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2020 Duck River Quantitative

Mussel Survey

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INTRODUCTION

The Duck River watershed of south-central Tennessee is one of the most biologically diverse systems in North America covering approximately 8,099 km^2 of the Interior Low Plateau Physiographic Province (Fenneman 1938). Beginning in the Eastern Highland Rim physiographic region in Coffee County, the Duck River flows westward across the Central Basin for approximately 290 miles passing through six counties before joining Kentucky Reservoir on the Tennessee River in the Western Highland Rim region in Humphreys County. The Duck River is largely unimpounded for much of its length, except for the Tennessee Valley Authority (TVA) Normandy Dam near the headwaters and several mill dams including one near Milltown and one in Columbia. The Duck River is the longest river contained completely within Tennessee's boundaries and the watershed drains approximately 8% of Tennessee's land area.

Approximately 77 native freshwater mussel species are known from the Duck River watershed of which 62 are extant (TN SWAP 2015). However, the river has experienced a host of anthropogenic perturbations including habitat loss due to landscape clearing and conversion, increased impervious surfaces, impoundment, agricultural runoff, phosphate mining, gravel dredging, and municipal and industrial wastewater discharge. As a result, 25 mussel species occurring in the watershed are federally listed as endangered or threatened under the Endangered Species Act (ESA) of 1973 (87 Stat. 884, as amended; 16 U.S.C. 1531 et seq.) with 6 species presumably extinct.

Globally, the Duck River contains the only known population of *Epioblasma ahlstedti* (Jones and Neves 2010) and the largest known population of *Lemiox rimosus* (Jones et al. 2009) and the last viable population of *Theliderma intermedia* (USFWS 2011). Fifteen federally endangered or threatened mussel species remain extant in the Duck River including: *Cumberlandia monodonta*, *Cyprogenia stegaria**, *E. ahlstedti*, *E. brevidens**, *E. triquetra**, *Lampsilis abrupta**, *Lemiox rimosus*, *Plethobasus cyphus*, *Pleuronaia dolabelloides*, *Ptychobranchus subtentus**, *Quadrula fragosa**, *Theliderma cylindrica*, *T. intermedia*, *Toxolasma* *cylindrellus**, and *Villosa fabilis** with several of these species recently reintroduced or augmented (marked by * above) into the river. These animals were introduced through captive propagation and/or translocation from the closest viable populations throughout their ranges. The Duck River plays an important role in the global conservation for a number of freshwater mussel species in Tennessee and the southeastern United States and is a vital component in multiple management or species recovery plans (e.g., Plan for the Population Restoration and Conservation of Imperiled Freshwater Mollusks of the Cumberlandian Region and Tennessee Freshwater Mollusk Strategic Plan), as the river is a primary source for many brood and translocation stock, as well as a recipient stream of previously extirpated mussel species (i.e., reintroductions or translocations).

A substantial portion of the Duck River was designated as critical habitat by the U.S. Fish and Wildlife Service for five federally protected mussel species. The most expansive designation was made for *P. subtentus* and *P. dolabelloides* (78 FR 59556). This designation extends from the confluence with Kentucky Reservoir in Humphreys County upstream through Perry, Hickman, Maury, Marshall, and Bedford Counties, Tennessee. This reach includes approximately 216 river miles of the Duck River from its inundation at DRM 11.7 in Kentucky Reservoir in Humphreys County, TN, upstream to its confluence with Flat Creek (DRM 228) near Shelbyville in Bedford County, TN. Critical habitat has also been designated for *E. ahlstedti*, *E. brevidens*, and *T. cylindrica* (69 FR 53136, 78 FR 57076). Additionally, *Obovaria subrotunda* was recently proposed for listing as threatened by the U.S. Fish and Wildlife Service and the Duck River was proposed as critical habitat in 59 river miles in Bedford, Marshall, and Maury Counties (85 FR 61384). These proposed actions, designations, and listings give TWRA opportunity to pursue ESA prescribed aquatic species recovery actions in this reach of the Duck River.

Watters (2000) recognized freshwater mussels as being excellent indicators of water quality and habitat stability due to their sensitivity to water quality and anthropogenic disturbance. Mussels provide a number of realized and unrealized ecosystem services as nutrient cyclers, biodepositors, ecosystem engineers, and sediment bioturbators, thus enhancing habitat and food resources for the entire aquatic food web (Gutiérrez et al. 2003; Howard and Cuffey 2006; Vaughn et al. 2008; Spooner and Vaughn 2006, 2008; Vaughn 2010). Their total biomass may exceed that of any other aquatic group (Howard and Cuffey 2006). The important role that mussels play in aquatic ecosystems cannot be overstated.

Mussels have received extensive attention from resource managers over the past 30 years due to their declining conservation status. In order to adequately manage and assess the conservation status this fauna, researches depend on periodic sampling to assess the abundance and distribution of species of interest. Beginning in 1979, the TVA established 22 quantitative mussel monitoring sites upstream of Columbia in association with the development of the Columbia Dam project (Ahlstedt 1991). TVA revisited some of these sites and added additional sites in 1988 (Jenkinson 1988) but ceased monitoring efforts before dismantling its defunct Columbia Dam project in 1999. These surveys provide a historical reference of the river's mussel population. Trend data indicates that populations of most mussel species have increased in recent years (Ahlstedt et al. 2004). Since 1991, reservoir release improvement measures implemented by TVA at Normandy Dam (Duck River Mile 248.6) have resulted in higher dissolved oxygen concentrations and minimum flow releases. Along with point source regulation and riparian habitat restoration activities, these management improvements are considered instrumental factors influencing the river's ongoing mussel population recovery (Ahlstedt et al. 2004).

To continue monitoring efforts in the Duck River, The Tennessee Wildlife Resources Agency (TWRA) in collaboration with the United States Fish and Wildlife Service (USFWS) Tennessee Ecological Services Field Office and the Tennessee Chapter of The Nature Conservancy (TNC) established a long-term monitoring program to assess mussel densities at 6 sites in the Duck River between Shelbyville and Littlelot. This program was established in 2010 with the intent of resampling at 5-year intervals with the sites revisited in 2015 and most recently during this occasion. The purpose of this report is to share the findings of the 2020 sampling effort and assess any changes in mussel densities or trends from previous surveys. In addition, we examined length data to provide insight into the viability of the Duck River's mussel populations by documenting recent recruitment and distribution of the various species across multiple year classes.

METHODS

Six long-term monitoring stations were established in 2010 and sampling was conducted in 2010, 2015 (Hubbs et al. 2010, Hubbs et al. 2015). Sites were selected based on their proximity to previous quantitative survey sites, distribution within the watershed, extent of available shoal habitat, and accessibility. These sites were located between Tarpley Bluff in Bedford County (DRM 207.3) and Littlelot in Hickman County (DRM 88.9; Figure 1).

Because riverine habitat is dynamic, each site was delineated immediately before sampling to ensure that only potentially suitable habitat was included in the survey area. We defined suitable habitat as gravel/cobble shoal habitat with limited bedrock. In addition, only habitat less than 1 meter in depth was included to facilitate sampling without the use of SCUBA. The upstream and downstream limit coordinates were recorded using a Global Positioning System (GPS) and the site length and site widths were measured using a laser range finder and tape measures. Total site area (m^2) was calculated by multiplying mean river width by the total length of the site. Stream stages at the USGS gage stations nearest to each site were recorded for each survey date (Table 1).

Sample size was set at approximately 80, 0.25-m^2 quadrats per site to facilitate sufficient statistical power for comparative trend analysis and to provide a reasonable probability of detecting rare mussel species. After a site was delineated, lateral transects were systematically distributed over the site and quadrats were randomly distributed along each transect as necessary to equally distribute effort across the site. Quadrats were sampled beginning at the downstream end of the site and working in an upstream direction. Substrate in each quadrat was excavated to approximately 15 cm depth by hand or garden-trowel and placed in a uniquely tagged mesh bag. Bags were maintained in flowing river water until processed. The contents of each bag were sorted through a series of sieves, mussels were removed, and the substrate was discarded into the river channel. Recovered mussels were sorted by species and sex (when possible), enumerated, measured along the longest axis parallel to the hingeline using dial calipers, and returned to their approximate location of collection. All data were recorded by transect and quadrat.

Data were entered into Microsoft Excel for analysis preparation. Estimation and comparison of mean densities were completed in R (R Core Team 2020) using ANOVA for comparisons of mean densities for each species among years. Tukey's Honest Significant Difference test was used to determine differences in densities between specific years at α=0.05). Site level population sizes for each species were estimated by multiplying estimated mussel density (number/m^2) by total site area.

Boxplots were generated for species having sufficient length data available among the 3 sampling occasions to show any distributional changes in length over time. Evidence of recent recruitment was subjectively defined as mussels less than 25-mm in length. In addition, mussels less than 35 mm in length were defined as individuals likely recruited since the previous survey occasion. Density data collected from the four sites surveyed during the current sampling was compared to the corresponding sites surveyed in 2010 and 2015.

RESULTS

A total of 1,157 mussels representing 38 species were collected from 4 sites in 2020, yielding a mean density of 13.94 mussels/m^2 (Table 2 and 3). Eleven federally endangered and one threatened species were collected during the 2020 survey. *Lemiox rimosus* (n=223) was the most abundant species collected and represented 19.27% of the total mussels collected during the survey despite being collected at 3 of the 4 sampled sites. *Cyclonaias tuberculata* (n=146) represented 12.62%, *Eurynia dilatata* (n=122) represented 10.54%, and *O. subrotunda* (107) represented 9.25% of the total mussels collected, respectively. Results of the 2020 survey show a declining trend in the total mussel population at the four sampled sites since 2010. However, some sites and species showed increasing trends between 2010 and 2020.

Tarpley Bluff

Tarpley Bluff, located at Duck River Mile (DRM) 207.3 in Bedford County was not sampled in 2020 due to elevated water levels due during September.

Lillard's Mill

The Lillard's Mill site (DRM 179.2) in Marshall County was sampled on 8 September 2020. Stream stage and discharge at the USGS gage station at the Duck River above Milltown, TN (USGS gage 03599240) was 11.24 ft and 315 ft^3/s, respectively and water was clear to slightly turbid (Table 1). Site set-up and sampling took approximately 1.5 and 8 hours to complete, respectively. The survey crew varied throughout the day with 3-5 individuals collecting samples and 2-3 individuals processing samples and recording data. Ten transects were spaced at 10-meter intervals in the channel between the left descending bank and the mid-channel island beginning 90 meters downstream from the island head (Figure 2). Nine transects were spaced at 10-meters intervals in the channel between the right descending bank and the mid-channel island beginning 80 meters downstream of the island head. Four quadrats were sampled along each transect. Eight quadrats were sampled along an additional transect spanning the width of the river approximately 10 meters upstream of the island head. The mean channel width along the left descending channel was approximately 25 meters and the mean width along the right descending channel was approximately 15 meters. The channel width on the upstream most transect was approximately 73 meters. Substrates were primarily gravel and cobble but approximately half of the right descending channel was bedrock and was subsequently not included in sampling. *Justicia americana* was present along both sides of the channel on the left descending side of the island. The shoal area sampled represented approximately 3,400 m^2 which was approximately equal to the area of the 2010 survey and approximately 33% larger than the area sampled in 2015.

During 2020 sampling, 381 mussels representing 33 species were collected from 84 total quadrats. *Ellipsaria lineolata*, *Cyprogenia stegaria*, *Epioblasma brevidens*, *E. obliquata*, *E. triquetra*, *Lampsilis abrupta*, *Theliderma metanevra*, and *Toxolasma cylindrellus* were all detected in 2020 but had not been previously detected in 2010 or 2015. *Lampsilis ovata*, *L. teres*, *Pleurobema rubrum*, *Strophitus undulatus*, *Utterbackia imbecillis*, and *Villosa iris* were not detected in 2020 although they had been detected in either or both 2010 and 2015. Twelve federally listed mussel species were collected in 2020 including *C. stegaria*, *E. ahlstedti*, *E.*

brevidens, *E. obliquata*, *E. triquetra*, *L. abrupta*, *Lemiox rimosus*, *Pleuronaia dolabelloides*, *Ptychobranchus subtentus*, *Theliderma cylindrica*, *T. intermedia*, and *T. cylindrellus*. In addition, federally proposed or petitioned species *Medionidus conradicus*, *O. subrotunda*, and *P. barnesiana* were collected at the site.

Estimated density (95% CI) among all species in the 2020 sampling was 18.1 mussels/m^2 (13.4-23.9 mussels/m^2) with *L. rimosus* representing the most frequently detected species with an estimated density of 4.4 individuals/m^2 (3.0-5.8 individuals/m^2) followed by *E. dilatata* (2.8 individuals/m^2; 1.9-3.6 mussels/m^2) and *C. tuberculata* (2.5 individuals/m^2; 1.6-3.4 mussels/m^2; Table 4). *C. stegaria*, *E. obliquata*, *E. triquetra*, *L. abrupta*, *L. fasciola*, *Leptodea fragilis*, *Megalonaias nervosa*, *Obliquaria reflexa*, *T. metanevra*, and *T. lividum* were each represented by one individual. Estimated density of *Corbicula fluminea* was 62.0 individuals/m^2 (48.5-75.4 individuals/m^2).

Estimated densities of all species except *C. tuberculata, E. ahlstedti*, *E. dilatata*, *L. costata*, *P. barnesiana*, and *V. taeniata* were similar among all three sampling occasions (Figure 3). *Cyclonaias tuberculata* densities were similar between 2010 and 2015 (p=0.79) but decreased by 49% from 2015 to 2020 (p=0.01). *Epioblasma ahlstedti* densities decreased by 84% from 2010 to 2015 (p<0.01) but were similar between 2015 and 2020 (p=0.95). *Eurynia dilatata* density decreased by 43% from 2010 to 2015 (p<0.01) and decreased an additional 47% from 2015 to 2020 (p<0.02). *Lasmigona costata* densities were similar between 2010 and 2015 (p=0.22) but decreased by 59% from 2015 to 2020 (p=0.02). Similarly, *P. barnesiana* densities were also similar between 2010 and 2015 (p=0.59) but decreased by 73% between 2015 and 2020 (p<0.01). *Villosa taeniata* densities were similar between 2010 and 2015 (p=0.24) and between 2015 and 2020 (p=0.13) but showed a 67% decline between 2010 and 2020 (p<0.01).

Recent reproduction (<25 mm in length) was evident for 9 species including *Amblema plicata*, *C. pustulosa*, *E. ahlstedti*, *E. dilatata*, *L. costata*, *L. rimosus*, *O. reflexa*, *P. subtentus*, and *Truncilla truncata*. In addition, *C. tuberculata*, *M. conradicus*, *O. subrotunda*, *P. barnesiana*, *T. lividum*, *V. taeniata*, and *V. vanuxemensis* were represented by at least one individual less than 40 mm in length, suggesting recruitment to the population since the 2015 survey. Although less than 40 mm, *E. obliquata* and *T. cylindrellus* retained a mark and *E. triquetra* was PIT-tagged indicating that these animals were recently released from captive propagation programs. Length data for each sampling occasion was only available for *E. ahlstedti* and *L. rimosus*. Lengths of *E. ahlstedti* was represented by multiple individuals less than 25 mm in length on all three sampling occasions at Lillard's Mill (Figure 4). However, the length range and 25th percentiles for 2015 and 2020 appeared to represent a smaller and younger population structure. The mean lengths of *L. rimosus* was similar among years and the 25th percentile lengths for each year was less than 35 mm or 5 years (Figure 5).

Venable Spring

The Venable Spring site (DRM 176.8) in Marshall County was sampled on 9 September 2020. Stream stage and discharge at the USGS gage station at the Duck River above Milltown, TN (USGS gage 03599240) was 11.22 ft and 305 ft^3/s, respectively and water was clear to slightly turbid (Table 1). Site set-up and sampling took approximately 1 hour and 8 hours to complete, respectively. The survey crew varied throughout the day with 3-4 individuals collecting samples and 2-3 individuals processing samples and recording data. Nine transects were spaced at 5-meter intervals in the channel beginning at the spring run on the right descending bank and extending 80 meters upstream (Figure 6). Five quadrats were sampled along each transect. The mean channel width was approximately 45 meters with a maximum width of approximately 55 meters. Substrates were primarily gravel and cobble but substrate in the lower half of the site along the right descending bank was a mix of gravel and cobble mixed with silt. *Justicia americana* was sporadic along the right descending bank with the greatest density in the upper half of the site. The area sampled represented approximately 3,600 m^2 which was approximately 20% larger than the 3,000 m^2 area sampled in 2010 and 2015.

During 2020 sampling, 293 mussels representing 24 species were collected from 83 total quadrats (Table 5). *Medionidus conradicus* was detected in 2020 but had not been previously detected in 2010 or 2015. *Arcidens confragosus*, *L. cardium*, *Potamilus alatus*, *Tritogonia verrucosa*, and *Utterbackia imbecillis* were not detected in 2020 although they had been detected in either or both 2010 and 2015. Five federally listed mussel species were collected in 2020 including *E. ahlstedti*, *L. rimosus*, *P. dolabelloides*, *T. cylindrica*, and *T. intermedia*. In addition, the petitioned species *M. conradicus*, *O. subrotunda*, *P. barnesiana* were collected at the site.

Estimated density (95% CI) among all species in the 2020 sampling was 14.1 mussels/m^2 (11.8-16.4 mussels/m^2) with *O. subrotunda* representing the most frequently detected species with an estimated density of 3.8 individuals/m^2 (2.3-5.3 individuals/m^2) followed by *L. rimosus* (2.3 individuals/m^2; 1.5-3.1 mussels/m^2). *Cyclonaias tuberculata* and *V. taeniata* were the third most frequently detected species with 1.0 individuals/m^2 (0.6-1.5 mussels/m^2) and 1.0 individuals/m^2 (0.5-1.6 mussels/m^2). *Lampsilis ovata*, *L. fragilis, M. conradicus, M. nervosa, and P. oviforme* were each represented by one individual. Estimated density of *C. fluminea* was 24.0 individuals/m^2 (19.1-29.0 individuals/m^2).

Estimated densities of all species except *E. ahlstedti* and *O. subrotunda* were similar among all three sampling occasions (Figure 6). *Ebioblasma ahlstedti* density decreased by 82%

between 2010 and 2015 (p<0.01) but was similar between 2015 and 2020 (p=0.99). *Obovaria subrotunda* densities were similar between 2010 and 2015 (p=0.77) but increased by 376% from 2010 to 2020 and 193% from 2015 to 2020 (p<0.01).

Recent reproduction (<25 mm in length) was evident for 9 species including *E.dilatata*, *C. pustulosa*, *E. ahlstedti*, *P. barnesiana, P. dolabelloides, O. subrotunda, T. cylindrica, T. truncata, T. lividum, and V. taeniata*. In addition, *A. plicata, C. tuberculata, L. costata*, *L. rimosus*, *M. conradicus, P. rubrum,* and *T. intermedia* were represented by at least one indvidual less than 40 mm in length, suggesting recruitment to the population since the 2015 survey. *Epioblasma ahlstedti* was represented by several individuals less than 25 mm in length in 2010 and 2020 but only one individual was detected in 2015. Overall lengths of the 2015 sampling occasion were skewed towards larger sizes whereas the 2020 sampling even was well represented by all sizes (Figure 4). Recent recruits of *L. rimosus* were not represented in the 2015 or 2020 survey but were represented in the 2010 survey. However, several animals in each of 2015 and 2020 were less than 35 mm in length suggesting that they were less than 5 year old (Figure 5).

Hooper Island

The Hooper Island site is located at DRM 163.1 in Maury County, just upstream of Carpenters Bridge Road. Stream stage and discharge at the USGS gage station at on the Duck River at Columbia, TN (USGS gage 03599500) was 2.30 ft and 362 ft^3/s, respectively and water was clear to slightly turbid (Table 1). Site set-up and sampling took approximately 1.0 and 8 hours to complete, respectively. The survey crew varied throughout the day with 2-3 individuals collecting samples and 3-4 individuals processing samples and recording data. Eleven transects were spaced at 10-meter intervals in the channel between the left descending bank and the mid-channel island beginning approximately 150 meters upstream from the lower extent of the island (Figure 8). Eight quadrats were sampled along each transect depending on the habitat. The mean channel width of the site was approximately 23 meters. Substrates were primarily gravel and cobble with clay along the first 2 meters of the left descending bank. *Justicia americana* was present along the island on the right descending bank. The shoal area sampled represented approximately 2,300m^2 which was approximately equal to the area of the 2010 survey and approximately 16% smaller than the area sampled in 2010 and approximately 11% larger than the areas sampled in 2015.

During 2020 sampling, 415 mussels representing 21 species were collected from 88 total quadrats (Table 2 and 3). *Obliquaria reflexa was* detected in 2020 but had not been previously detected in 2010 or 2015. *Lampsilis ovata*, *M. nervosa*, *P. alatus*, *P. fasciolaris*, and *V. vanuxemensis* were not detected in 2020 although they had been detected in either or both

2010 and 2015. Five federally listed mussel species were collected in 2020 including *E. ahlstedti*, *L. rimosus*, *P. dolabelloides*, *T. cylindrica*, and *T. intermedia*. In addition, the petitioned species *O. subrotunda*, *P. barnesiana,* and *T. lividum* were collected at the site.

Estimated density (95% CI) among all species in the 2020 sampling was 19.1 mussels/m^2 (16.3-21.8 mussels/m^2; Table 6) with *L. rimosus* representing the most frequently detected species with an estimated density of 4.9 individuals/m^2 (3.6-6.3 individuals/m^2) followed by *E. dilatata* (2.5 individuals/m^2; 1.8-3.3 mussels/m^2) and *C. tuberculata* (1.7 individuals/m^2; 1.2-2.3 mussels/m^2). *Leptodea fragilis*, *O. reflexa*, and *T. lividum* were each represented by one individual. Estimated density of *C. fluminea* was 93.6 individuals/m^2 (80.9-106.3 individuals/m^2).

Estimated densities for 11 of 21 species differed among the three sampling occasions (Figure 9). *Eurynia dilatata* densities were similar between 2010 and 2015 (p=0.80) but decreased by 46% in 2020 (p=0.02). *Pleurobema rubrum* densities were similar between 2010 and 2015 (p=0.12) but decreased by 73% between 2010 and 2020 (p=0.01). *Pleuronaia barnesiana* and *T. intermedia* densities both decreased by 71% between 2010 and 2015 (p<0.01) but were similar between 2015 and 2020 (p=0.99). In contrast, *E. ahlstedti* densities were similar between 2010 and 2015 (p=0.77) but increase by 233% between 2015 and 2020 (p<0.01). *Theliderma cylindrica* densities increased by 867% between 2010 and 2015 (p<0.01) but were similar between 2015 and 2020 (p=0.61). *Villosa taeniata* densities also increased by 68% between 2010 and 2015 (p=0.03) but was similar between 2015 and 2020 (p=0.94). *Lasmigona costata* densities increased by 141% from 2010 to 2015 (p=0.02) but decreased by 69% between 2015 and 2020 (p<0.01; Figure 10). *Lemiox rimosus* densities decreased by 56% from 2010 to 2015 (p<0.01) but increased by 91% between 2015 and 2020 (p=0.02). *Tritogonia verrucosa* densities decreased from 2010 to 2015 (p=0.02) but were similar between 2010 and 2020 (p=0.10). Densities for each of these species were similar between 2010 and 2020 (p>0.05).

Recent reproduction (<25 mm in length) was evident for 10 species including *C. pustulosa, C. tuberculata*, *E. ahlstedti*, *E. dilatata*, *L. costata*, *L. rimosus, L. fragilis, M. conradicus, O. subrotunda, and P. barnesiana*. In addition, *A. plicata, O. reflexa, P. oviforme, P. rubrum, P. dolabelloides, T. cylindrica, T. intermedia, and T. lividum*, were represented by at least one indvidual less than 40 mm in length, suggesting recruitment to the population since the 2015 survey. Length measurements for *E. ahlstedti* were not available for Hooper Island but recent recruits of *L. rimosus* were collected from the site during all three sampling occasions with the 25th percentile lengths less than 35 mm for each year (Figure 5).

<u>Columbia Mill Dam</u>

The Columbia Mill Dam site (DRM 133.5) in Maury County is 0.37 miles downstream from the dam and approximately 200 meters downstream of the TWRA boat ramp off Riverside Drive (Figure 10). The site was sampled 11 September 2020. Stream stage and discharge at the USGS gage station at on the Duck River at Columbia, TN (USGS gage 03599500) was 2.23 ft and 344 ft^3/s, respectively and water was clear to slightly turbid (Table 1). Site set-up and sampling took approximately 1.5 and 6 hours to complete, respectively. The survey crew varied throughout the day with 3-5 individuals collecting samples and 3 individuals processing samples and recording data. Nine transects were spaced at 5-meter intervals in each of the channels surrounding the mid-river island beginning 40 meters downstream from the island head. An additional transect spanned the river channel approximately 5-meters upstream of the island. Three quadrats were sampled in each of transects 1-4, 4 quadrats were sampled in transects 5-7, and 5 quadrats were sampled in transects 8 and 9 of the right descending channel. Four quadrats were sampled in each of transects 1-7 and transect 9 and 5 quadrats were sampled in transect 8 of the left descending channel. Six quadrats were sampled in the transect immediately upstream of the island. The mean channel width along the left descending channel was approximately 21 meters and the mean width along the right descending channel was approximately 20 meters. The channel width on the upstream most transect was approximately 35 meters. Substrates were primarily gravel and cobble. *Justicia americana* was present along both sides of the channel along the island and on the left side of the left descending channel. The shoal area sampled represented approximately 1,845 m^2 which was approximately 16% smaller than the area sampled in 2010 and 2015.

During 2020 sampling, 96 mussels representing 15 species were collected from 77 total quadrats. *Potamilus alatus* was detected in 2020 but had not been previously detected in 2010 or 2015. *Actinonaias ligamentina*, *A. pectorosa*, *E. lineolata*, *Elliptio crassidens*, *E. dilatata, Ligumia recta, and Q. quadrula*, were not detected in 2020 although they had been detected in either or both 2010 and 2015. *Pleuronaia dolabelloides* and T. cylindrica were the only federally listed mussel species were collected in 2020.

Estimated density (95% CI) among all species in the 2020 sampling was 5.0 mussels/m^2 (3.8-6.1 mussels/m^2; Table 7) with *C. tuberculata* representing the most frequently detected species with an estimated density of 1.8 individuals/m^2 (1.2-2.5 individuals/m^2) followed by *C. pustulosa* (1.0 individuals/m^2; 0.6-1.4 mussels/m^2), and *A. plicata* (0.6 individuals/m^2; 0.3-1.0 mussels/m^2). *Lampsilis fasciola*, *L. costata*, *M. nervosa*, *P. alatus*, and *T. cylindrica* were each represented by one individual. Estimated density of *C. fluminea* was 4.6 individuals/m^2 (3.3-5.8 individuals/m^2).

Estimated densities of all species except *A. plicata, L. costata*, *L. fragilis*, *O. reflexa, and Q. quadrula* were similar among all three sampling occasions (Figure 11). *Amblema plicata* densities were similar between 2010 and 2015 (p=0.16) but decreased by 57% from 2010 to 2020 (p=0.03). *Lasmigona costata* density decreased by 89% between 2010 and 2020 (p=0.01), *L. fragilis* density decreased by 70% between 2010 and 2020 (p=0.04), *O. reflexa* density decreased by 43% between 2010 and 2020 (p=0.02), and *Q. quadrula* density decreased by 83% between 2010 and 2015 (p=0.03). However, each of these species were represented by 10 or fewer individuals in the 2010 collection and were represented by 4 or fewer individuals in 2020.

Recent reproduction (<25 mm in length) was evident for 5 species including *C. pustulosa*, *C. tuberculata*, *P. dolabelloides*, *L. fragilis*, *and O. reflexa*. In addition, *T. truncata* was represented by one individual less than 40 mm in length, suggesting recruitment to the population since the 2015 survey.

<u>Littlelot</u>

Littlelot, located at DRM 88.9 in Hickman County, 20 m upstream from State Highway 230 Bridge was not sampled in 2020 due to elevated water levels during September.

DISCUSSION

The Duck River continues to harbor one of the most diverse and abundant assemblages of freshwater mussels in North America. We collected 38 of the 71 known mussel species from the Duck River among 4 sites in 2020 with 33 of the species collected at Lillard's Mill. Species richness at this site was the highest recorded at any sites within in Duck River over the past 30 years, in large part due to the reintroduction of several species. The Tennessee Wildlife Resources Agency stocked 4,962 *P. subtentus* into Lillards's Mill from 2006 until 2014. Six *P. subtentus* were collected during the 2010 survey and 26 individuals were collected in 2015 ranging from 42 to 110 mm in length (TWRA 2015). We collected 16 individuals ranging from 9 to 120 mm in length in the 2020 survey indicating that the species has successfully recruited at the site over multiple years and possibly multiple generations. In addition, unmarked *C. stegaria* (48 mm), *E. brevidens* (50 mm), *E. triquetra* (36 mm), and *L. abrupta* (89 mm) were collected in 2020 documenting the first collections of these species since their reintroductions were initiated in 2013. An individual tagged *E. obliquata* was also collected in 2020. The collections of these reintroduced animals indicate that some animals have survived multiple years since their release.

The Duck River between Lillard's Mill and the Columbia Mill Dam likely contains the densest populations of *E. ahlstedti*, *L. rimosus*, *P. dolabelloides*, *O. subrotunda*, *T. cylindrica*, and *T. intermedia* throughout their global ranges. The enhancement of the status of these species in the Duck River is presumably a result of TVA's Reservoir Release Improvement Program at Normandy Dam, improvements to water supply and wastewater treatment facilities, and coordination among conservation agencies and organizations that have sought to improve the water quality and aquatic habitat within the watershed (Ahlstedt et al. 2004; Jones et al. 2009; TWRA 2015). The success of these conservation actions are best exemplified by statistically significant increases in density of *E. ahlstedti*, *T. cylindrica*, and *V. taeniata* between 2010 and 2020 at Hooper Island. However, these increases were not nearly as substantial as the 376% increase in density of *O. subrotunda* at Venable Spring. Estimated densities of most other species did not change between 2010 and 2020 which should be perceived positively for this highly imperiled fauna which is generally in decline across North America.

Although densities of some species increased, the trajectories of some population trends appear to differ among sites. *Epioblasma ahlstedti* appeared to decline by 84% and 72% at Lillard's Mill and Venable Spring, respectively between the two previous surveys but no difference in density was detected at Hooper Island during the same time. TWRA (2015) attributed this decline to increased flows between 2010 and 2015 which may have impacted reproduction and recruitment. This decline appears to have stabilized at both sites between 2015 and 2020 but the estimated density at Hooper Island has increased by 200% since 2015. Similarly, *V. taeniata* density decreased by 67% between 2010 and 2020 at Lillard's Mill but had increased by 214% from 2010 to 2015 at Hooper Island and remained steady from 2015 to 2020. These inconsistent trends in density suggest that factors influencing these populations may be affecting reaches differently. This is further supported by that fact that several species had detectable changes in density over time at one or more sights but showed no detectable changes at other sites.

Sampling bias is likely contributing to the inconsistent trends observed across and within sites since 2010 as species detection and capture are typically imperfect in field sampling (MacKenzie 2006). Detection/capture probabilities are often assumed as perfect when conducting quadrat sampling with excavation as the contents of an individual quadrat is removed and sorted through sieves which greatly improves the probability of seeing small and or cryptic individuals that may have otherwise been missed due to small size or similarity to substrate. In addition, buried mussels, which may be inadvertently omitted from surface counts may be included in the contents. Although this assumption may be valid at the individual quadrat scale, density is estimated at the site scale which is subject to incomplete detection/capture due to heterogeneity in spatial distribution of the species as well as the sample size and distribution of sampling units across sites (Downing and Downing 1992; Strayer and Smith 2003; Pooler and Smith 2005). The spatial coverage of samples among sites from 2010 to 2020 ranged from 0.59% to 1.04% of the total site areas and differed among years. In addition, the sampling site limits were not fixed but varied among years to accommodate changes in habitat distribution over time. Therefore, it is probable that different portions of the overall superpopulations were sampled each year, including or missing portions of population aggregations that could have weighted density estimates differently. This may explain differences in estimated density of *L. costata*, *L. rimosus*, and *T. verrucosa* at Hooper Island which showed statistically significant changes in density from 2010 to 2015 but similar densities between 2010 and 2020. These changes are biologically questionable unless they resulted from emigration or immigration of animals to or from adjacent populations through active or passive movement from bed scour followed by high mortality event. Although possible, we are unable to provide any empirical evidence explain these changes. We have omitted discussions of the overall site abundances of each species as they may not be statistically meaningful or useful for temporal comparisons because they do not characterize the same overall site. However these values are provided in the tables for each site.

Under the current sampling design, 80 0.25-m^2 quadrat samples provided sufficient power (0.80) to detect a 20% change in the density of mussels among sampling years. Such a change could be deleterious to the persistence of some rare species and therefore may require higher precision to detect changes as they occur. Increasing the sample size to 144 0.25-m^2 quadrats per site would provide power to detect a 15% change and 322 quadrats would be needed to detect a 10% change between sampling occasions. Such efforts may aid in determining finer scale changes in the population but may not be worth the additional costs as they would be resource intensive and provide limited return for managing the mussel populations of the Duck River. Alternatively, other sampling and modeling methodologies are available that allow for the estimation of apparent survival, per capita recruitment, and other demographic parameters in response to environmental variables while accounting for recapture probability. Specifically, apparent survival and per capita recruitment provide the necessary parameters to estimate population growth over time while providing insight into the ecological mechanisms affecting these parameters. Such approaches have been successfully implemented for freshwater mussels across North America (Villella et al. 2004; Meador et al. 2011; Peterson et al. 2011; Inoue et al. 2014; Wisniewski et al. 2013; Wisniewski et al. 2015; Carey et al. 2019) and allow resource managers to develop the necessary understanding of the species ecology to proactively manage the resources. This is particularly important in the Duck River due to the expected increase in demand for municipal and industrial water use.

MANAGEMENT RECOMMENDATIONS

1. Continue monitoring these sites at 5-year intervals
    - Consider increasing sample size to increase precision of density estimates
    - Use same spatial limits as 2020 sampling which are similar to 2015 to minimize over or under-representation of different portions of the super-population

2. Initiate a long-term capture-mark-recapture study to estimate population demographics
    - Estimation of apparent survival and recruitment in response to stream-flow and other variables will allow for a better understanding of the mechanisms responsible for changes in populations
    - This may also be used to validate density estimates by allowing for the estimation of capture probability.
3. Continue propagation and restoration activities
    - Successful reintroduction and augmentation activities require multiple years of releases to build the population for greater probability of natural reproduction.
    - Additional species should be considered for reintroduction or augmentation if they were historically known from the Duck River watershed.

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TABLES

Table 1. Total area (m^2) sampled and stream flow metrics of the 2010, 2015, and 2020 long-term monitoring surveys of the Duck River.

2010 Site	Duck River Mile	Shoal Habitat Surveyed (m^2)	USGS gage and observed flow (ft^3/s)	Gage Height (elevation, ft.)
Tarpley Bluff	207.3	960	Shelbyville, 167	2.72
Lillard's Mill Dam	179.2	3,350	Milltown, 172	10.94
Venable Spring	176.8	3,000	Milltown, 168	10.93
Hooper Island	163.1	2,740	Columbia, 160	1.38
Columbia Mill Dam	133.5	2,170	Columbia, 160	1.38
Littlelot	88.9	1,750	Columbia, 120	1.37

2015 Site	Duck River Mile	Shoal Habitat Surveyed (m^2)	USGS gage and observed flow (ft^3/s)	Gage Height (elevation, ft.)
Tarpley Bluff	207.3	1,160	Shelbyville, 251	3.20
Lillard's Mill Dam	179.2	2,040	Milltown, 263	11.16
Venable Spring	176.8	3,000	Milltown, 285	11.50
Hooper Island	163.1	2,800	Milltown, 220	11.05
Columbia Mill Dam	133.5	2,200	Columbia, 186	1.59
Littlelot	88.9	Not sampled in 2015		

2020 Site	Duck River Mile	Shoal Habitat Surveyed (m^2)	USGS gage and observed flow (ft^3/s)	Gage Height (elevation, ft.)
Tarpley Bluff	207.3	Not Sampled in 2020		
Lillard's Mill Dam	179.2	3,400	Milltown, 315	11.24
Venable Spring	176.8	3,600	Milltown, 305	11.22
Hooper Island	163.1	2,740	Milltown, 291	11.19
Columbia Mill Dam	133.5	1,845	Columbia, 344	2.23
Littlelot	88.9	Not sampled in 2020		

Table 2. Mussel density, sampling effort, and species richness among long-term monitoring sites in the Duck River from 2004 to 2020. NS denotes not sampled

Survey	Ahlstedt et al. 2004			TWRA 2010			TWRA 2015			TWRA 2020		
Site	#/m^2	# Quads	# Spec.	#/m^2	# Quads	# Spec.	#/m^2	# Quads	# Spec.	#/m^2	# Quads	# Spec.
Tarpley Bluff	2.2	20	6	4.85	80	10	1.80	80	9	NS		
Lillard's Mill Dam	36.6	20	17	37.4	80	29	30.26	80	27	18.14	84	33
Venable Spring	19.6	30	16	12.65	80	25	14.15	80	23	14.12	83	24
Hooper Island	24.4	20	19	22.2	80	23	20.15	80	21	19.08	88	21
Columbia Mill Dam	NS			10.5	80	19	7.4	80	18	4.98	77	15
Littlelot	NS			17	80	17	NS			NS		

Table 3. Number of individuals collected by site during 2020 survey and % collection. LM=Lillard's Mill, VS=Venable Spring, HI=Hooper Island, CD=Columbia.

Mussel Species	LM	VS	HI	CD	Total	% Collection
Amblema plicata	35	8	5	12	60	5.19
Cyclonaias pustulosa	13	2	15	19	49	4.24
Cyclonaias tuberculata	52	21	38	35	146	12.62
Cyprogenia stegaria (E)	1	0	0	0	1	0.09
Ellipsaria lineolata	2	0	0	0	2	0.17
Epioblasma ahlstedti (E)	19	16	33	0	68	5.88
Epioblasma brevidens (E)	3	0	0	0	3	0.26
Epioblasma obliquata (E)	1	0	0	0	1	0.09
Epioblasma triquetra (E)	1	0	0	0	1	0.09
Eurynia dilatata	58	9	55	0	122	10.54
Lampsilis abrupta (E)	1	0	0	0	1	0.09
Lampsilis fasciola	1	4	8	1	14	1.21
Lampsilis ovata	0	1	0	0	1	0.09
Lasmigona costata	15	9	10	1	35	3.03
Lemiox rimosus (E)	92	47	107	0	223	19.27
Leptodea fragilis	1	1	1	3	6	0.52
Medionidus conradicus	3	1	25	0	29	2.51
Megalonaias nervosa	1	1	0	1	3	0.26
Obliquaria reflexa	1	0	0	4	5	0.43
Obovaria subrotunda	2	79	26	0	107	9.25
Pleurobema oviforme	3	1	9	0	13	1.12
Pleurobema rubrum	0	6	5	0	11	0.95
Pleuronaia barnesiana	6	17	8	0	31	2.68
Pleuronaia dolabelloides (E)	5	17	11	3	36	3.11
Potamilus alatus	0	0	0	1	1	0.09
Ptychobranchus fasciolaris	0	0	0	2	2	0.17
Ptychobranchus subtentus (E)	16	0	0	0	16	1.38
Reginia ebena	0	0	0	2	2	0.17
Strophitus undulatus	0	4	0	0	4	0.35
Theliderma cylindrica (T)	4	16	23	1	44	3.80

Table 3 Continued

Theliderma intermedia (E)	2	4	10	0	16	1.38
Theliderma metanevra	1	0	0	0	1	0.09
Toxolasma cylindrellus (E)	2	0	0	0	2	0.17
Toxolasma lividum	1	4	1	0	6	0.52
Tritogonia verrucosa	6	0	2	7	15	1.30
Truncilla truncata	2	2	0	4	8	0.69
Villosa taeniata	27	21	22	0	70	6.05
Villosa vanuxemensis	2	2	0	0	2	0.17
Total	**379**	**293**	**414**	**96**	**1157**	5.19
# Species	**32**	**24**	**20**	**15**	**38**	

Table 4. Lillard's Mill site summary statistics of 84, 0.25-m^2 quadrat samples.

Species	Totals	Estimated Density (#/m^2)	Stand. Dev. (SD)	Stand. Error (SE)	CV of SE (Precision)	95% LCI	95% UCI	Estimated Site Pop. (3400 m^2)	95% LCI	95% UCI
Amblema plicata	35	1.67	3.18	0.35	0.21	0.99	2.35	5667	3355	7978
Cyclonaias pustulosa	13	0.62	1.91	0.21	0.34	0.21	1.03	2105	714	3496
Cyclonaias tuberculata	52	2.48	4.30	0.47	0.19	1.56	3.40	8419	5294	11544
Cyprogenia stegaria	1	0.05	0.44	0.05	1.00	-0.05	0.14	162	-155	479
Ellipsaria lineolata	2	0.10	0.87	0.10	1.00	-0.09	0.28	324	-311	958
Epioblasma ahlstedti	19	0.90	1.90	0.21	0.23	0.50	1.31	3076	1696	4457
Epioblasma brevidens	3	0.14	0.97	0.11	0.74	-0.06	0.35	486	-220	1192
Epioblasma obliquata	1	0.05	0.44	0.05	1.00	-0.05	0.14	162	-155	479
Epioblasma triquetra	1	0.05	0.44	0.05	1.00	-0.05	0.14	162	-155	479
Eurynia dilatata	58	2.76	4.12	0.45	0.16	1.88	3.64	9390	6397	12384
Lampsilis abrupta	1	0.05	0.44	0.05	1.00	-0.05	0.14	162	-155	479
Lampsilis cardium	2	0.10	0.61	0.07	0.70	-0.04	0.23	324	-122	770
Lampsilis fasciola	1	0.05	0.44	0.05	1.00	-0.05	0.14	162	-155	479
Lasmigona costata	15	0.71	1.88	0.21	0.29	0.31	1.12	2429	1062	3795
Lemiox rimosus	92	4.38	6.50	0.71	0.16	2.99	5.77	14895	10168	19622
Leptodea fragilis	1	0.05	0.44	0.05	1.00	-0.05	0.14	162	-155	479
Medionidus conradicus	3	0.14	0.75	0.08	0.57	-0.02	0.30	486	-57	1029
Megalonaias nervosa	1	0.05	0.44	0.05	1.00	-0.05	0.14	162	-155	479
Obliquaria reflexa	1	0.05	0.44	0.05	1.00	-0.05	0.14	162	-155	479
Obovaria subrotunda	2	0.10	0.61	0.07	0.70	-0.04	0.23	324	-122	770
Pleurobema oviforme	3	0.14	0.75	0.08	0.57	-0.02	0.30	486	-57	1029
Pleuronaia barnesiana	6	0.29	1.04	0.11	0.40	0.06	0.51	971	218	1725
Pleuronaia dolabelloides	5	0.24	1.44	0.16	0.66	-0.07	0.55	810	-235	1854
Ptychobranchus subtentus	16	0.76	3.37	0.37	0.48	0.04	1.48	2590	139	5042
Theliderma cylindrica	4	0.19	0.86	0.09	0.49	0.01	0.37	648	25	1271
Theliderma intermedia	2	0.10	0.61	0.07	0.70	-0.04	0.23	324	-122	770
Theliderma metanevra	1	0.05	0.44	0.05	1.00	-0.05	0.14	162	-155	479
Toxolasma cylindrellus	2	0.10	0.87	0.10	1.00	-0.09	0.28	324	-311	958
Toxolasma lividum	1	0.05	0.44	0.05	1.00	-0.05	0.14	162	-155	479
Tritogonia verrucosa	6	0.29	1.21	0.13	0.46	0.03	0.54	971	93	1850
Truncilla truncata	2	0.10	0.61	0.07	0.70	-0.04	0.23	324	-122	770
Villosa taeniata	2	1.29	4.78	0.52	0.41	0.26	2.31	4371	899	7844
Villosa vanuxemensis	2	0.10	0.61	0.07	0.70	-0.04	0.23	324	-122	770
Corbicula fluminea	1301	61.95	62.75	6.85	0.11	48.53	75.37	210638	165014	256262
Total Unionids	**381**	**18.14**	**22.16**	**2.42**	**0.13**	**13.40**	**22.88**	**61686**	**45575**	**77797**

Table 5. Venable Spring site summary statistics of 83, 0.25-m^2 quadrat samples.

Species	Totals	Estimated Density (#/m^2)	Stand. Dev. (SD)	Stand. Error (SE)	CV of SE (Precision)	95% LCI	95% UCI	Estimated Site Pop. (3600 m^2)	95% LCI	95% UCI
Amblema plicata	8	0.39	1.34	0.15	0.38	0.10	0.67	1388	349	2427
Cyclonaias pustulosa	2	0.10	0.62	0.07	0.70	-0.04	0.23	347	-131	825
Cyclonaias tuberculata	21	1.01	2.56	0.28	0.28	0.46	1.56	3643	1658	5629
Epioblasma ahlstedti	16	0.77	1.71	0.19	0.24	0.40	1.14	2776	1455	4097
Eurynia dilatata	9	0.43	1.25	0.14	0.32	0.16	0.70	1561	592	2531
Lampsilis fasciola	4	0.19	0.86	0.09	0.49	0.01	0.38	694	26	1362
Lampsilis ovata	1	0.05	0.44	0.05	1.00	-0.05	0.14	173	-167	514
Lasmigona costata	9	0.43	1.53	0.17	0.39	0.10	0.76	1561	375	2748
Lemiox rimosus	47	2.27	3.76	0.41	0.18	1.46	3.07	8154	5244	11065
Leptodea fragilis	1	0.05	0.44	0.05	1.00	-0.05	0.14	173	-167	514
Medionidus conradicus	1	0.05	0.44	0.05	1.00	-0.05	0.14	173	-167	514
Megalonaias nervosa	1	0.05	0.44	0.05	1.00	-0.05	0.14	173	-167	514
Obovaria subrotunda	79	3.81	6.84	0.75	0.20	2.34	5.28	13706	8408	19004
Pleurobema oviforme	1	0.05	0.44	0.05	1.00	-0.05	0.14	173	-167	514
Pleurobema rubrum	6	0.29	1.22	0.13	0.46	0.03	0.55	1041	100	1982
Pleuronaia barnesiana	17	0.82	1.85	0.20	0.25	0.42	1.22	2949	1517	4381
Pleuronaia dolabelloides	17	0.82	1.62	0.18	0.22	0.47	1.17	2949	1692	4207
Strophitus undulatus	4	0.19	0.86	0.09	0.49	0.01	0.38	694	26	1362
Theliderma cylindrica	16	0.77	2.29	0.25	0.33	0.28	1.26	2776	1001	4551
Theliderma intermedia	4	0.19	0.86	0.09	0.49	0.01	0.38	694	26	1362
Toxolasma lividum	4	0.19	0.86	0.09	0.49	0.01	0.38	694	26	1362
Truncilla truncata	2	0.10	0.62	0.07	0.70	-0.04	0.23	347	-131	825
Villosa taeniata	21	1.01	2.06	0.23	0.22	0.57	1.45	3643	2050	5237
Villosa vanuxemensis	2	0.10	0.62	0.07	0.70	-0.04	0.23	347	-131	825
Corbicula fluminea	499	24.05	23.03	2.53	0.11	19.09	29.00	86573	68734	104413
Total Unionids	**293**	**14.12**	**10.75**	**1.18**	**0.08**	**11.81**	**16.43**	**50834**	**42505**	**59163**

Table 6. Hooper Island site summary statistics of 88, 0.25-m^2 quadrat samples.

Species	Totals	Estimated Density (#/m^2)	Stand. Dev. (SD)	Stand. Error (SE)	CV of SE (Precision)	95% LCI	95% UCI	Estimated Site Pop. (2300 m^2)	95% LCI	95% UCI
Amblema plicata	5	0.23	0.94	0.10	0.44	0.03	0.43	529	76	981
Cyclonaias pustulosa	15	0.69	1.64	0.18	0.25	0.35	1.03	1586	795	2378
Cyclonaias tuberculata	38	1.75	2.57	0.28	0.16	1.21	2.29	4018	2778	5259
Epioblasma ahlstedti	33	1.52	2.68	0.29	0.19	0.95	2.08	3490	2196	4783
Eurynia dilatata	55	2.53	3.51	0.38	0.15	1.79	3.27	5816	4119	7513
Lampsilis fasciola	8	0.37	1.16	0.12	0.34	0.12	0.61	846	284	1408
Lasmigona costata	10	0.46	1.55	0.17	0.36	0.13	0.78	1057	310	1805
Lemiox rimosus	107	4.92	6.42	0.69	0.14	3.57	6.27	11315	8213	14417
Leptodea fragilis	1	0.05	0.43	0.05	1.00	-0.04	0.14	106	-102	313
Medionidus conradicus	25	1.15	2.19	0.23	0.20	0.69	1.61	2644	1584	3703
Obovaria subrotunda	26	1.20	2.21	0.24	0.20	0.73	1.66	2749	1682	3817
Oliquaria reflexa	1	0.05	0.43	0.05	1.00	-0.04	0.14	106	-102	313
Pleurobema oviforme	9	0.41	1.37	0.15	0.35	0.13	0.70	952	290	1613
Pleurobema rubrum	5	0.23	0.94	0.10	0.44	0.03	0.43	529	76	981
Pleuronaia barnesiana	8	0.37	1.31	0.14	0.38	0.09	0.64	846	211	1481
Pleuronaia dolabelloides	11	0.51	1.47	0.16	0.31	0.20	0.81	1163	453	1874
Theliderma cylindrica	23	1.06	2.55	0.27	0.26	0.52	1.59	2432	1200	3664
Theliderma intermedia	10	0.46	1.42	0.15	0.33	0.16	0.76	1057	371	1744
Toxolasma lividum	1	0.05	0.43	0.05	1.00	-0.04	0.14	106	-102	313
Tritogonia verrucosa	2	0.09	0.60	0.06	0.70	-0.03	0.22	211	-80	503
Villosa taeniata	22	1.01	1.95	0.21	0.21	0.60	1.42	2326	1384	3269
Corbicula fluminea	2021	93.58	60.49	6.49	0.07	80.87	106.29	215237	185999	244475
Total Unionids	**415**	**19.08**	**13.18**	**1.41**	**0.07**	**16.31**	**21.85**	**52280**	**37516**	**50254**

Table 7. Columbia site summary statistics of 77, 0.25-m^2 quadrat samples.

Species	Totals	Estimated Density (#/m^2)	Stand. Dev. (SD)	Stand. Error (SE)	CV of SE (Precision)	95% LCI	95% UCI	Estimated Site Pop. (3400 m^2)	95% LCI	95% UCI
Amblema plicata	12	0.62	1.60	0.18	0.29	0.27	0.98	1150	492	1809
Cyclonaias pustulosa	19	0.99	1.74	0.20	0.20	0.60	1.37	1821	1106	2536
Cyclonaias tuberculata	35	1.82	2.87	0.33	0.18	1.18	2.46	3355	2172	4537
Lampsilis fasciola	1	0.05	0.46	0.05	1.00	-0.05	0.15	96	-92	284
Lasmigona costata	1	0.05	0.46	0.05	1.00	-0.05	0.15	96	-92	284
Leptodea fragilis	3	0.16	0.78	0.09	0.57	-0.02	0.33	288	-34	609
Megalonaias nervosa	1	0.05	0.46	0.05	1.00	-0.05	0.15	96	-92	284
Obliquaria reflexa	4	0.21	0.89	0.10	0.49	0.01	0.41	383	15	752
Pleuronaia dolabelloides	3	0.16	0.78	0.09	0.57	-0.02	0.33	288	-34	609
Potamilus alatus	1	0.05	0.46	0.05	1.00	-0.05	0.15	96	-92	284
Ptychobranchus fasciolaris	2	0.10	0.64	0.07	0.70	-0.04	0.25	192	-72	456
Reginia ebena	2	0.10	0.64	0.07	0.70	-0.04	0.25	192	-72	456
Theliderma cylindrica	1	0.05	0.46	0.05	1.00	-0.05	0.15	96	-92	284
Tritogonia verrucosa	7	0.36	1.33	0.15	0.42	0.07	0.66	671	124	1218
Truncilla truncata	4	0.21	0.89	0.10	0.49	0.01	0.41	383	15	752
Corbicula fluminea	88	4.57	5.53	0.63	0.14	3.34	5.81	8434	6154	10715
Total Unionids	**96**	4.99	5.16	0.59	0.12	3.84	6.14	9201	7076	11326

Figure 1. Long-term mussel monitoring sites in the Duck River, Bedford, Hickman, Maury, and Marshall counties, Tennessee.

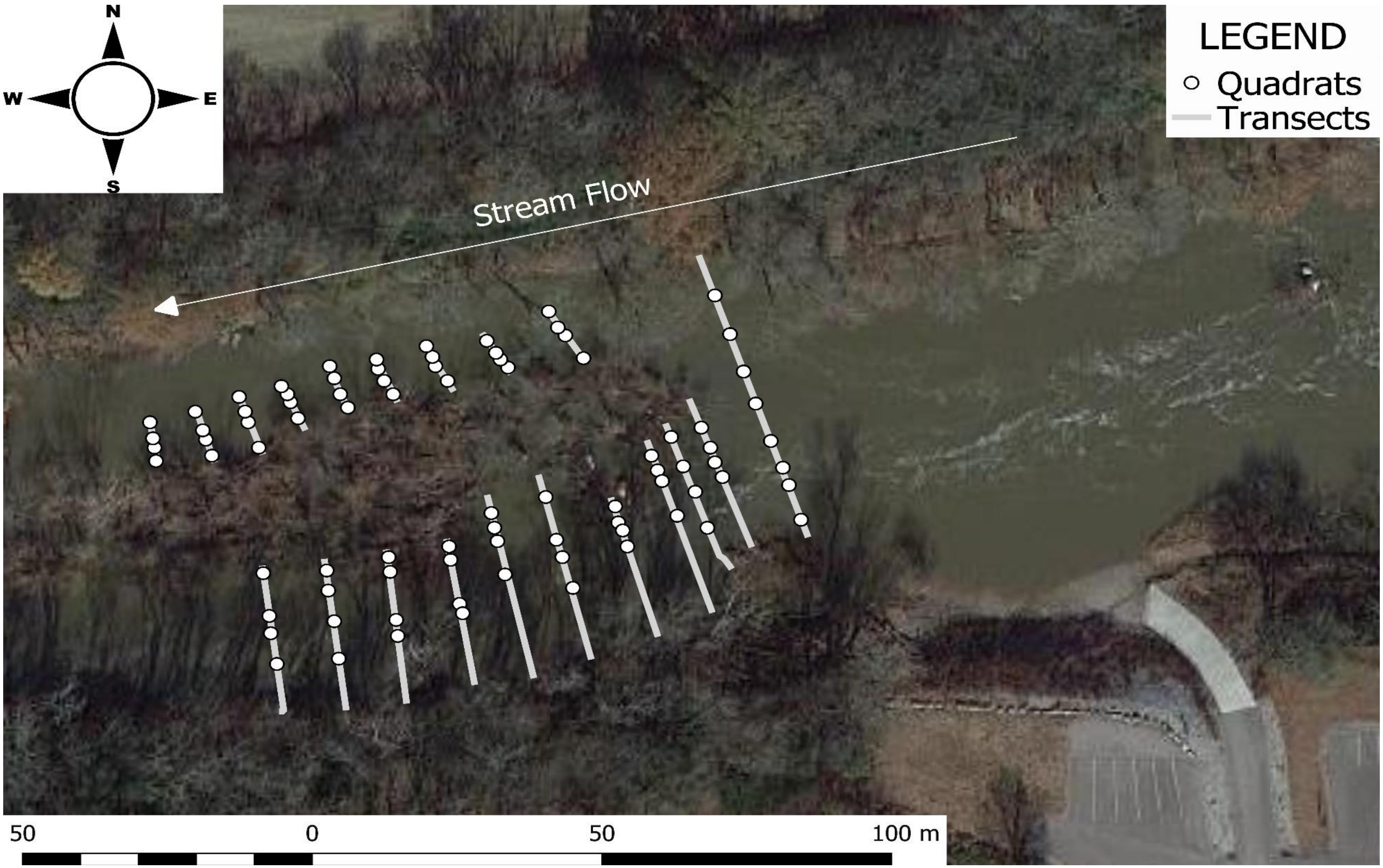

Figure 2. Survey site location including transect and quadrat positions at Lillard's Mill, Marshall County, Tennessee during the 8 September 2020 sampling occasion.

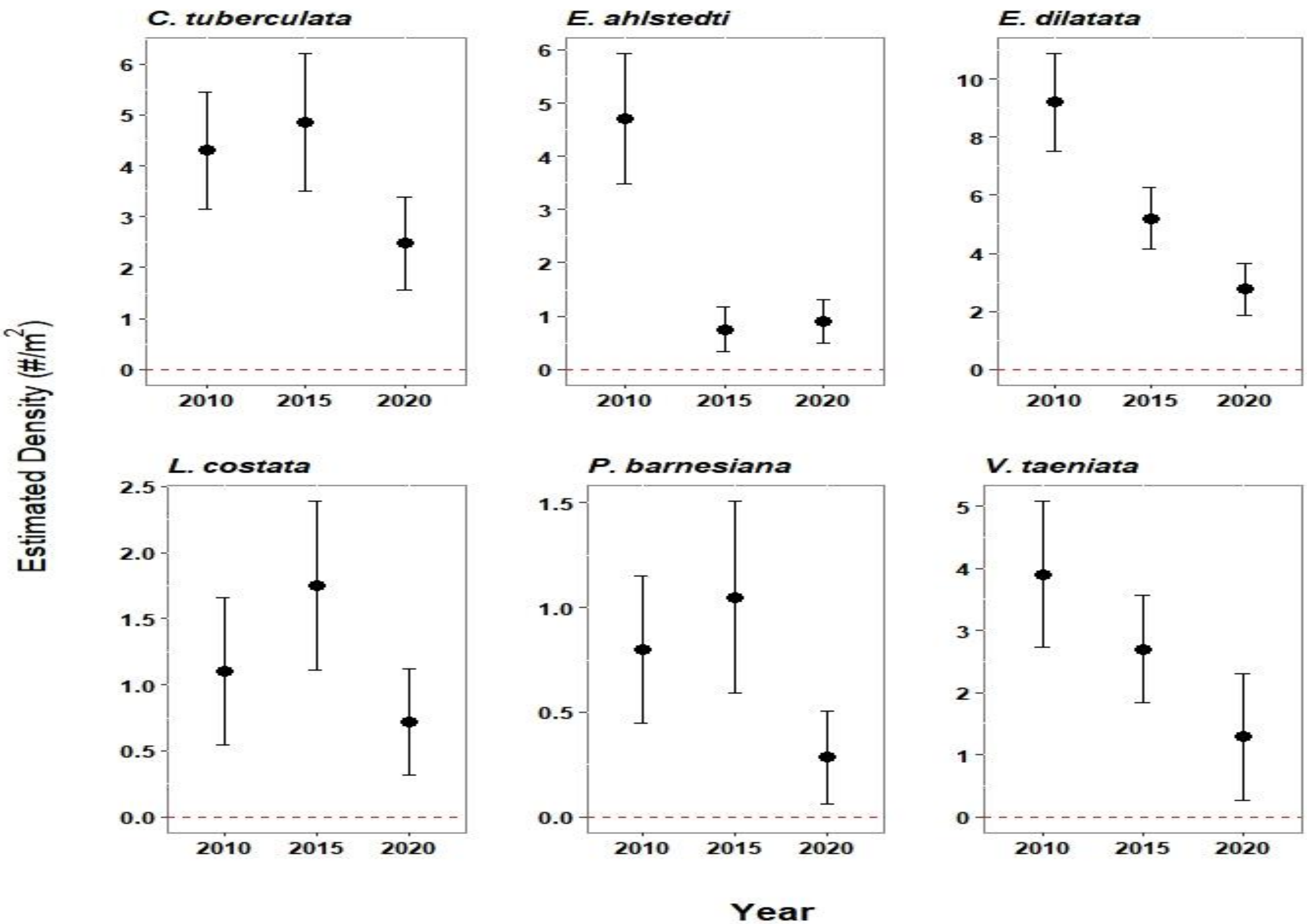

Figure 3. Mussel species exhibiting changes in estimated densities with 95% confidence intervals among sampling conducted from 2010 and 2020 in the Duck River at Lillard's Mill. Differences were significant at α=0.05.

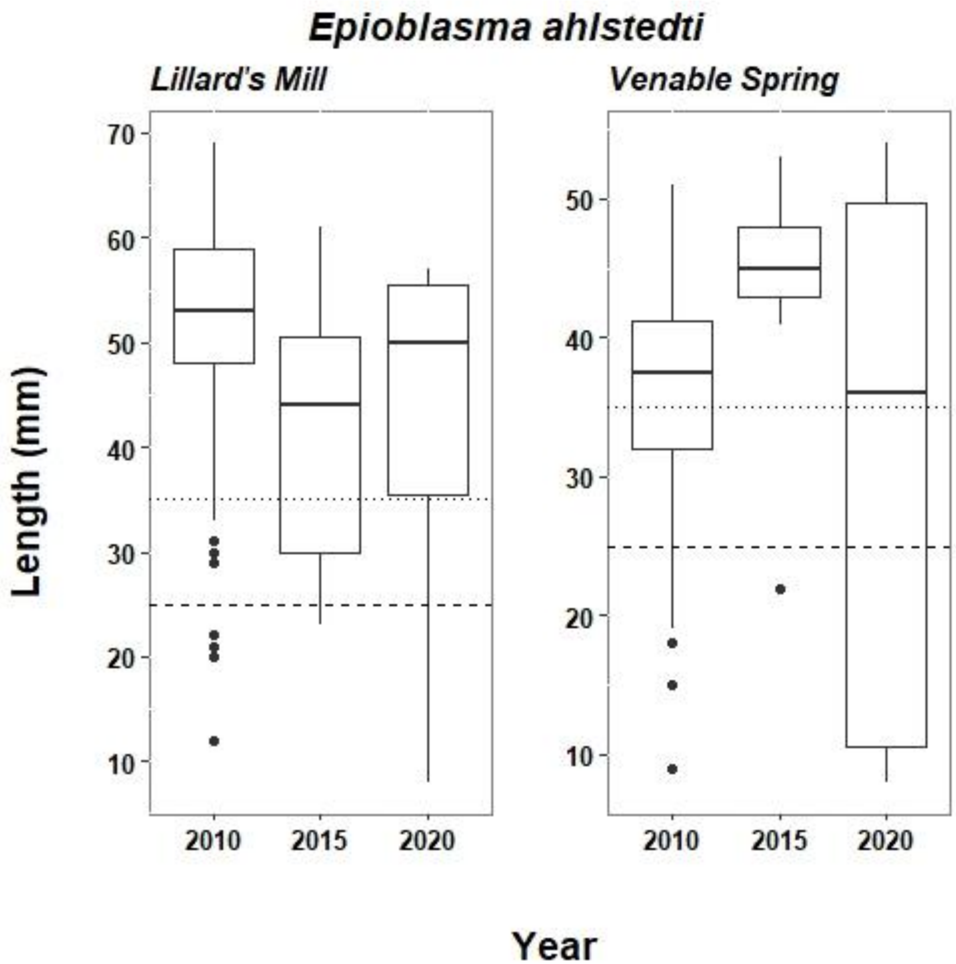

Figure 4. Boxplot of lengths of *Epioblasma ahlstedti* among sampling years for Lillard's Mill and Venable Spring. Boxplots contain the mean length, 25th and 75th percentile lengths, minimum lengths, and maximum lengths for each year. Dashed line represents 25 mm length and dotted line represents the 35 mm length.

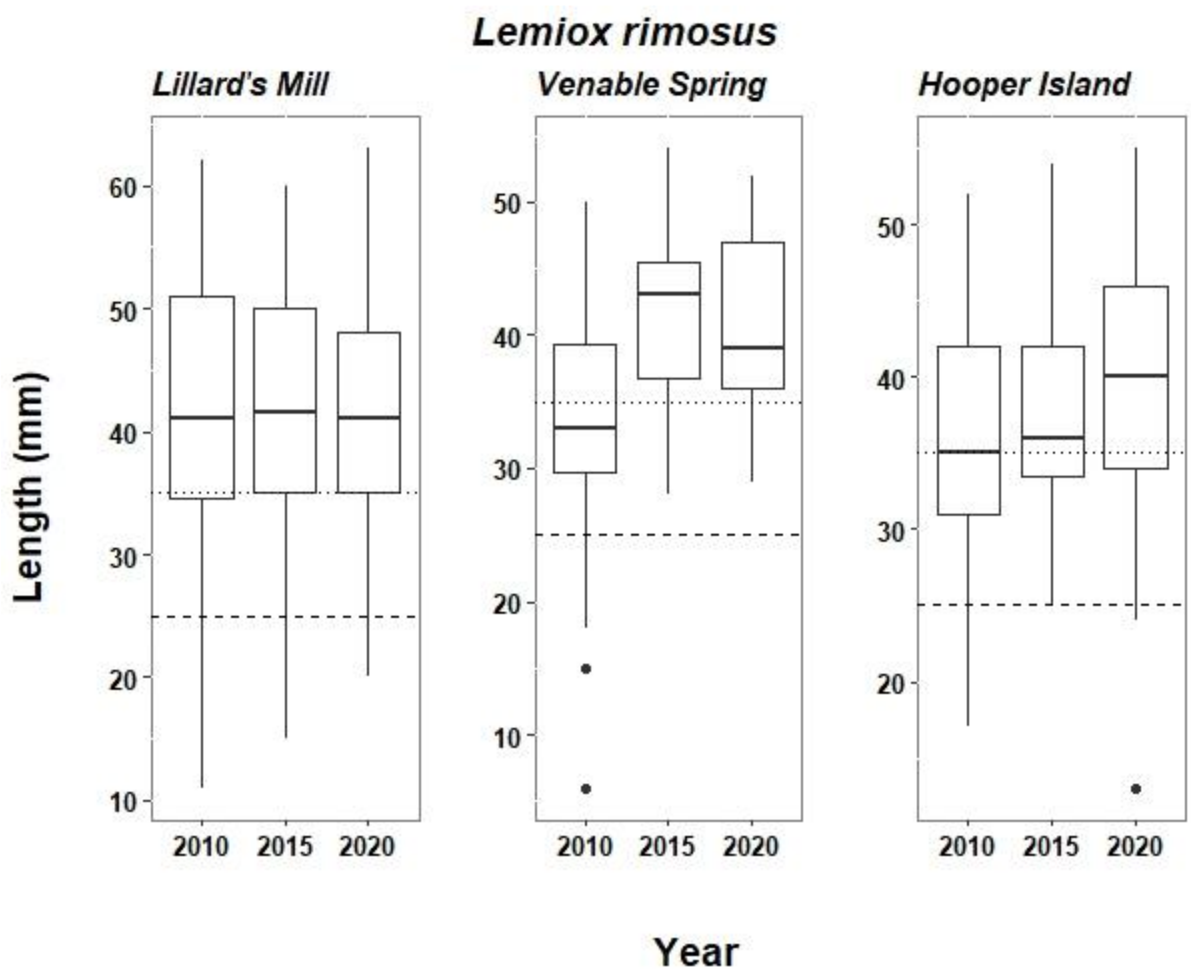

Figure 5. Boxplot of lengths of *Lemiox rimosus* among sampling years for Lillard's Mill, Venable Spring, and Hooper Island. Boxplots contain the mean length, 25th and 75th percentile lengths, minimum lengths, and maximum lengths for each year. Dashed line represents 25 mm length and dotted line represents the 35 mm length.

Figure 6. Survey site location including transect and quadrat positions at Venable Spring, Marshall County, Tennessee during the 9 September 2020 sampling occasion.

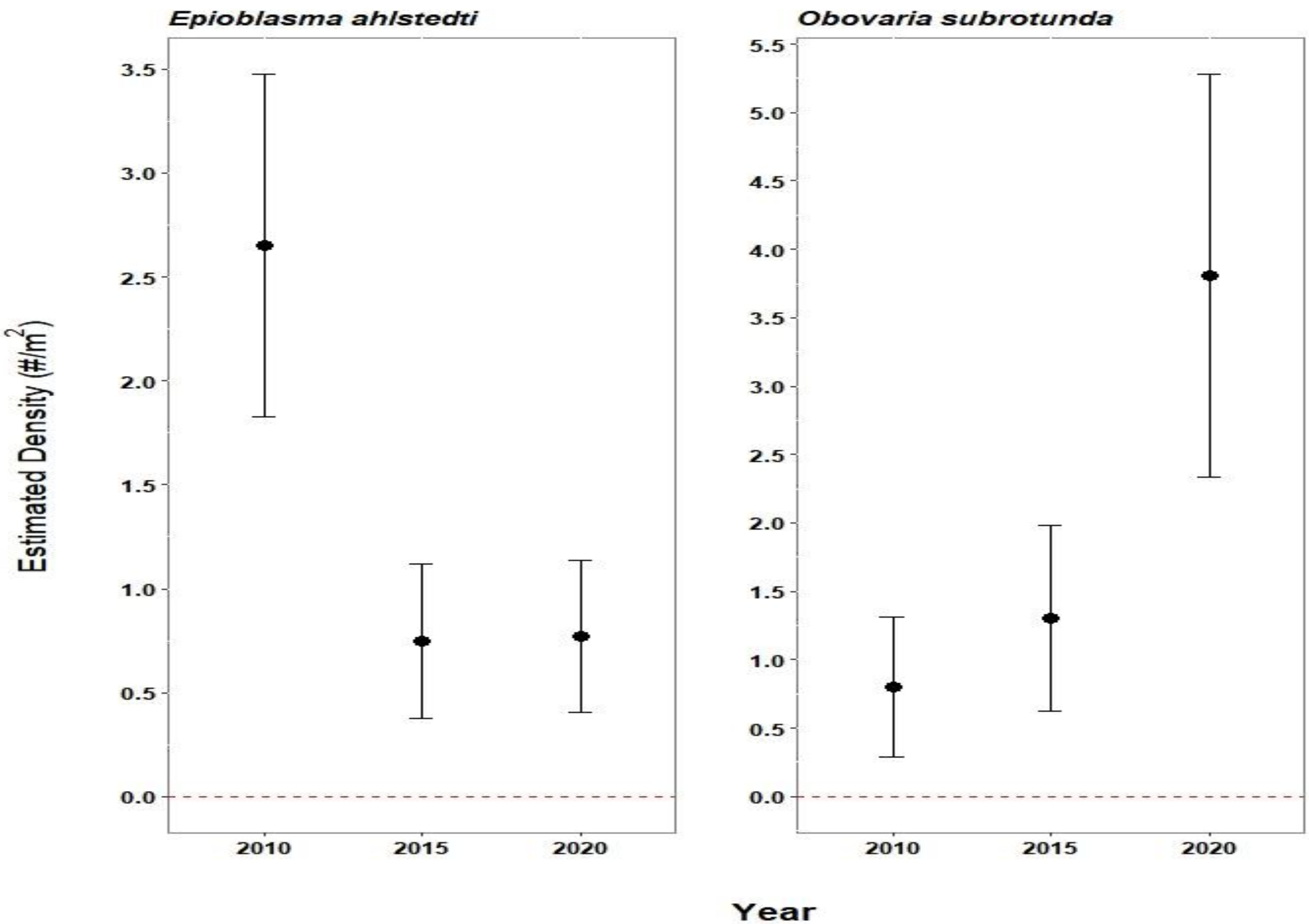

Figure 7. Mussel species exhibiting changes in estimated densities with 95% confidence intervals among sampling conducted from 2010 and 2020 in the Duck River at Venable Spring. Differences were significant at α=0.05.

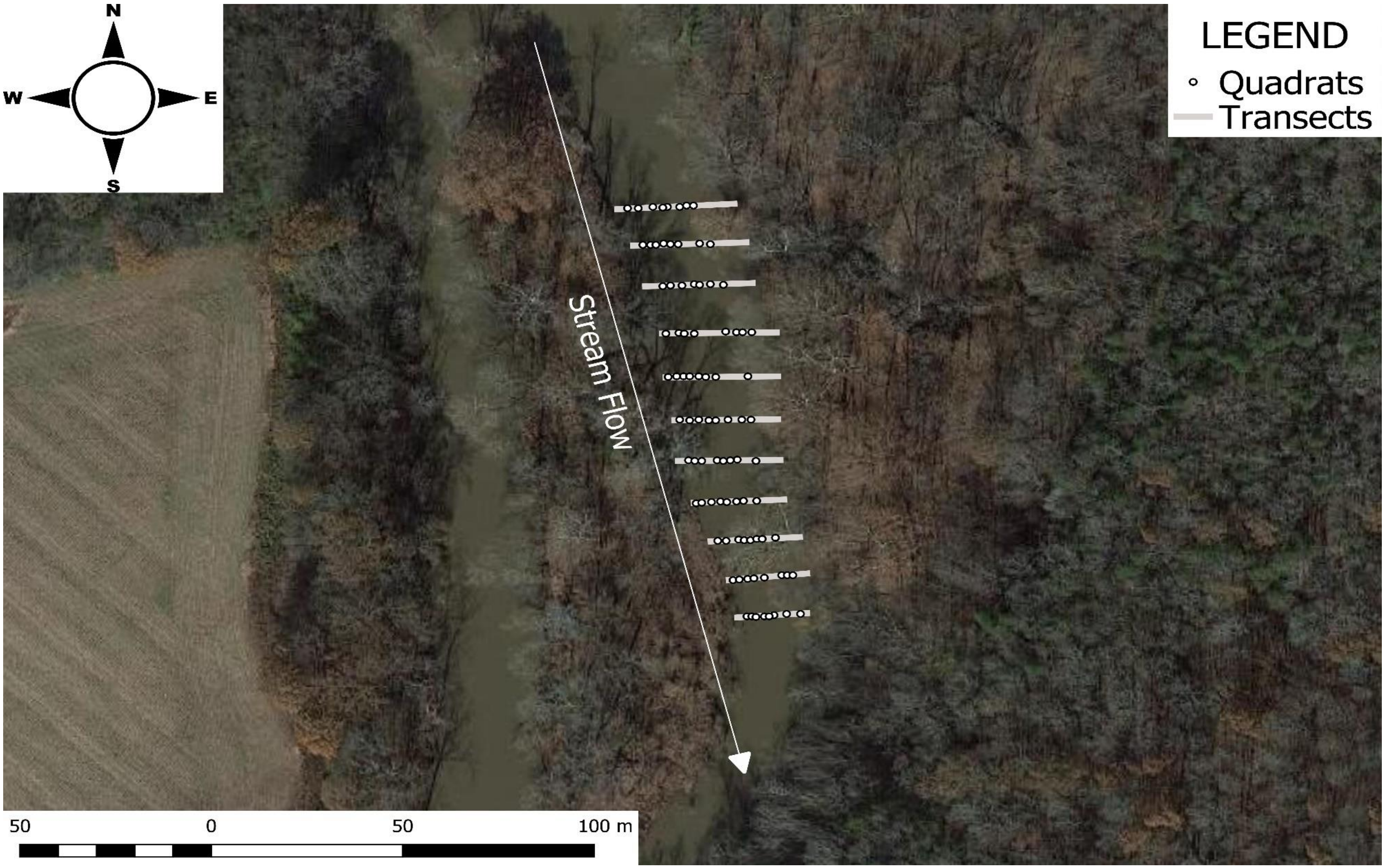

Figure 8. Survey site location including transect and quadrat positions at Hooper Island, Maury County, Tennessee during the 10 September 2020 sampling occasion.

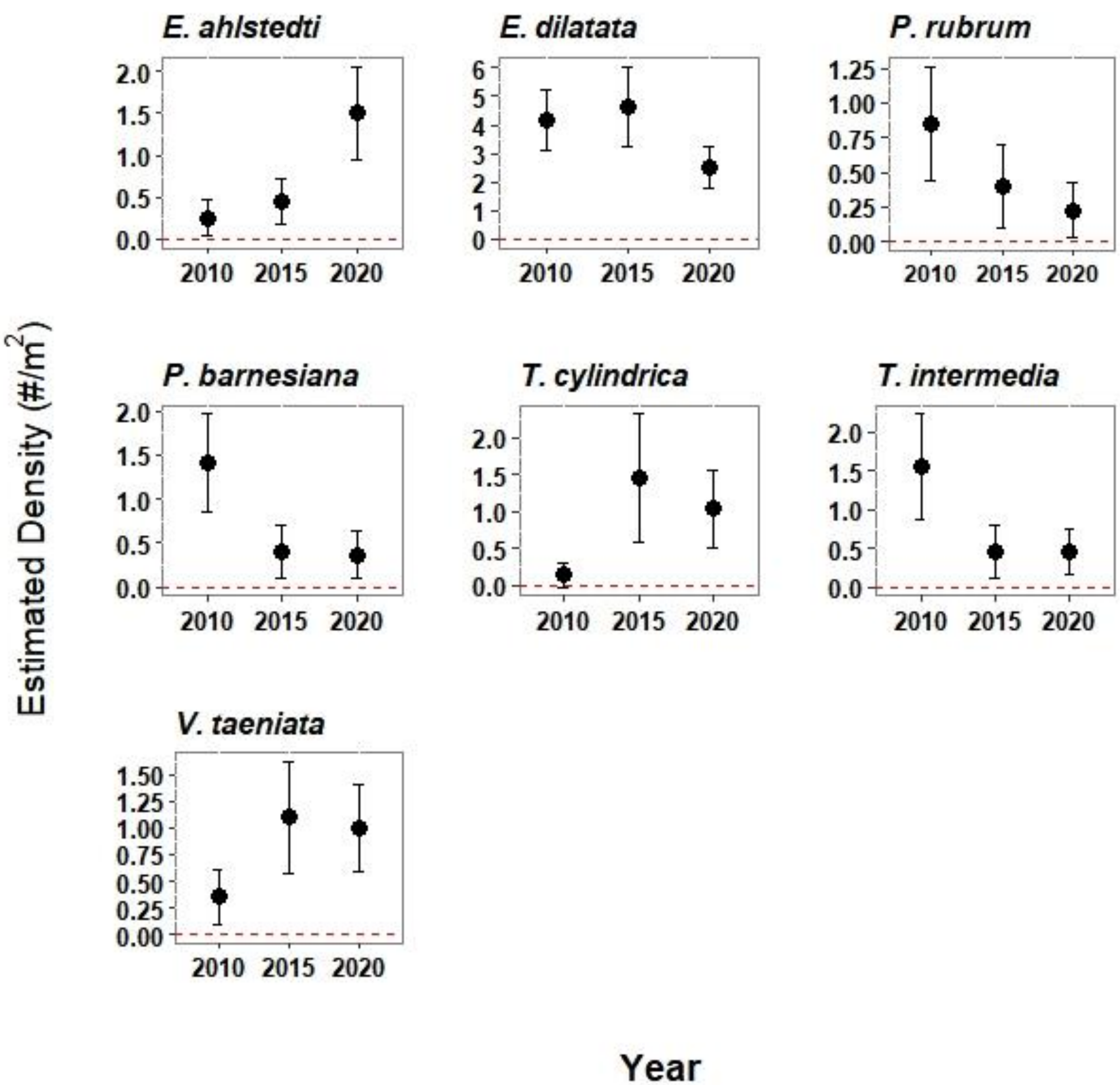

Figure 8. Mussel species exhibiting changes in estimated densities with 95% confidence intervals among sampling conducted from 2010 and 2020 in the Duck River at Hooper Island. Differences were significant at α=0.05.

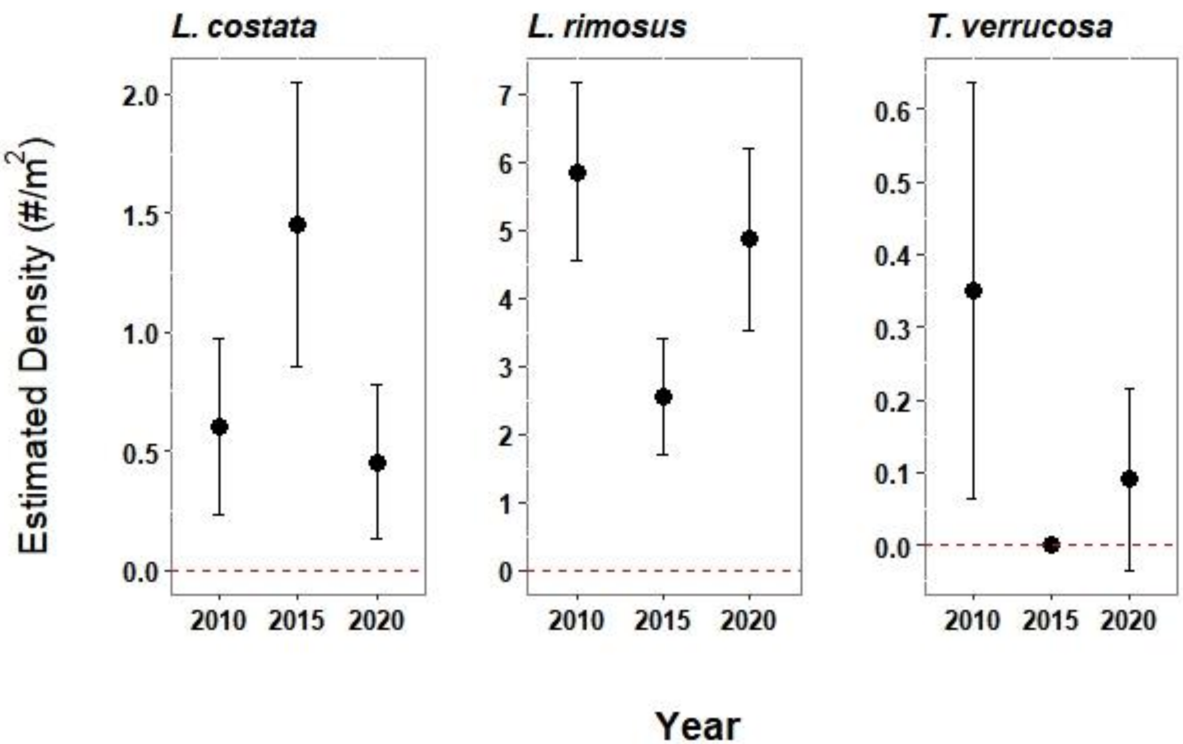

Figure 9. Mussel species exhibiting changes in estimated densities with 95% confidence intervals among sampling conducted from 2010 and 2020 in the Duck River at Hooper Island. Differences were significant at α=0.05.

Figure 10. Survey site location including transect and quadrat positions at Columbia, Maury County, Tennessee during the 11 September 2020 sampling occasion.

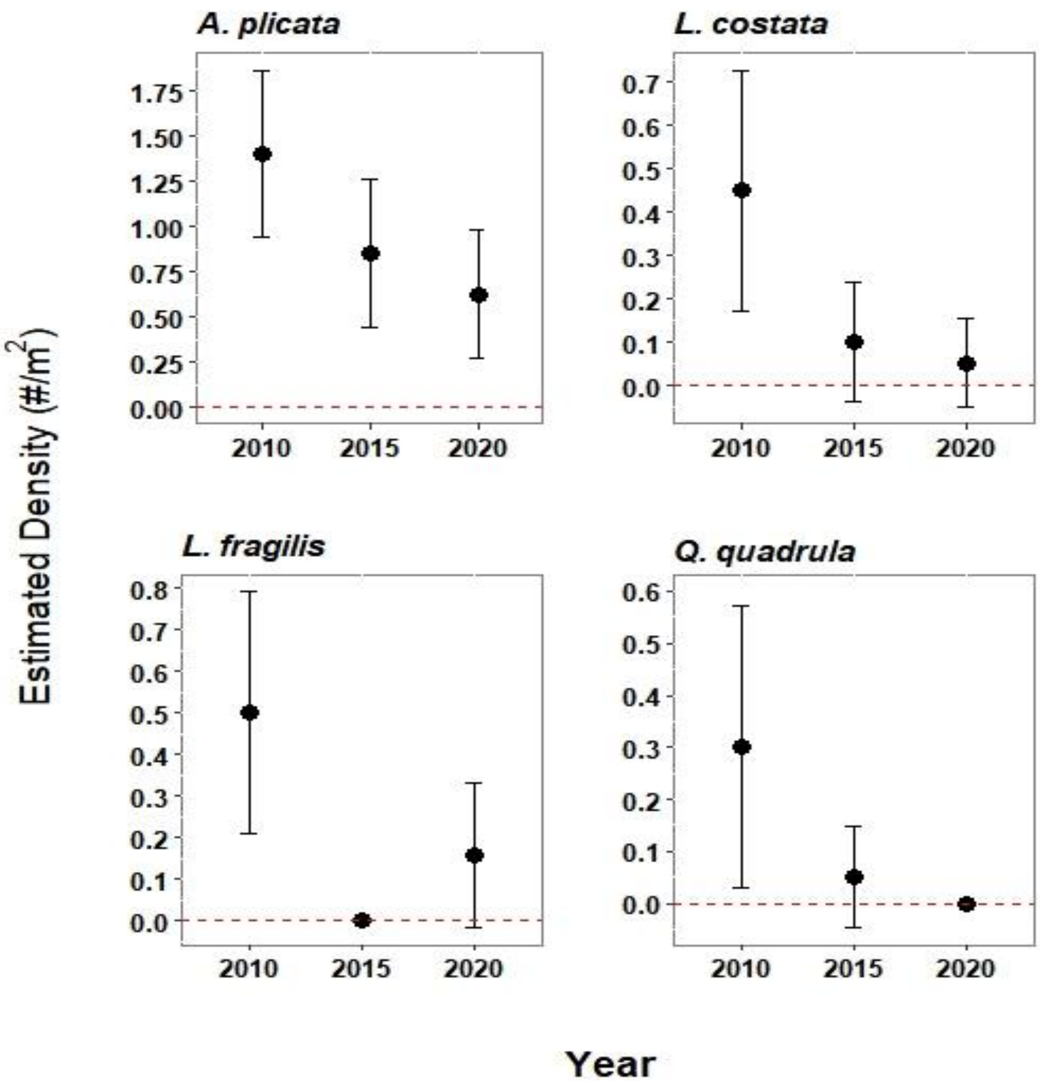

Figure 11. Mussel species exhibiting changes in estimated densities with 95% confidence intervals among sampling conducted from 2010 and 2020 in the Duck River at Columbia. Differences were significant at α=0.05.