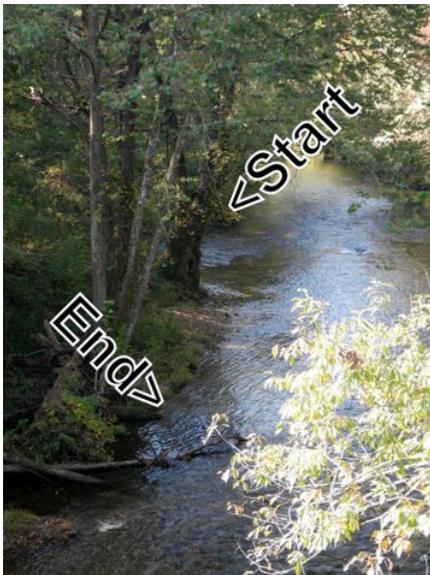
Species: *Elliptio* spp.

Downstream start: Start at dying sycamore tree with large wood (size 3) on top of it.

Upstream end: Stop at the upstream end of the gravel bar on right bank, which is just downstream of a small pile of large wood.



Looking downstream



Looking downstream



Looking upstream

Site: 14, Lick Log Creek

Access: From Hwy 28 take SC45 (Village Creek Rd.), turn left (sharp turn) on 50 (Nicholson Ford Rd), stay right at fork, parking in small parking area at end of road. Follow Foothills Trail out back of parking area, trail will cross 2 foot bridges, when close to river after Lick Log Falls, drop down to river by campsites; 20 min walk. NOTE: Parking area is small and crowded on weekends; best to do this site on a week day.

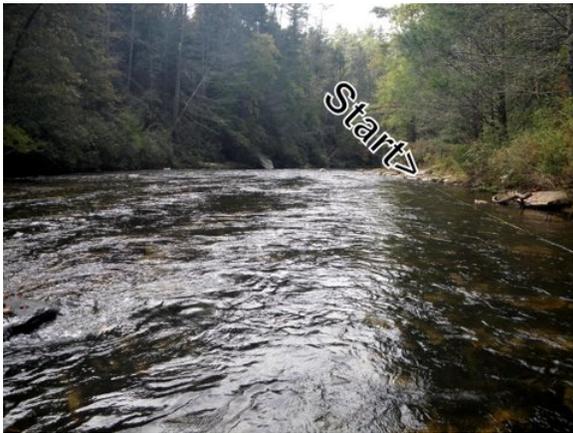
Length x Width: 43 x 20 m (sample width extends the width of the channel)

Side of river: GA

Species: Elliptio spp.

Downstream start: Site starts downstream of campsites (sample area is a run with swift water). Start at crest of riffle approx. 60 m downstream of large boulders in the middle of the river.

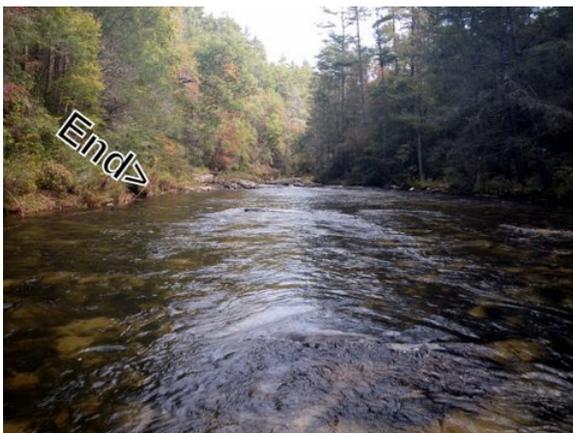
Upstream end: Stop approx. 15 m downstream of the large boulders in the middle of the river and before depth becomes too deep.



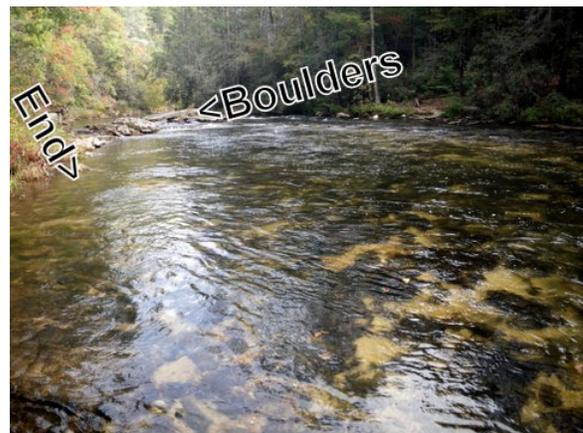
Looking downstream



Looking downstream



Looking upstream



Looking upstream

Appendix C – Mussel Sampling Methods

Methods Summary for Mussel Inventory at Field Sample-Site:

Site Layout:

1. Measure wadeable shoal length and width (m)
2. Enter length and width into “Mussel_Transect_Calculator.xls” on Netbook
3. Record random start number (1,2,3) with corresponding transect location (m) from “Mussel_Transect_Calculator.xls” onto paper datasheet (Figures C1 and C2)
4. Place tape measure along water’s edge for the length of the shoal (place 0 m end of tape at downstream end)
5. Mark each transect location with a flag

Quadrat Searches:

6. Starting at the downstream most flag, place quadrat on streambed
 - a. If transect distance is **odd**, place quadrat next to bank
 - b. If transect distance is **even**, place quadrat one quadrat length from bank
7. Search quadrat before and after light disturbance of substrate
 - a. Search the surface visually using viewing bucket or snorkel gear
 - b. Remove large gravels, cobbles, wood; visually search the surface again
 - c. Sift fine substrates with fingers to a depth of 5-10 cm
8. Extract located mussels, record species and length (mm), carefully return all live mussels to substrate
9. Flip quadrat twice (in-line with transect) to find new location
10. Record number of quadrats searched per transect
11. All relics found on the shoal (not just in quadrats) are collected and placed in a labeled bag
12. Note presence/absence of Asian clams (*Corbicula fluminea*)

| | |
|---|--|
| Site: #6, Fall Creek | Crew: Colin Krause, Ashley Lewis, Rachel Goodpaster, James Hudson, Jacoby Lipscomb, Mike Knoerr |
| Date: 10/11/2012 | |
| # of bags of relics: 0, no relics found | Notes: 70x10m; 14C; Corbicula absent; 23 of the 24 mussels in transect 39.3m were found within in 1 of the 10 quadrats searched in this transect |

Live Mussels:

*AV = *Alasmidonta varicosa*, ES = *Elliptio spp.*

| RS #1,2,3 | Transect (m) | # Quads | Species* & Lengths (mm) |
|-----------|--------------|---------|--|
| 2 | 3.5 | 10 | ES = 45, 29 |
| 3 | 9.5 | 10 | ES = 59 |
| 1 | 16.0 | 10 | ES = 51, 37 |
| 2 | 26.8 | 10 | None |
| 3 | 32.8 | 10 | AV = 45 ES = 73 |
| 1 | 39.3 | 10 | AV = 45, 42, 55, 51, 47, 47, 51, 63, 39, 38, 48, 54, 53 ES = 64, 58, 34, 49, 52, 43, 51, 56, 59, 49, 61 |
| 2 | 50.2 | 10 | None |
| 3 | 56.2 | 10 | None |
| 1 | 62.7 | 10 | ES = 62, 59 |
| | | | |
| | | | |
| | | | |

Figure C2. Example paper datasheet containing actual data from 2012 inventory of Site 6.

Methods Summary for Site Layout and Results Calculations (Wendell Haag, pers. comm.):

Site Layout Calculations

Sample sites are established in wadeable shoal areas. Wadeable shoals do not span the entire wetted channel in many areas. Shoal location and size is thoroughly documented with written descriptions, measurements, site drawings, digital photographs, and GPS points. Detailed site descriptions are necessary so that sites can be accurately located and re-sampled during future inventories.

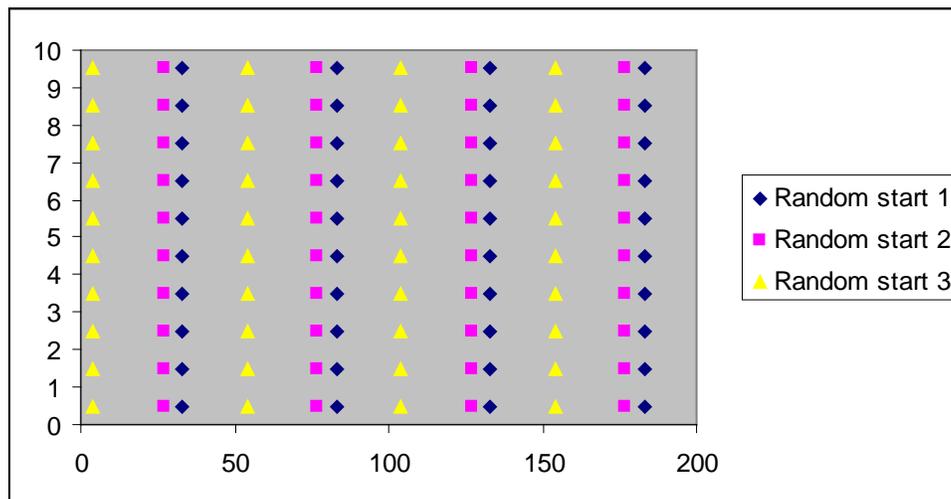
Once the total wadeable area available for sampling is known the sampling layout for each site can be determined in the field with a portable computer (i.e. laptop or netbook). Sample layout will proceed for each site as follows:

- Sampling unit: quadrat = 0.25 m^2 , 0.5 m x 0.5 m
- Example site: length = 100 m, width = 10 m, area = 1000 m^2 , 4000 possible quadrats
- Sample 2.5% of site = $4000 * 0.025 = 100$ total quadrats
- Quadrats are 0.5 m on a side, so there are 200 possible transects along the 100 m length of the unit
- Within each transect sample a point every 1 m across the stream, so 10 quadrats/transect (sampling point will be in the middle of the quadrat, so after sampling a quadrat, flip quadrat twice to find new location, quadrats are 0.5 m on each side so two flips = 1.0 m)
- 100 total quadrats desired \div 10 quadrats/transect = 10 total transects
- 10 transects/3 random starts = 3.3 transects/random start, round up to 4
- 200 possible transects \div 4 transects/random start = 50 possible transect arrays
- Draw 3 random numbers between 0 and 49, these are the starting points for the first transect in each random start. Example: 33, 27, 4. **(It is important to record the random start number associated with each transect)**
- Add 50 to each point to give the position of the next transect in each random start. Example: $33+50=83$, $27+50=77$, $4+50=54$
- Add 50 to each point two more times. This will give you the locations of all transects as follows:
Random start 1: 33, 83, 133, 183
Random start 2: 27, 77, 127, 177
Random start 3: 4, 54, 104, 154
- Divide each of these numbers by 2 to give the transect starting position in meters:
Random start 1: 16.5, 41.5, 66.5, 91.5
Random start 2: 13.5, 38.5, 63.5, 88.5
Random start 3: 2, 27, 52, 77

- If the transect starting number is odd, then place the first quadrat of each transect in that random start next to the bank. If the starting number is even, place the first quadrat of all transects one quadrat length from the bank. Example: Random start 3 (starting number=2) begins one quadrat length from shore

Although this gives 12 transects and 120 total quadrats, slightly more than planned, it is an easy way to allocate effort and gives the desired number of samples as a minimum. It is important to note that $n=3$ here not 12, 40, or 120. Each random start constitutes a single sample because the placement of all subsequent quadrats in the sample is dependent on the placement of the first one. With this worked out for each site, the only remaining layout necessary in the field is to stretch a meter tape, find the starting points and begin sampling

Below is a map of the hypothetical site; stream length is along the x-axis and width is represented by the y-axis (stream flow is parallel to the x-axis).



Quadrats are searched visually using viewing bucket or snorkel gear and with light disturbance of substrate; no comprehensive excavation of substrate will be undertaken. Once a mussel is located, it is extracted from the substrate for identification and measurement. All live mussels are carefully returned to the substrate. All relics found on the shoal (not just in quadrats) are collected and placed in a labeled bag. Relics are brought back to the lab for identification and measurement. For insurance purposes, it is best to record the number of quadrats searched in each transect, but with good average width measurements, the number of quadrats is not needed in computation.

Results Calculations

Computation of an estimate of total number of mussels at the site (τ), is straight-forward. Using the previous example, there are 50 possible transect arrays and 2 possible bank starting positions (next to bank or 1 quadrat length offshore), so there is a total of 100 possible random starts at the site (N). There are $n = 3$ samples, and y = the number of mussels in each sample.

$$\tau = \frac{N}{n} \sum_{i=1}^n y_i$$

As an example consider the following dataset:

| Site | Random start | Transect | Number of <i>Elliptio</i> spp. |
|------------|--------------|----------|--------------------------------|
| Camp Creek | 1 | a | 8 |
| | | b | 2 |
| | | c | 3 |
| | | d | 5 |
| | | total | 18 |
| | 2 | a | 7 |
| | | b | 1 |
| | | c | 0 |
| | | d | 6 |
| | | total | 14 |
| | 3 | a | 9 |
| | | b | 2 |
| | | c | 5 |
| d | | 2 | |
| total | | 18 | |

$$\tau = \frac{100}{3}(18 + 14 + 18) = \mathbf{1667} \text{ mussels at the site}$$

The variance is computed as follows:

$$\text{var}(\tau) = \frac{N(N-n)}{n} \times \frac{\sum_{i=1}^n (y_i - \bar{y})^2}{n-1}$$

The variance for the example above is 115,428.

The confidence intervals are computed as follows:

$$\exp\left(\ln(\hat{\tau}) \pm t_{\alpha/2, df} \sqrt{\frac{\text{var}(\hat{\tau})}{\hat{\tau}^2}}\right)$$

The confidence intervals for the example above is 3,296 (+) and 843 (-).

This approach allows us to calculate the number of mussels at each sample site. Density is calculated as the number of mussels divided by the total area of the sample site. Repeating this sampling protocol at the same sample sites at some time in the future allows us to detect temporal changes in mussel population sizes using t-tests (Strayer and Smith 2003, pages 79-81).