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<p>Abstract</p> <p>Three spined, mussel species occur in the United States along the Atlantic slope: James spiny mussel (<i>Pleurobema collina</i>), Tar spiny mussel (<i>Elliptio steinstansana</i>), and Altamaha spiny mussel (<i>E. spinosa</i>). The James spiny mussel was listed as endangered in 1988 and was until recently considered to be endemic to the James River basin. Biologists with the North Carolina Department of Transportation discovered spiny mussel populations in the Dan and Mayo rivers in North Carolina in 2000 and 2001, respectively. The U.S. Fish & Wildlife Service (USFWS) tentatively identified this species as <i>Pleurobema collina</i>. Two working hypotheses regarding the range of <i>Pleurobema collina</i> in the Dan River, North Carolina, have been proposed subsequent to more than 380 person-hours spent conducting surveys. The species was found in a 57-rkm reach of the Dan River and in a 19-rkm reach of the Mayo River, Stokes and Rockingham counties. The overall catch per unit effort (CPUE) varied from 0.08/hr to 1.48/hr.</p> <p>The purpose of this project was to determine where <i>P. collina</i> resides in Virginia and the extent of its range within the state. The USFWS requires surveys by the Virginia Department of Transportation for this species at all roadway projects on rivers and tributaries in Virginia until the range of this species is defined by adequate survey work. An informal preliminary survey design for <i>P. collina</i> was used during the summer of 2002 to improve the future survey design. Simple random sampling was deployed in surveys conducted in 2003 and 2004 to provide a good basis for comparison to gauge the efficiency of the informal sampling design used.</p> <p>In 2002, 116 person-hours were spent surveying 39 localities on the Mayo, Dan, and Smith rivers. The species was observed only in the South Fork of the Mayo River, Patrick and Henry counties, Virginia. During the summers of 2003 and 2004, 228 person-hours were spent surveying 38 equal-area river reaches (10,000 m²) on the mainstems of the Dan, Smith, South Mayo, and Banister rivers. No specimens of <i>P. collina</i> (live or relic shells) were detected. However, two species, <i>Elliptio complanata</i> and <i>Villosa constricta</i>, were detected at almost every site surveyed. Water levels and flows were moderate to extremely high throughout the spring, summer, and early fall of both years, with water temperatures remaining low during the summer of 2003.</p> <p>A simple random sampling approach was designed to be easy, relatively quick, and cost-effective, applicable to most rivers, and to provide actual numbers for comparison. Negative results were reported after only 6 person-hours of searching within each randomly selected, equal-area river reach had been expended. <i>P. collina</i> was declared absent from the Virginia random sites surveyed in 2003 and 2004 with a confidence of approximately 90%. This information should eliminate the need to perform surveys for future work done in Dan and Mayo river basins.</p>				

FINAL CONTRACT REPORT

SURVEY FOR THE NEWLY DISCOVERED DAN SPINY MUSSEL IN THE DAN, MAYO, AND SOUTH MAYO RIVERS, VIRGINIA

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ABSTRACT

Three spined, mussel species occur in the United States along the Atlantic slope: James spiny mussel (*Pleurobema collina*), Tar spiny mussel (*Elliptio steinstansana*), and Altamaha spiny mussel (*E. spinosa*). The James spiny mussel was listed as endangered in 1988 and was until recently considered to be endemic to the James River basin. Biologists with the North Carolina Department of Transportation discovered spiny mussel populations in the Dan and Mayo rivers in North Carolina in 2000 and 2001, respectively. The U.S. Fish & Wildlife Service (USFWS) tentatively identified this species as *Pleurobema collina*. Two working hypotheses regarding the range of *Pleurobema collina* in the Dan River, North Carolina, have been proposed subsequent to more than 380 person-hours spent conducting surveys. The species was found in a 57-rkm reach of the Dan River and in a 19-rkm reach of the Mayo River, Stokes and Rockingham counties. The overall catch per unit effort (CPUE) varied from 0.08/hr to 1.48/hr.

The purpose of this project was to determine where *P. collina* resides in Virginia and the extent of its range within the state. The USFWS requires surveys by the Virginia Department of Transportation for this species at all roadway projects on rivers and tributaries in Virginia until the range of this species is defined by adequate survey work. An informal preliminary survey design for *P. collina* was used during the summer of 2002 to improve the future survey design. Simple random sampling was deployed in surveys conducted in 2003 and 2004 to provide a good basis for comparison to gauge the efficiency of the informal sampling design used.

In 2002, 116 person-hours were spent surveying 39 localities on the Mayo, Dan, and Smith rivers. The species was observed only in the South Fork of the Mayo River, Patrick and Henry counties, Virginia. During the summers of 2003 and 2004, 228 person-hours were spent surveying 38 equal-area river reaches (10,000 m²) on the mainstems of the Dan, Smith, South Mayo, and Banister rivers. No specimens of *P. collina* (live or relic shells) were detected. However, two species, *Elliptio complanata* and *Villosa constricta*, were detected at almost every site surveyed. Water levels and flows were moderate to extremely high throughout the spring, summer, and early fall of both years, with water temperatures remaining low during the summer of 2003.

A simple random sampling approach was designed to be easy, relatively quick, and cost-effective, applicable to most rivers, and to provide actual numbers for comparison. Negative results were reported after only 6 person-hours of searching within each randomly selected, equal-area river reach had been expended. *P. collina* was declared absent from the Virginia random sites surveyed in 2003 and 2004 with a confidence of approximately 90%. This information should eliminate the need to perform surveys for future work done in Dan and Mayo river basins.

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INTRODUCTION

Three spined mussel species occur in the United States along the Atlantic slope: James spiny mussel (*Pleurobema collina*), Tar spiny mussel (*Elliptio steinstansana*), and Altamaha spiny mussel (*E. spinosa*). Both the James spiny mussel and the Tar spiny mussel are federally endangered. The James spiny mussel (*P. collina*) was listed as endangered in 1988, and was until recently considered to be endemic to the James River basin (Clarke and Neves 1984; U.S. Fish and Wildlife Service [USFWS] 1990).

When the recovery plan for this species (USFWS 1990) was completed, *P. collina* was estimated to have been extirpated from 90% of its historic range in the James River basin, principally due to anthropogenic alteration of habitat. Changes in land use (e.g., increased development, cattle grazing, and road construction) have increased sediment loads and decreased water quality (Virginia Department of Environmental Quality 2002). Potential competition with the introduced Asian clam (*Corbicula fluminea*), predation by muskrats, and increasing spatial separation (fragmentation) among populations have exacerbated the effects of habitat alteration. Although definitive reasons for the decline of *P. collina* in the James River basin are unclear, it seems reasonable to assume that industrial and agricultural development have been major contributors to its decline (Clarke and Neves 1984; Hove 1990; USFWS 1990).

Newly Discovered Distribution in the Dan River Sub-basin

Biologists with the North Carolina Department of Transportation discovered spiny mussel populations in the Dan and Mayo rivers in North Carolina in 2000 and 2001, respectively. The USFWS tentatively identified this species as the James spiny mussel (*Pleurobema collina*). These streams are part of the Dan River sub-basin of the Roanoke River system, which flows into Albemarle Sound in northeastern North Carolina.

PURPOSE AND SCOPE

The purpose of this project was to determine where *P. collina* resides in Virginia and the extent of its range within the state. The Virginia reaches of the Dan and Mayo rivers likely were historic habitat, but no data existed to confirm or refute the presence or extirpation of *P. collina* until summer 2002 surveys revealed its presence. Surveys conducted by Virginia Polytechnic Institute & State University (Virginia Tech) during the summer of 2002 revealed the presence of the same species of spiny mussel in the South Fork Mayo River, Patrick and Henry counties, Virginia. The South Fork Mayo empties into the Mayo River, a major tributary of the Dan River.

The USFWS requires surveys by the Virginia Department of Transportation (VDOT) for this species at all roadway projects on rivers and their tributaries in Virginia until the range of this species is defined by adequate survey work. Prior to this study, populations of *P. collina* were known to exist only in isolated tributaries of the upper James River system (see Appendix A). The following two sections provide brief reviews of recent survey work conducted to define the overall range of *P. collina* in Virginia and North Carolina.

Survey in the James River Drainage, Virginia

Distribution of *P. collina* in the James River drainage recently was found to be broader than the original descriptions provided by Clarke and Neves (1984) and Hove (1990). Extensive surveys conducted since 1989 by the Virginia Department of Game and Inland Fisheries (VDGIF) identified additional populations in the James River drainage (B. Watson, 2005, personal communication). Although *P. collina* is widely distributed throughout the basin, its populations are isolated and rare, and some populations appear to be in decline (B. Watson, 2005, personal communication). Since the James River mainstem no longer supports populations of *P. collina*, and all of the sub-drainages containing *P. collina* are isolated, it is likely that the loss of *P. collina* from any sub-drainage would not be followed by natural colonization unless and until reasons for population decline are remedied in the mainstem James River (Hove 1990).

Survey and Current Distribution in the Dan River Sub-basin, North Carolina

Upon discovery of the James spiny mussel (*P. collina*) in the Dan River in October 2000, the North Carolina Department of Transportation coordinated an extensive multi-agency survey of the Dan River sub-basin. Participants included the USFWS, Catena Consulting Group, North Carolina Wildlife Resources Commission, North Carolina State University, North Carolina Natural Heritage Program, and Virginia Tech. Survey efforts have concentrated in Stokes, Rockingham, and Caswell counties, North Carolina. Over 380 person-hours (p-h) were spent surveying the Dan River and its tributaries. In addition to the mainstem of the Dan River, the James spiny mussel was discovered in the Mayo River, a tributary to the Dan River at approximately river kilometer (rkm) 175, in northwest Rockingham County. The species has not been found in any other tributaries of the Dan River. In fact, the majority of tributaries in the Dan River drainage appear to be devoid of mussels (Savidge 2002).

Although surveys in the basin have not been completed, two working hypotheses regarding the range of *P. collina* in the Dan River, North Carolina, have been proposed. The species has been found in a 57-km reach of the Dan River and a 19-km reach of the Mayo River. Within the Dan River, catch per unit effort (CPUE) varied from 0.08/hr to 1.48/hr but was lowest at the upstream and downstream limits. One stretch of river was surveyed 3 times before the species was detected. The current distribution in the Dan River extends from below the North Carolina/Virginia border, near the first bridge crossing in North Carolina (Flippin Road, SR 1416) in northwest Stokes County, down to at least SR 1695 (Dodgetown Road) below the town of Danbury in central Stokes County (Figure 1). The species was not found in the reach between the SR 103 crossing in Patrick County, Virginia, and SR 1416 in North Carolina (Flippin Road). This reach will be resurveyed, however, because *P. collina* was not found in the reach below Danbury from SR 1652 (Moir Farm Road) down to SR 1695 (Dodgetown Road) until the third survey of the reach. All the places where *P. collina* is thought not to occur have received similar repeated sampling effort by biologists from the various agencies in North Carolina. Survey work will continue above and below the documented range in North Carolina to establish the actual distribution of this species in this drainage (Savidge 2002).

In the upper part of the documented range, *P. collina* is rare and is known from only one individual. A small impoundment at Jessups Mill, located on the Dan River just above North Carolina SR 1432, may restrict the dispersal of this species. Dams, even lowhead structures as small as 1 m high, are obstacles to the distribution of some fishes and may contribute to the overall depletion of unionoids by artificially restricting their distributions and isolating populations from each other (Watters 1995). Distribution and movement patterns of fish hosts have been shown to play an important role in the distribution of mussels (Watters 1992; Vaughn 1997; Haag and Warren 1998; Vaughn and Taylor 1999). Because of its small size (approximately 32 mm in length), the one individual found above the dam cannot be considered an old relict adult. However, the CPUE is very low (0.08/hr) in this reach compared to the reach immediately below the dam (0.43/hr). It is likely that the dam influences the species' distribution in this section of the river (Watters 1995; Vaughn and Taylor 1999; Kelner and Seitman 2000). Below Jessups Mill, *P. collina* continuously occurs in densely aggregated multi-species "beds" separated by areas where mussels occur sporadically in the river. It becomes patchier in occurrence (i.e., beds separated by areas where mussels do not occur at all) below Danbury. According to Savidge (2002), it seems to be most abundant (based on CPUE) in the reach between North Carolina 704 and North Carolina 89.

A distribution of 19 rkm in the Mayo River, North Carolina, also has been established for *P. collina* (Figure 1), from the North Carolina/Virginia border to just downstream of SR 770 in northwest Rockingham County, North Carolina. Below this point in the Mayo River, there is approximately 4.8 km of the river where *P. collina* has not been found in repeated sampling, presumably due to a point-source discharge (Stoneville Wastewater Treatment Plant), a sand/gravel mine (Stoneville Sand Mine), and an impoundment (Avalon Dam) (Starnes and Gasper 1996; Goudreau et al. 1988; Brown et al. 1998; Vaughn and Taylor 1999). The species has been found in a short reach (approximately 0.8 km) of the Mayo River between Avalon Dam and Mayo Dam. Further surveys are needed below Mayo Dam to determine its presence/absence in this reach of the river. The overall CPUE in the Mayo River is 0.98/hr (Savidge 2002).



Figure 1. Current distribution of *Pleurobema collina* in the Dan River sub-basin, Patrick and Henry counties, Virginia, and Stokes and Rockingham counties, North Carolina.

A description of chemical and physical conditions at sites currently and historically supporting *P. collina* in the James River basin was given in Boss and Clench (1967) and Clarke and Neves (1984). The habitat was generally described as runs of moderate current, with sand, gravel, and cobble substrates. Individuals from the Dan River population have been found in a variety of substrates, from silt/sand to sand, gravel, cobble, bedrock crevices and sand surrounded by boulders with a variety of flow patterns; from slack pools, to runs of moderate to swift currents. A minimum hardness value of 50 mg/L as CaCO₃ is believed to be a requirement for this species (Clarke and Neves 1984).

Informal Vs. Probability-Based Survey Design

Many species of mussels, such as *P. collina*, are increasingly studied because they are endangered or have been extirpated by human activities (Williams et al. 1993; Master et al. 2000; Strayer and Smith 2003). Consequently, biologists frequently need to estimate presence

and extent of range to evaluate the status of endangered mussel populations and to research mussel biology or ecology (e.g., Downing et al. 1989; Strayer et al. 1997). Mussel sampling designs differ widely in cost, ease of use, and suitability to address various objectives. Biologists should use a survey design that is well suited to their objectives, thereby reducing cost and effort while increasing the quality and applicability of the resulting data (Strayer and Smith 2003).

A survey is any procedure used in an observational study to sample a population for the purpose of estimating occurrence patterns, or other population parameters (Strayer and Smith 2003). There are many designs used for mussel surveys. Designs to meet these objectives should have a high and known probability of detecting mussel species. Designs that have a high probability of species detection (i.e., informal sampling designs) do not allow estimation of that detection probability, and designs that readily allow estimation of detection probabilities (i.e., formal sampling designs) usually do not have high detection probabilities (Strayer and Smith 2003). Informal designs, though not statistically robust, are widely used by field biologists because they require little or no planning, are flexible, and are easy to execute in the field. They rely largely on expert judgment, often by a single expert. Survey sites for sampling mussels or sediments within a site are selected without a formal design for the convenience of the investigator. Examples include most timed searches, in which field biologists use visual searches to locate mussels at convenient or suspected places (e.g., riffles near bridges).

Informal surveys offer very good detection of mussel species (Hornbach and Deneka 1996; Strayer et al. 1997; Vaughn et al. 1997; Obermeyer 1998). However, the inability to estimate the detection probabilities of informal designs limits their use in determining the absence of mussel species. It is not possible to say with certainty that a mussel species is absent from an area unless the entire study area can be completely searched (Strayer and Smith 2003). It also is not possible to draw any inferences about an entire mussel assemblage from informal sampling without accepting the untested assumption that samples are representative of the target population. The data collected will be biased to an unknown extent (Strayer and Smith 2003). Also, there is no valid method to assess sampling variance from informal sampling. Thus, results from informal samples are reported without measures of uncertainty and will not be reliable for assessing population density, relative abundance of species among sites, and assessing temporal changes in mussel populations. Informal sampling is most useful in preliminary surveys and for determining the presence, but not absence, of a mussel species at a site (Strayer and Smith 2003).

Probability-based methods, such as simple random sampling, allow for the estimation of sampling probabilities, which then are used to estimate a population parameter (e.g., abundance) and the variance of the estimate (Strayer and Smith 2003). In simple random sampling, the spatial area of interest is divided into N non-overlapping units, which are numbered consecutively. The investigator then randomly selects n of these units adequate to detect the presence of rare mussel species with some specified probability of detection (i.e., a power analysis), often using statistical software or tables of random numbers. Simple random designs produce unbiased estimates over entire study areas of mussel abundance and other attributes. However, estimates of overall mussel population size or density may be imprecise because many of the random samples will contain no mussels, and just a few will contain many mussels. One solution to this problem is to increase the area sampled by increasing the size or number of units sampled (Strayer and Smith 2003).

Virginia Tech conducted surveys in 2002-2004 to determine whether *P. collina* occurs in the Virginia portion of the Dan River sub-basin and what the extent of its range is within the sub-basin. VDOT requires surveys for this endangered species at all roadway projects on both rivers and tributaries in Virginia, until the range is defined by adequate survey work. An informal preliminary survey design for *P. collina* used during the summer of 2002 revealed the presence of this species in the South Fork Mayo River, Virginia, a major tributary of the Dan River. Because good survey design is based on knowledge of the target population and site characteristics, the goal was to use the estimated mean CPUE (i.e., sampling effort defined as the encounter rate) from the informal survey design to improve future survey design. Therefore, the main goal for surveys in 2003-2004 was to detect the presence-absence of *P. collina* using timed searches in the Dan River sub-basin. The objective was to achieve the most precise estimate given the resources available to conduct the surveys. Precision was determined by two factors: abundance or density (CPUE) and survey design (i.e., sample size n = number of sites) (Downing and Downing 1992; Strayer et al. 1997). The challenge was to design a survey that reduced the chance of missing the presence of *P. collina* to an acceptable level. The chance of missing a species that is actually present at a site (equivalent to a type II error) decreases with increased species density and with increased sampling effort and spatial coverage (Green and Young 1993; Strayer et al. 1997; Metcalfe-Smith et al. 2000).

Using an estimate of the mean abundance (CPUE) of *P. collina* from informal preliminary surveys, a simple random sampling design was deployed in 2003-2004 surveys to allow for a probability statement to be made about species absence and maximum abundance (CPUE) at a site even if no mussels were found (Green and Young 1993). A power analysis incorporating conservative estimates of rare species density was used to determine if sampling effort was likely to detect *P. collina* presence with sufficient certainty (Green and Young 1993). The importance of a rigorous survey design for determining species presence is imperative when considering endangered species assessment. Suppose *P. collina* was not detected at a site of a potential impact under implementation of an informal and untested survey design. The finding of absence could be challenged because of the ambiguity of “species absence” and the inadequacy of an informal survey design (Strayer and Smith 2003). Methods and results for both informal preliminary surveys and formal simple random surveys are reported in subsequent sections.

Study Area

Roanoke River Basin

The Roanoke River basin covers 16,529 square kilometers of Virginia (i.e., 64% of the total watershed area). The Virginia portion of the Roanoke River basin is bounded on the north by the James River basin, on the east by the Chowan River basin, and the west by the New River basin. The southern boundary of the basin in Virginia is the state line. The headwaters begin in the mountains of eastern Montgomery County and flow southeast to the Virginia/North Carolina state line. In Virginia, the Roanoke River passes through three physiographic provinces, the Valley and Ridge Province to the northwest, and the Blue Ridge and Piedmont provinces to the southeast. The topography ranges from steep slopes and valleys in the Valley and Ridge Province to gently sloping terrain east of the Blue Ridge Mountains in the Piedmont Province.

In Virginia, the Roanoke watershed includes four major impoundments, Smith Mountain and Leesville lakes to the north, and Kerr Reservoir and Lake Gaston located at the junction of the Roanoke River and the North Carolina state line. These reservoirs range in size from the 19,830 hectare Kerr Reservoir to the 1,376-hectare Leesville Lake. The Dan River system (193 rkm) has four major tributary systems. From east to west, they are the Banister River, Smith River, Mayo River, and Dan headwaters on the Blue Ridge (Figure 2). Over 62% of the basin is forested, nearly 25% is in cropland and pasture, and approximately 10% is urban. The human population in the Virginia portion of the Roanoke River basin in 1994 was approximately 669,681 (VADEQ 2002).

The North Carolina portion of the Roanoke River basin is composed of two major parts: (1) Dan River and its tributaries in the western section, upstream of Kerr Reservoir, and (2) Roanoke River as it enters North Carolina in the eastern section. The Roanoke River mainstem enters Kerr and Gaston lakes in North Carolina and then flows into Roanoke Rapids Lake before regaining its riverine form and flowing into Albemarle Sound. The entire Roanoke River watershed is approximately 25,035 square kilometers, with about 7,770 square kilometers in North Carolina (i.e., 16% of total watershed area). Flow in the Roanoke River in North Carolina is regulated by the operation of Kerr Reservoir and Lake Gaston (North Carolina Division of Water Quality [NCDWQ] 2001).

Based on 1990 census data, the North Carolina population of the sub-basin is 263,691 people. Over half of the land in the river basin is forested (NCDWQ 2001). Statistics provided by the U.S. Department of Agriculture, Natural Resources Conservation Service, indicate that during the last decade, there has been an increase in the amount of developed land and a decrease in the amount of cultivated cropland (Savidge 2002).



Figure 2. Dan, South Mayo, Smith, and Banister rivers of the Roanoke River basin study area, Virginia.

Dan River Sub-basin in the Roanoke River

The Dan River arises in the uplands of the Blue Ridge Province in Patrick County, Virginia and flows south through the Blue Ridge Escarpment before crossing into North Carolina in northwestern Stokes County at approximately rkm 260. It then flows southeast across most of Stokes County before turning sharply to the northeast near Walnut Cove, flowing through most of Rockingham County, North Carolina. The river flows into southern Pittsylvania County, Virginia, back into Rockingham County, North Carolina, east into Caswell County, North Carolina, then north back into Pittsylvania County, Virginia. The river then flows east through the city of Danville, turns to the south and re-enters North Carolina in north-central Caswell County. It flows east before turning back to the north, re-entering Virginia, flowing generally to the northeast before entering Kerr Reservoir. From its origin to the confluence with the Roanoke River at Kerr Reservoir, the Dan River is 320 rkm long and drains 6600 km² (Rohde et al. 2001).

The Dan River flows through four physiographic subdivisions: (1) Upland (rkm 320-312), (2) Blue Ridge Escarpment (rkm 312-266), (3) Inner Piedmont (rkm 265-197), and (4) Fault Basin (rkm 196-0) (Rohde et al. 2001). Most of the land in this basin is forested (73%), but a significant portion is cultivated cropland and pasture (25%). Many tributaries and sections of the Dan River are deeply entrenched, suggesting the effects of long-term erosion. Soil erosion rates as great as 21 tons/0.4 hectare/yr have been documented for cultivated cropland in the upper Dan River (Natural Resources Conservation Service 1995). The upper Dan River is classified as trout waters, and part of the area is also designated a State Water Trail by the North Carolina Division of Parks and Recreation. Characteristics of this sub-basin are transitional between mountain and piedmont regions, resulting in moderately steep topography. Headwater reaches of most tributaries are forested, while riparian lands in many downstream sections are intensively farmed (Savidge 2002).

METHODS

Informal Preliminary Survey in the Roanoke River Drainage, Virginia

Virginia Tech designed and conducted preliminary survey work in the mainstem and major tributaries of the Dan, Mayo, and Smith rivers in Virginia, in 2002. Efforts were focused in Patrick and Henry counties, where these drainages occur. Because no previous mussel surveys had been conducted in these rivers and their major tributaries, summer 2002 was spent doing informal reconnaissance surveys in these systems to identify reaches with freshwater mussels and habitat suitable for *P. collina*.

Stream reaches that were accessible at primary and secondary road crossings in Patrick and Henry counties were surveyed in 2002 to identify locations with suitable habitat for this species. The Virginia Atlas and Gazetteer (DeLorme, Yarmouth, Maine 2000) and USGS quadrangle maps were used to select accessible sites to be surveyed, beginning near the North Carolina/Virginia border and progressing north in these rivers. Sites were qualitatively surveyed for presence of mussels in a minimum of 200 m or 3 p-h (arbitrary threshold) by at least three

experienced biologists. On average, one person searched approximately 100 m² over 1 hour per site. Most sites were snorkeled; however, due to drought conditions in late summer 2002, waterscoping and/or collection of mussels by hand were used to detect mussels. Some reaches of the South Fork Mayo and Dan rivers were on private land and inaccessible; therefore, these areas were surveyed by canoe float trips. If mussels were present, species composition, relative abundance (i.e., CPUE = number of mussels encountered per hour), and location within the reach were recorded (latitude/longitude in decimal degree format). Stream margins were searched for mussel shells and muskrat middens to supplement the instream list of species at each site. Sufficient effort was expended at each site to state with reasonable confidence that *P. collina* was present or absent at that location.

Survey results and habitat information for each site were recorded on a Standard Survey Record Form, as standardized for all mussel surveys conducted for VDOT. Survey conditions and habitat features recorded included weather and river conditions, typical river width and depth, general substrate type (visually assessed), primary land-use, general nature of bankside vegetation, evidence of disturbance features (e.g., cattle grazing or in-stream gravel mining), mean water column velocity, and near-bed velocity. Each site was photographed with a digital camera.

Simple Random Survey in the Roanoke River Drainage, Virginia

Virginia Tech designed and conducted survey work in the mainstem and major tributaries of the Dan, Mayo, Smith, and Banister rivers, Virginia, in 2003 and 2004. Survey efforts were focused in Patrick, Henry, Pittsylvania, and Halifax counties, where these drainages occur. To more objectively define distribution (i.e., occurrence) and abundance, a probability-based sampling design (Strayer and Smith 2003) was followed to assess the range of *P. collina* across the Roanoke drainage. A simple random survey design was used because there is no standard procedure for defining the range of a mussel species.

Two quantities must be specified to define the range of a species: the grain size at which the range is defined and the minimum density within each site/locality that is required for the species to be considered “present” (Strayer and Smith 2003). “Grain size” is used here, in the sense of landscape ecology, to mean the finest level of spatial resolution in the data set (Turner et al. 2001). Grain size is the spatial extent of the sampling units (e.g., riffle, reach, watershed). Within the Roanoke River system, grain size was set as a 1 km river reach with 10,000 m² of the length or area of stream bottom as the sub-sample (Strayer and Smith 2003).

Once the grain size was set, a threshold density below which the species is considered to be absent from a site was determined. It rarely is possible to do a complete census of sites or be certain that a mussel species actually is absent (as opposed to rare) from a site. This threshold could have been set in quantitative terms (e.g., species *X* is defined as present if $>T$ individuals exist in a 1-km reach, or if the mean density exceeds D/m^2) if quantitative sampling methods such as excavation of quadrats along a transect line were used. However, quantitative methods are so inefficient at detecting rare species, especially burrowed specimens, that such methods are unsuitable for defining a species’ range (Strayer and Smith 2003). Instead, timed searches were deemed more appropriate to establish this species’ range, and detection threshold was set in

terms of minimum encounter rates (e.g., species *X* is defined as absent if no live mussel is detected in *Y* p-h of searching) (Strayer and Smith 2003).

The precision of presence-absence data presents a special problem. Conservation biologists who work with large vascular plants or vertebrates consider presence-absence data to be robust, but for cryptic animals like mussels, absence data rarely can be definitive, except for small study areas. Stating that a mussel species is “present” is equivalent to saying that the mussel population density is above some detection threshold, and can be estimated only with error (Strayer and Smith 2003).

It is complicated to estimate the probability of detecting a mussel population using timed searches. Following Green and Young (1993)

$$p(\text{detection}) = 1 - e^{-R}$$

where *R* is the mean number of animals detected in a timed search; i.e., length of the search times the encounter rate or CPUE (Strayer et al. 1996; Strayer 1999). Nevertheless, it is clear that the probability of detecting a rare mussel population in a timed search depends on the encounter rate and the length of time spent searching, and is usually an unknown function of population density (Strayer and Smith 2003). As Metcalfe-Smith et al. (2000) and Lellis (2001) have shown, mussel populations may be so sparse and cryptic that they are detected only with long (>5 p-h), timed searches. Green and Young (1993) state that any species having true density > 0.1 per sample unit size is *not* rare. Therefore, Green and Young’s (1993) probability of detection formula and extremely conservative estimates of CPUE (i.e., 0.01, 0.05, and 0.1) were used to calculate the probability of detection for *P. collina* over *n* = 1-100 sites for 6 p-h, 4 p-h, and 2 p-h. The number of samples (*n*) needed to detect the presence of this rare species with power 1 – β was determined using the formula: $n = - (1/m) \log \beta$. Mean density (*m*) was defined as mean CPUE. Two assumptions were made: (1) that mussels were uniformly distributed throughout the rivers, and (2) that in 1 p-h it was reasonable to assume that an area of 100 m² was searched (i.e., estimated length of search).

Because good survey design is based on knowledge of the target population and site characteristics, informal preliminary surveys of poorly known sites almost always improve survey design, often substantially (Strayer and Smith 2003). The study area of this project is large and diverse, which made conducting the extensive preliminary survey work in 2002 worthwhile before attempting the formal survey. Species biology and preliminary survey results in Virginia and North Carolina were used to eliminate portions of the drainage where the species was unlikely to be found, such as streams smaller than fourth order. Approximately 30 streams of <4° were searched in 2002 preliminary surveys with no occurrences of *P. collina* detected.

Initial survey efforts focused on major tributaries of the Roanoke River in Virginia and North Carolina (e.g., Dan, Smith, Mayo, and Banister rivers) and a major tributary to the Mayo River, the South Mayo River. Rivers were divided into roughly equal-area reaches (10,000 m²) by assigning each reach an identification number referenced by 1 km reaches (*N* = 249 total rkm). The 89 rkm in Virginia and North Carolina with known “presence” of *P. collina* were not included. Using results from the power curve analysis (see Figure 3), 100 reaches (*n* =

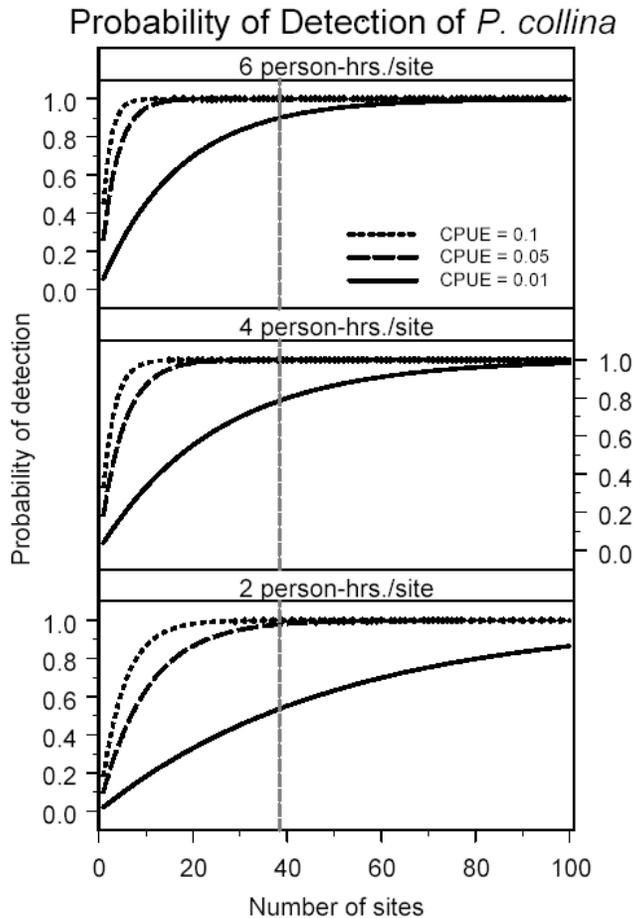


Figure 3. Power curves showing probability of detection for *P. collina* over $n = 1-100$ sites for 6 p-h, 4 p-h, and 2 p-h. The number of samples (n) needed to detect the presence of this rare species with power $1 - \beta$ was determined using the formula: $n = - (1/m) \log \beta$. Mean density (m) was defined as mean CPUE. Two assumptions were made: (1) that mussels were uniformly distributed throughout the rivers, and (2) that in 1 p-h it was reasonable to assume that an area of 100 m^2 was searched. Dashed gray line denotes the actual random sites surveyed in VA in 6 p-h of searching ($n = 38$).

100) were randomly selected for study with a probability of detecting *P. collina* at 99.7% (SAS Institute 2001; S-PLUS 2003). Due to extreme flood events and high flow conditions during the summers of 2003 and 2004, the sample size (n) had to be modified. The Virginia random survey sites ($n = 56 \text{ rkm}$) became the main focus of study with the probability of detection still high at 96%. Two-hour timed searches with at least three biologists (total of 6 p-h) on each reach ($10,000 \text{ m}^2$ per rkm) were conducted.

CPUE data are sensitive to sampling conditions and workers' skill; therefore, these effects were minimized by sampling at low flow, when water was clear, and by deploying experienced field crews. At least two of the same individuals were always present during all sampling conducted to standardize "persons." *P. collina* was declared as *absent* if no live animal or shell was detected in 6 p-h of searching (Metcalf-Smith et al. 2000; Lellis 2001). Methods for detecting mussels were visual searches by observers wearing mask and snorkel or viewsopes.

Simple random sampling with three equidistant starting points in relation to each river (based on three biologists at each 1-km sampling unit) was applied to select sites for this broad-scale survey. When possible, areas from upper, middle, and lower reaches were included. Multiple equidistant starts were included to estimate catch-per-unit-effort variance and to guard against the interval between samples corresponding to a periodicity in the mussel density (e.g., riffle-pool spacing). Biologists snorkeled in a slow upstream zigzag search pattern to maximize chances of finding mussels (Figure 4). Sampling started in early summer and continued into early fall since high proportions of some mussels are at the substrate surface in summer during periods of low flow. Evidence suggests mussels bury more, and so are less visible, in cold water with high water levels (Amyot and Downing 1991; Balfour and Smock 1995).

Occurrence data for *P. collina* were plotted on detailed ArcView quadrangle maps to define upstream and downstream extent of this and other mussel species encountered. GIS points were plotted on county maps to better define the range of the spiny mussel and other species. A geographic description of occurrence and range per tributary and mainstem was provided to VDOT and the USFWS using roadways as landmarks. Preparation of a distribution and range map was coordinated with the GIS staff at the Conservation Management Institute at Virginia Tech.

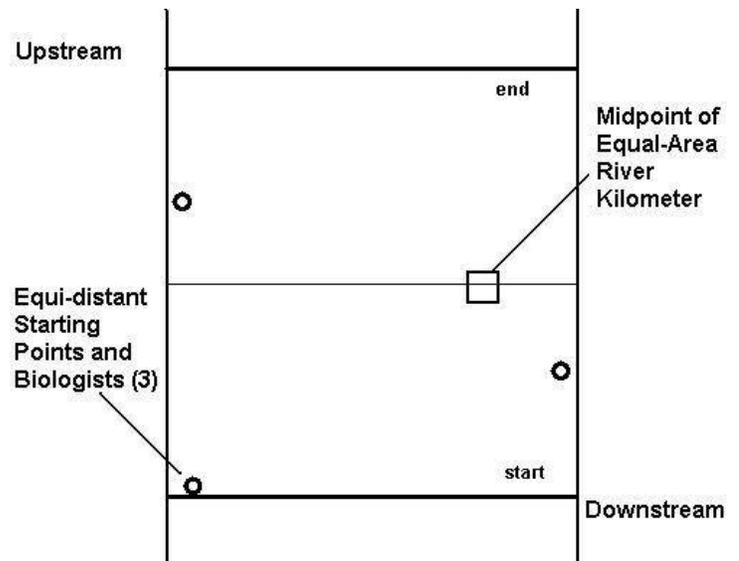


Figure 4. Diagram of simple random sampling methodology used in 2003-2004.

RESULTS AND DISCUSSION

Informal Preliminary Survey in the Roanoke River Drainage, Virginia

In 2002, a total of 116 p-h were spent surveying 39 localities on the Mayo, Dan, and Smith rivers. The species was observed only in the South Fork of the Mayo River, Patrick and

Henry counties, Virginia, confirming its occurrence near the North Carolina/Virginia state line. A total of 96 *P. collina* was observed in the South Fork Mayo River. Estimated mean CPUE was 1.5 specimens/hr. On average, at least 1 individual was found per every hour assuming that one person searched approximately 100 m² over 1 hour per site. Thus, by Green's and Young's (1993) definition of rare (<0.1/m²), *P. collina* was far above that density level in the South Fork Mayo River. The mean CPUE calculated was an estimate of density. At this time, there is no accurate measure of the true density of *P. collina* in Virginia or North Carolina. Consequently, if the distribution was restricted to habitat patches and clustered in the South Fork Mayo River, *P. collina* may still be rare over the whole state of Virginia, and thus, the United States. A range of 24 km was documented in the South Fork Mayo River at 12 localities (Figure 5). Localities surveyed in Virginia, dates, and species composition are listed in Table B-1 in Appendix B.

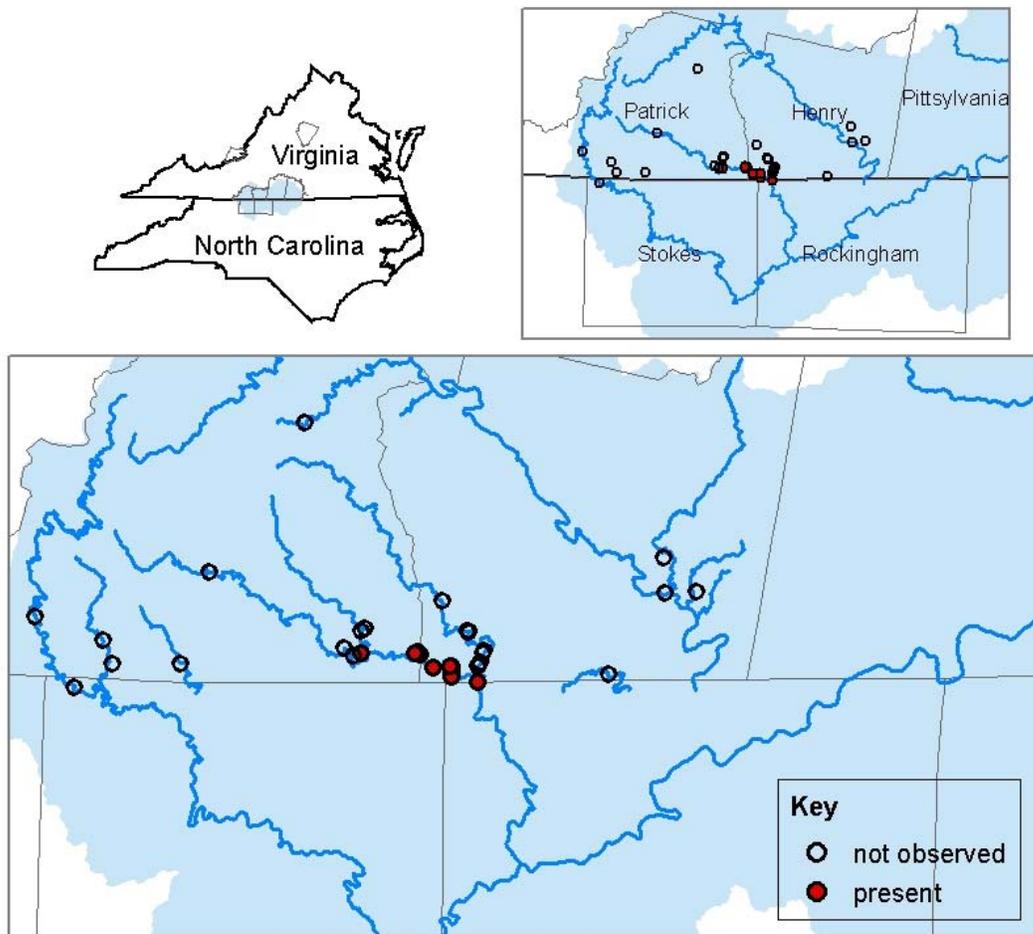


Figure 5. Preliminary, informal surveys conducted for *Pleurobema collina* throughout the Dan River sub-basin, Virginia, 2002. Mussels present throughout 24 rkm of the South Mayo River, Patrick and Henry counties, Virginia (denoted by closed dots).

The range extension of *P. collina* includes a 57-km reach of the Dan River, Stokes and Rockingham counties, North Carolina, which is separated from the 19-km distribution in the Mayo River, Rockingham County, North Carolina. There is approximately 40 rkm separating the downstream extent of *P. collina* in the Dan and Mayo rivers (Savidge 2002). The documented distribution of *P. collina* in the Mayo River continues upstream into Virginia, extending for 24 rkm into the South Fork of the Mayo River, Patrick and Henry counties. A contiguous range of 43 rkm for *P. collina* in the Mayo River has been delineated thus far by surveys in North Carolina and Virginia. A large, extensive falls area (height approximately 1.2 m) occurs where the distribution of *P. collina* appears to end in the South Fork Mayo River (uppermost end of the range). The falls may act as a barrier to dispersal of host fishes for *P. collina*. Banks of the South Fork Mayo River at sites occupied by the species are very stable. The woodland area of the riparian zone is intermediate to extensive, with occasional pasture and cattle grazing present. Nonetheless, the buffer width of the riparian zone is moderate to wide. The species was found in a range of habitats in the South Fork Mayo River. No immediate threats to the South Fork Mayo River habitat are evident at this time.

Age-class structure of 98 live specimens of *P. collina* measured at the South Fork Mayo River ranged from 0-18 years, with a mean age of approximately 5 years (38.1 mm standard length). Standard lengths ranged from 16.9-66.8 mm. Four juveniles less than 15 mm in length were not measured (age-class 0-1 year). Relatively high numbers of mussels between the ages 4 and 7 provide evidence of recent recruitment and good reproduction. The age-classes applied in the above assessment were determined by using Hove's (1990) age-class structure estimations.

The species was found in a range of habitat types in the South Fork Mayo River, including shallow riffle, run, slack or low-velocity areas and pool (50-70% < 61 cm depth) with abundant sand/gravel bars present in the riffle, run, and slack stream segments. James spinymussels appeared more abundant in slack water or low-velocity areas with sand/gravel bars present. Substrates in low-velocity areas were predominantly silt, sand, cobble and gravel. Water level was average to low according to the USGS gauging station on this river, with river width ranging from 10-30 m. Banks of the South Fork Mayo River at sites occupied by *P. collina* were very stable. The woodland area of the riparian zone was intermediate to extensive, with active cattle grazing occasionally present. The buffer width was moderate to wide (approximately 50-200 m) in most reaches.

Simple Random Survey in the Roanoke River Drainage, Virginia

During the summers of 2003 and 2004, 228 p-h were spent surveying 38 equal-area river reaches (10,000 m²) on the mainstems of the Dan, Smith, South Mayo, and Banister rivers (Figure 6; closed dots denote 2002 occurrences). No *P. collina* (live or relic shells) was detected. However, two species, *Elliptio complanata* (mean CPUE = 6.08, SD = 10.1) and *Villosa constricta* (mean CPUE = 0.45, SD = 0.98), were collected at almost every site surveyed (Table B-2 in Appendix B). Water levels and flows were moderate to extremely high throughout spring, summer and early fall of both years, with water temperatures remaining low during the summer of 2003 (ranging between 11-22°C) (refer to www.USGS.gov for 2002-2004 hydrographs).

Due to unfavorable river conditions, $n = 100$ or 56 planned surveys were not completed. However, $n = 38$ -km sites were completed with approximately 90% probability of detecting one mussel in 6 p-h of searching, assuming the most conservative estimate of CPUE = $0.01/m^2$ (Figure 3). Planned survey trips frequently were cancelled due to months of above-normal precipitation and severe and extensive flooding, which resulted in river conditions too dangerous and/or turbid to conduct sampling. Even after moderate rain events, low visibility from turbid conditions remained for at least 1 week or until the next heavy rain event, which made the rivers unsuitable for visual searches. Based on the discharges measured at the USGS gauging station at the following locations; Dan River near Francisco, North Carolina; South Mayo River near Nettleridge, Virginia; and Banister River at Halifax, Virginia; when flows exceeded 200+ cubic feet per second (cfs), the river at that station was too high and fast flowing, or just simply too turbid for survey.

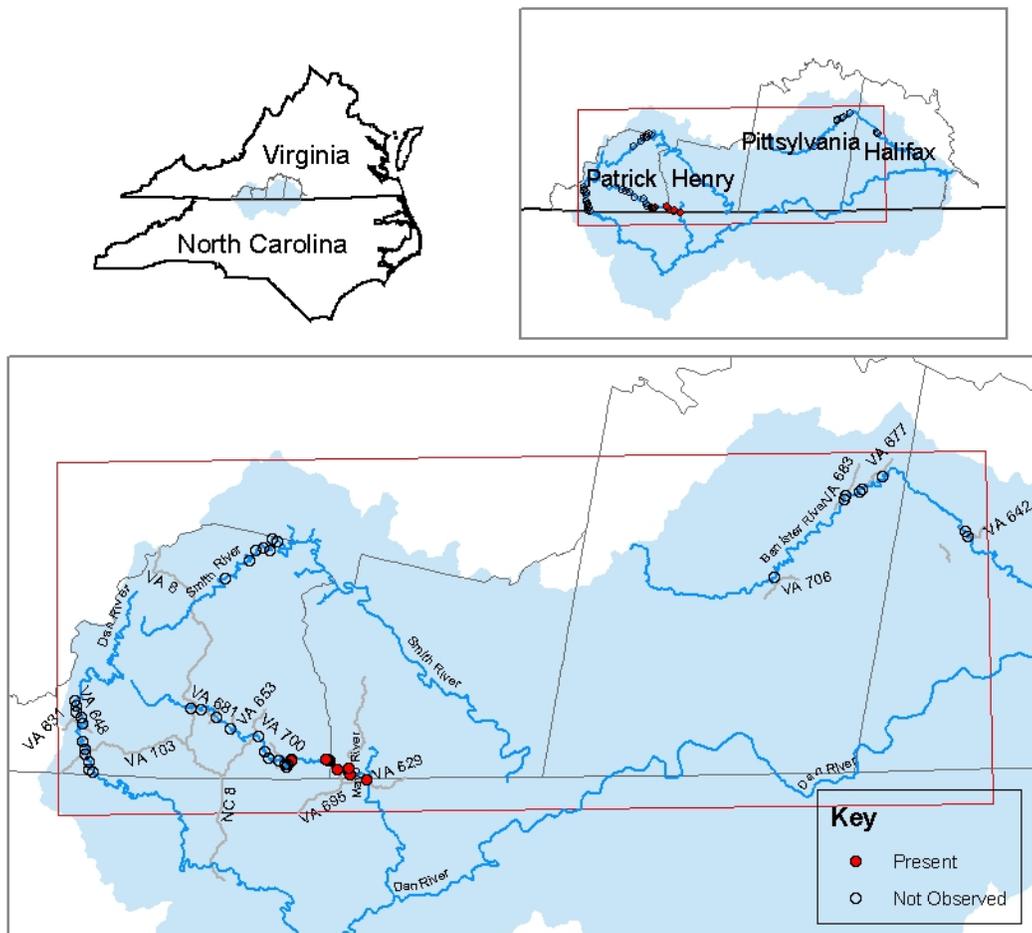


Figure 6. Formal simple random surveys (present/not observed) for *Pleurobema collina* in the Roanoke River basin (Dan River sub-basin), Virginia, 2003-2004. Closed dots denote 2002 occurrences of *P. collina*.

Of the 56-km sites that were randomly selected for survey in Virginia in the Dan, South Fork Mayo, Smith, and Banister rivers, *P. collina* was not detected despite 228 p-h of sampling effort over 38-km sites. Negative results were reported only after 6 p-h of searching within each

randomly selected, equal-area river reach (10,000 m²) had been expended. It can be stated with reasonable confidence (approximately 90%) that *P. collina* is absent from the remaining 18 random sites in Virginia (Table B-3 in Appendix B; Dan and Banister rivers; Figure 3).

Informal sampling was most useful in preliminary surveys for determining the presence, but not absence, of *P. collina* in the Virginia portion of the Dan River sub-basin. There was no valid method to assess sampling variance from the informal sampling and results were not reliable for assessing the true population density, mean CPUE (abundance) of species among sites, and assessing temporal changes in *P. collina* populations. Informal surveys offered very good detection of *P. collina* and other mussel species, however, the inability to estimate the detection probabilities of the informal design limited their use in determining the absence of *P. collina*. Using an estimate of the mean abundance (CPUE) of *P. collina* from informal preliminary surveys, the simple random sampling design used in 2003-2004 surveys allowed for a probability statement to be made about *P. collina* absence and maximum abundance (CPUE) of mussel species at a site even if no *P. collina* were found. Probability-based methods, such as simple random sampling, allowed for the estimation of sampling probabilities, which then were used to estimate a population parameter (e.g., mean CPUE) for *Elliptio complanata* and *Villosa constricta* and the variance (SD) of the estimate.

CONCLUSIONS

- *The distribution of P. collina is more widespread than previously recognized* (Clarke and Neves 1984; Hove 1990). Prior to this study, populations were known to exist only in isolated sub-drainages of the James River basin (Conrad 1837; Clarke and Neves 1984; Hove 1990; Watson 2005, personal communication). This species is no longer considered endemic to the James River system, following the discovery of populations in the Dan River sub-basin, Virginia, and North Carolina in 2000-2002.
- *The informal surveys conducted in 2002 indicated that P. collina has a wide and apparent patchy distribution in the Dan River sub-basin of Virginia.* This species was detected throughout a 24-km reach of the South Fork Mayo River, Virginia, in summer 2002, after using informal sampling, based on the discovery of this species in the Dan and Mayo Rivers in North Carolina, 2000-2001. The discovery occurred during drought conditions, which provided optimal sampling conditions; low flow and water levels, low turbidity, and high visibility. Estimated mean CPUE for *P. collina* was far above the density levels defined for rare species. However, since the CPUE was estimated from informal sampling data, it cannot be stated as the true density. For this reason extremely conservative estimates of density were used in the probability of detection power analysis before conducting the simple random surveys in 2003-2004.
- *Given that P. collina was not detected during the formal simple random surveys of 2003-2004, it was absent from the Dan, Smith, and Banister rivers in Virginia at a 90% confidence level.* The presence-absence (approximately 90% probability of detection) of the endangered *P. collina* was estimated using probability-based sampling in 2003-2004 to evaluate its status. The simple random sampling design will allow comparisons among studies. No

underlying distributions or models were assumed; therefore, the sampling approach allows nonparametric estimates of CPUE for the two species detected (*E. complanata*, *V. constricta*), because accuracy of estimates depended on random selection of sampling units, and not on underlying statistical models or spatial distribution of mussels within a site (Strayer and Smith 2003). Given this knowledge, mean CPUE data to provide estimates of population densities of *E. complanata* and *V. constricta* within the rivers sampled in Virginia. However, it is not straightforward to relate CPUE in timed searches to actual population densities. In the only case where this relationship was investigated for mussels, it had a very large scatter (Strayer et al. 1997).

- *Much remains to be learned about the potential for timed searches to produce repeatable, quantitative assessments of mussel populations.* Variance in CPUE statistics can arise from numerous sources, many of which have yet to be quantified. This variance can arise from day-to-day differences in search conditions and mussel behavior, differences among observers, visibility among species, and longer-term variation in efficiency of a single observer (Strayer et al. 1997). Variation from these uninvestigated sources may be substantial, and these problems will have to be addressed through further research on timed-search methodology before timed searches can be used with confidence to assess mussel populations. Adaptive sampling is a flexible probability-based design that helps allocate sampling effort to areas where mussels, i.e., *P. collina*, are present or at high density. In an adaptive design, sampling units are initially laid out using a conventional design (i.e., simple random or systematic). If the response variable (e.g., mussel density) in a sampling unit exceeds some predetermined threshold, then the investigator takes additional samples in the vicinity of this sampling unit. Adaptive sampling is easy to implement in the field and is a way to focus effort where large-scale patchiness is known and smaller-scale patchiness is unknown (Strayer and Smith 2003). Adaptive designs are described in detail by Thompson (1992) and Thompson and Seber (1996). Adaptive cluster sampling is an area of active research, and techniques to prevent excessive sampling effort are receiving attention (see Smith et al. 2003). Now that it is known where *P. collina* is present in the Dan River sub-basin, Virginia, adaptive sampling could be applied to delineate a complete distribution of this species.

RECOMMENDATIONS

1. *Surveys should be continued until the complete distribution of *P. collina* is delineated.* Any additional surveys could include completion of the other 62 random sites originally planned for this study, especially focusing on the North Carolina sites (n = 44). Based on the “6 p-h/site power analysis curve” (see Figure 3), the probability of detection then would increase to almost 100% (n = 100). If only the remaining 18 sites in Virginia are sampled, the probability of detection would be approximately 96% (n = 56). The range limits for *P. collina* have not been completely defined; therefore, it is recommended that adaptive sampling be applied to help allocate the sampling effort. Many of the sites that were surveyed possessed suitable habitat for most mussels and often had other unionids present.

2. *P. collina* should still be considered a rare and endangered species unless and until the true density is ascertained. A high density (based on estimated mean CPUE) of *P. collina* occurs in the South Fork Mayo River, a third-order tributary. However, because of the apparent patchy and clustered distribution, the Dan and Mayo river populations in North Carolina are separated by 40-km and therefore subject to reduced gene flow and the gradual loss of genetic variation. There is no potential for genetic exchange among populations due to pollution or distance barriers if fish hosts do not disperse between the two rivers. The continuity in the life history process has been disrupted (Noss and Csuti 1997). Without immigration through fish host-mediated dispersal of glochidia, no *P. collina* population may be large enough to avoid loss of genetic variability through genetic drift. This loss of genetic variation may reduce a population's ability to adapt and persist in a changing environment, and thereby reduce its viability over long time periods (Meffe and Carroll 1997). One practical way to reduce the threat of genetic drift is to promote immigration, both natural (fish host dispersal) and artificial (by captive propagation and augmentation). Defining current populations as either Evolutionarily Significant Units (ESUs) or Management Units (MUs) (as defined by Moritz 1994 and Waples 1991) can provide the mechanism to justify management strategies and recommendations.
3. *The recovery of the James spiny mussel should continue with two goals for establishing viable populations: (1) protect existing habitat and improve degraded habitat (i.e., pollution and siltation free), and (2) increase the size of each population to a level at which genetic, demographic, and normal environmental uncertainties are less likely to eliminate whole populations.*
4. *P. collina* should remain classified as endangered due to the patchiness of the distribution and uncertainty of the true population density in the Dan River sub-basin. One can infer from this study that populations of *P. collina* in the South Fork Mayo River, Virginia, are stable based on estimated mean CPUE, evidence of recent recruitment, and habitat. In addition, the populations are distributed widely enough such that it is unlikely that a single adverse event would result in the total loss of *P. collina* from the river. However, additional populations were not discovered during the simple random surveys conducted in the other rivers (i.e., Dan, Smith, and Banister).
5. *The management necessary to recover the species based on new research and insight into population viability should be outlined in detail.* Population viability analyses (PVAs) can determine extinction probabilities for *P. collina* by evaluating ways in which habitat loss, environmental uncertainty, demographic stochasticity, and genetic factors interact. Most PVAs combine field studies of important demographic parameters (see Clark and Neves 1987; Hove 1990) with simulation modeling of the possible effects of various extinction factors (Soulé 1987; Shaffer 1990). Endangered or threatened freshwater mussels frequently are restricted to a few habitat patches, but within those patches can reach high population densities. PVAs for these species will need to emphasize environmental uncertainty and catastrophic factors (Murphy et al. 1990). The population viability analyses of populations restricted to the Dan and Mayo rivers also should include genetic and demographic factors that affect small populations (Pulliam and Dunning 1997).

6. *A James spiny mussel recovery team should be assembled to determine whether populations within the Dan River sub-basin are declining or stable, perhaps by conducting population viability analyses in combination with a 5-yr period of monitoring to determine true population densities. If the populations are shown to be declining, then the agent(s) of decline should be determined and removed or neutralized. If the recovery team can confirm the cause of decline and remedy the factors, then captive propagation and augmentation of marginal populations could proceed for several years, along with monitoring, to bolster population numbers.*

COSTS AND BENEFITS ASSESSMENT

Currently the U.S. Fish and Wildlife Service requires that VDOT conduct surveys to determine the presence/absence of the James spiny mussel for any roadway project affecting the Dan or Roanoke Rivers and their tributaries. Just since 2000, a total of 59 surveys have been conducted at a total cost of approximately \$101,000. The information derived from this research project will eliminate the need to perform these surveys for future work done in these basins (exclusive of the areas where the James spiny mussel was determined to be present). In addition to the approximately \$2,000 per survey this will save, it will eliminate the common project delays of 6 to 9 months needed to perform a survey. The elimination of these delays is especially important for emergency projects.

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APPENDIX A

DISTRIBUTION OF *Pleurobema collina* IN THE JAMES RIVER BASIN

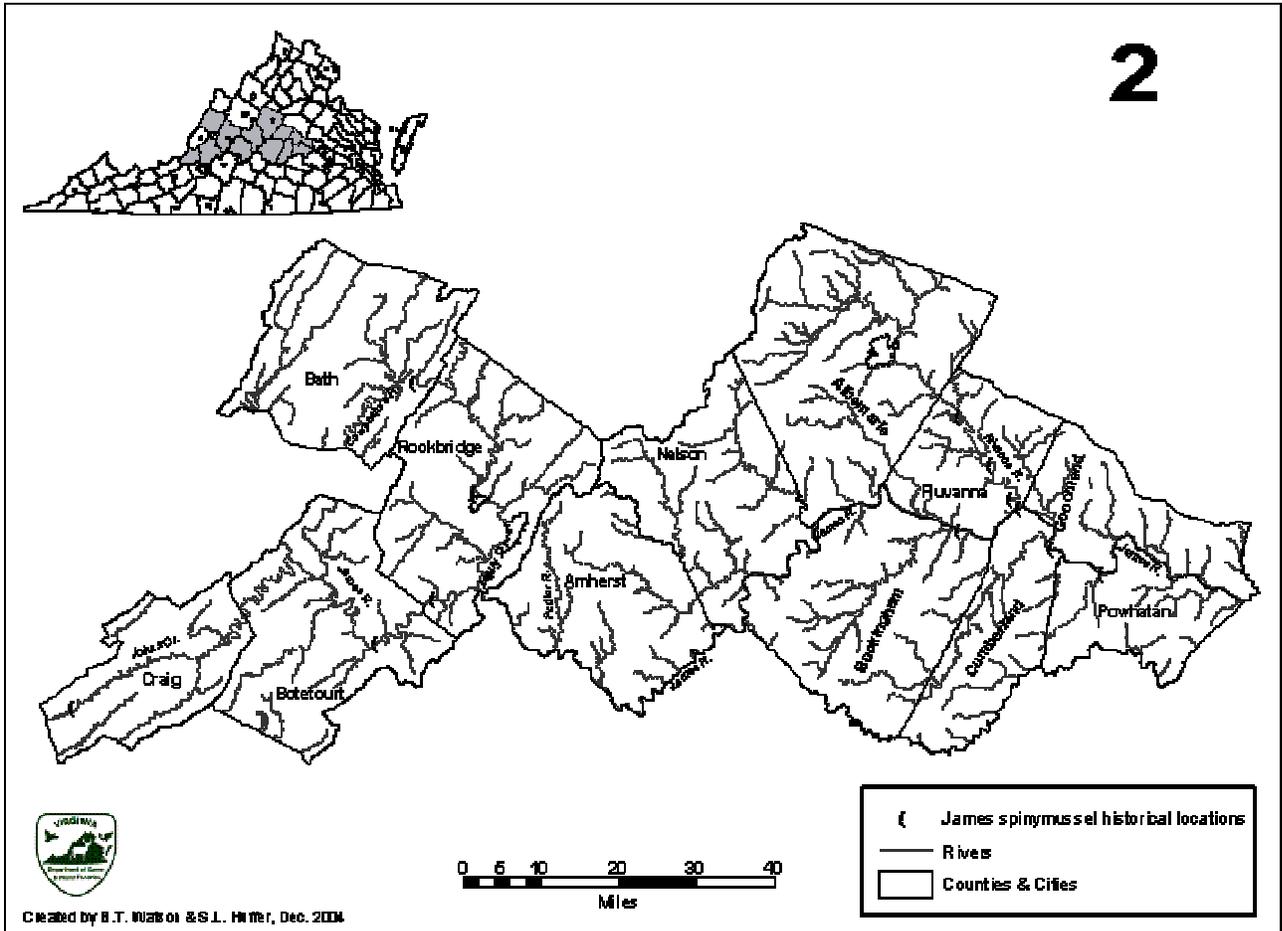


Figure A-1. Historical (e.g., 1837-1988) localities of *Pleurobema collina* in the James River basin. Waterways (County): Calpasture River type locality (Rockbridge); James River (Goochland, Powhatan, Cumberland, Buckingham, Fluvanna); Rivanna River (Fluvanna); Mill Creek (Bath); Johns Creek (Craig).

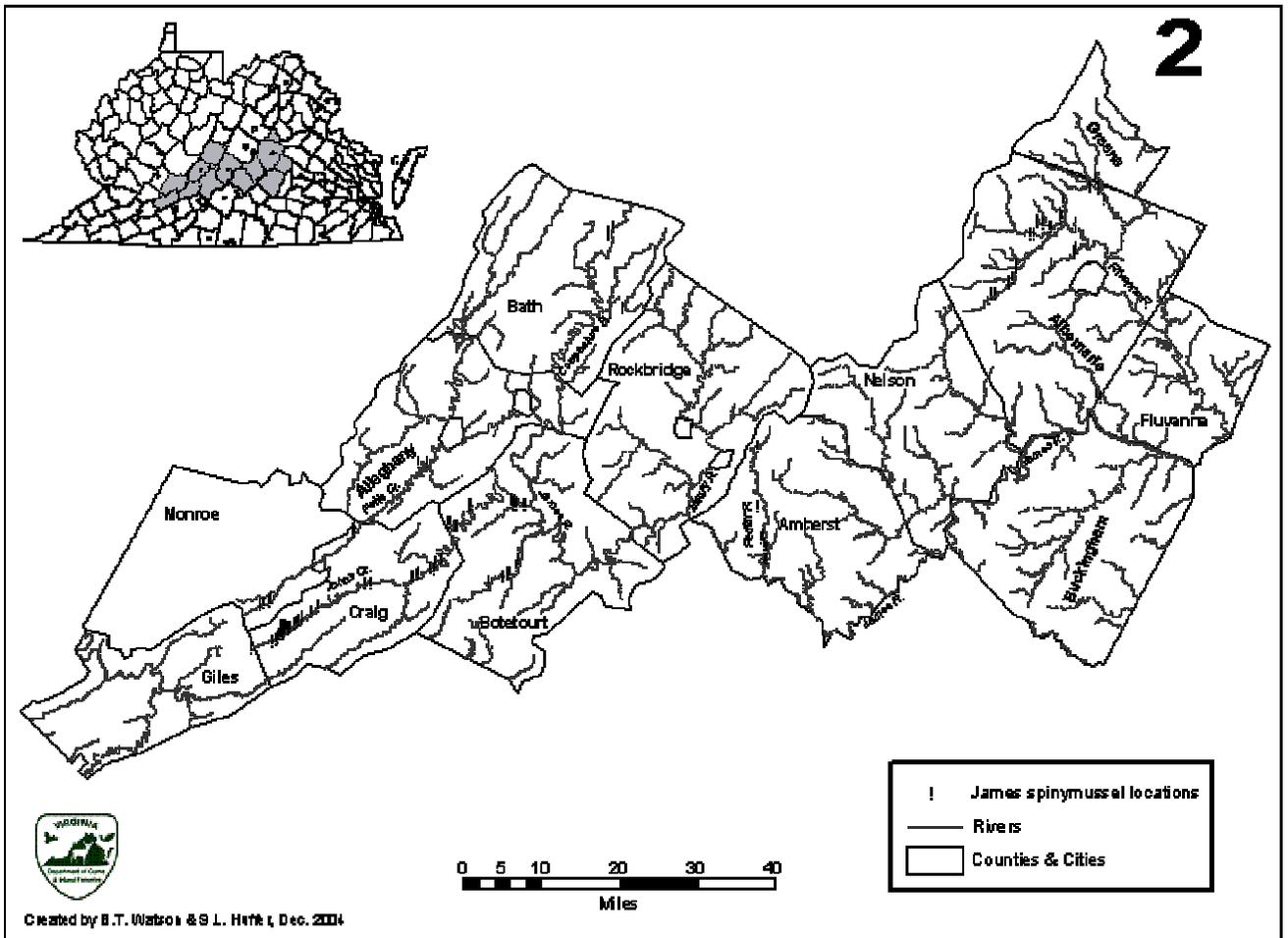


Figure A-2. Current distribution of *Pleurobema collina* in the James River basin, Virginia and West Virginia, 2004. Waterways (County): Buck Mtn. Creek (Albemarle); Catawba Creek (Botetourt); Cowpasture River (Bath); Craig Creek (Botetourt and Craig); Dicks Creek (Craig); Hardware River (Fluvanna and Albemarle); Ivy Creek (Albemarle); Johns Creek (Craig); Little Oregon Creek (Craig); North Maury River (Rockbridge); Mechums River (Albemarle); Mill Creek (Bath); Moormans River; North Fork Rivanna River (Albemarle); Patterson Creek (Botetourt); Pedlar Creek (Amherst); Potts Creek (Monroe); Piney Run; Rocky Creek (Albemarle); Rocky Run (Albemarle); South Fork Potts Creek (Monroe, WV); Swift Run (Albemarle and Greene); Wards Creek (Albemarle).

APPENDIX B

Survey Localities 2002-2004

Table B-1. Localities surveyed for *Pleurobema collina* during May through August of 2002. *Lat/Long coordinates provided by VDOT were an approximation, not an exact locality. Survey effort designated in person-hours (p-h). On average, one person searched ~100 m² over one hour per site.

Waterway	County	State	Locality or Lat/Long	Species Observed	Number of <i>P. collina</i>	Effort p-h
Smith River	Patrick	VA	Bluegrass Road, VA722, bridge #6342	0 mussels	0	2.0
Peters Creek	Patrick	VA	Five Forks Rd, VA660 bridge	0 mussels	0	2.0
Goblintown Creek	Patrick	VA	Thomas Farm Rd, VA788 bridge	0 mussels	0	2.0
Matrimony Creek	Henry	VA	N36.547 W079.8581	0 mussels	0	3.0
Leatherwood Creek	Henry	VA	I-73 bridge, *N36.6441 W079.7989	0 mussels	0	3.0
N. Fork Mayo River	Henry	VA	N36.568 W079.98575	0 mussels	0	3.5
N. Fork Mayo River	Henry	VA	N36.56593 W079.9878	0 mussels	0	4.0
N. Fork Mayo River	Henry	VA	N36.55878 W079.99001	<i>E. complanata</i> <i>V. constricta</i>	0	5.0
N. Fork Mayo River	Henry	VA	N36.5563 W079.99171	<i>V. constricta</i>	0	10.0
N. Fork Mayo River	Henry	VA	N36.55465 W079.99328	0 mussels	0	3.5
S. Fork Mayo River	Henry	VA	N36.55513 W080.020483	<i>E. complanata</i> <i>V. constricta</i>	5	4.75
S. Fork Mayo River	Henry	VA	N36.5514 W080.020533	<i>E. complanata</i> <i>V. constricta</i>	4	3.5
S. Fork Mayo River	Henry	VA	N36.54073 W080.019583	<i>E. complanata</i> <i>V. constricta</i>	2	3.5
S. Fork Mayo River	Rockingham	NC	N36.54156 W079.99253	<i>E. complanata</i> <i>V. constricta</i> <i>L. subviridus</i>	4	2.33
S. Fork Mayo River	Patrick and Henry	VA	N36.56551 W080.0521	<i>E. complanata</i> <i>V. constricta</i>	2	1.0
S. Fork Mayo River	Henry	VA	N36.55446 W080.03946	<i>V. constricta</i>	3	0.5
S. Fork Mayo River	Henry	VA	N36.555616 W080.021316	<i>E. complanata</i> <i>V. constricta</i>	11	6.0
S. Fork Mayo River	Henry	VA	N36.56528 W080.1216	<i>E. complanata</i> <i>V. constricta</i>	0	2.0
S. Fork Mayo River	Patrick	VA	N36.57093 W080.130383	<i>E. complanata</i>	0	8.0

Waterway	County	State	Locality or Lat/Long	Species Observed	Number of <i>P. collina</i>	Effort p-h
S. Fork Mayo River	Patrick	VA	N36.566 W080.0535	<i>E. complanata</i> <i>V. constricta</i>	57	6.0
S. Fork Mayo River	Patrick	VA	N36.5675 W080.054816	<i>E. complanata</i> <i>V. constricta</i>	4	1.0
S. Fork Mayo River	Patrick	VA	N36.56685 W080.0582	<i>E. complanata</i> <i>V. constricta</i>	2	1.5
S. Fork Mayo River	Patrick	VA	N36.63562 W080.26911	0 mussels	0	0.5
S. Fork Mayo River	Patrick	VA	N36.56717 W080.11321	<i>E. complanata</i> <i>V. constricta</i>	1	3.0
S. Fork Mayo River	Patrick	VA	N36.56647 W080.11311	<i>E. complanata</i> <i>V. constricta</i>	1	3.0
Dan River	Patrick	VA	VA Hwy103 canoe float to Flippin Rd, NC	<i>E. complanata</i> <i>V. constricta</i> <i>L. subviridus</i>	0	*
Dan River	Patrick	VA	VA773 bridge, Ararat Hwy	<i>E. complanata</i> <i>V. constricta</i>	0	6.0
N. Fork Mayo	Henry	VA	N36.58453 W080.0032	0 mussels	0	1.5
N. Fork Mayo	Henry	VA	N36.58433 W080.00263	0 mussels	0	1.5
N. Fork Mayo	Henry	VA	N36.58396 W080.0019	0 mussels	0	1.5
N. Fork Mayo	Henry	VA	N36.61017 W080.02903	0 mussels	0	2.25
Spoon Creek	Patrick	VA	N36.5877 W080.109483	0 mussels	0	3.0
Spoon Creek	Patrick	VA	N36.58556 W080.112933	0 mussels	0	3.0
Smith River	Henry	VA	*N36.6140 W079.7989	0 mussels	0	5.0
Middle Creek	Henry	VA	*N36.6156 W079.7661, Irisburg Rd, VA650 bridge	0 mussels	0	3.0
Fall Creek	Henry	VA	*N36.6156 W079.7661, Irisburg Rd, VA650 bridge	0 mussels	0	3.0
Little Dan River	Patrick	VA	VA Hwy 103 bridge	0 mussels	0	1.0
Little Dan River	Patrick	VA	Gammons Rd bridge	0 mussels	0	1.0

Table B-2. Simple random sampling sites (n=38 equal-area river reaches) surveyed for *Pleurobema collina* during the summers of 2003-2004 in the Banister, Dan, South Fork Mayo, and Smith rivers, Virginia. No *P. collina* was detected. *Elliptio complanata* (mean CPUE=6.08, SD=10.1) and *Villosa constricta* (mean CPUE=0.45, SD=0.89) were detected. Lat/Long coordinates reported in decimal degree format.

River	Random Site #	Rkm	Lat/Long	USGS Quad	County	Mussels Species Detected (n)	Person-Hours (CPUE)
Banister	B138	55	N 36.79423 W 079.33805	Spring Garden	Pittsylvania	<i>E. complanata</i> (13)	6 (2.2)
						<i>V. constricta</i> (2 shells)	6 (0.33)
Banister	B158	35	N 36.89183 W 079.22461	Mt. Airy	Pittsylvania	<i>E. complanata</i> (204)	6 (34.0)
						<i>V. constricta</i> (2)	6 (0.33)
Banister	B159	34	N 36.89738 W 079.22132	Mt. Airy	Pittsylvania	<i>E. complanata</i> (156)	6 (26.0)
Banister	B161	32	N 36.90101 W 079.20034	Mt. Airy	Pittsylvania	<i>E. complanata</i> (18)	6 (3.0)
Banister	B162	31	N 36.90508 W 079.19458	Mt. Airy	Pittsylvania	<i>E. complanata</i> (23)	6 (3.8)
						<i>V. constricta</i> (1 shell)	6 (0.17)
Banister	B168	25	N 36.92064 W 079.16283	Mt. Airy	Pittsylvania	<i>E. complanata</i> (67)	6 (11.2)
						<i>V. constricta</i> (7)	6 (1.2)
Banister	B185	8	N 36.84706 W 079.03135	Vernon Hill	Halifax	<i>E. complanata</i> (4)	6 (0.67)
Banister	B186	7	N 36.84078 W 079.02724	Vernon Hill	Halifax	<i>E. complanata</i> (3)	6 (0.5)
Dan	D2	279	N 36.64608 W 080.45752	Meadows of Dan	Patrick	No mussels	6
Dan	D3	278	N 36.63880 W 080.45466	Meadows of Dan	Patrick	No mussels	6
Dan	D4	277	N 36.63052 W 080.45428	Meadows of Dan	Patrick	No mussels	6

River	Random Site #	Rkm	Lat/Long	USGS Quad	County	Mussels Species Detected (n)	Person-Hours (CPUE)
Dan	D5	276	N 36.62479 W 080.44679	Claudville	Patrick	No mussels	6
Dan	D7	274	N 36.61611 W 080.44388	Claudville	Patrick	No mussels	6
Dan	D13	268	N 36.59313 W 080.44499	Claudville	Patrick	<i>E. complanata</i> (105)	6 (17.5)
						<i>V. constricta</i> (2)	6 (0.33)
Dan	D16	265	N 36.58237 W 080.44132	Claudville	Patrick	<i>E. complanata</i> (57)	6 (9.5)
						<i>V. constricta</i> (3)	6 (0.5)
Dan	D17	264	N 36.57559 W 080.44021	Claudville	Patrick	<i>E. complanata</i> (260)	6 (43.3)
						<i>V. constricta</i> (12)	6 (2.0)
Dan	D19	262	N 36.56743 W 080.43432	Claudville	Patrick	<i>E. complanata</i> (110)	6 (18.3)
						<i>V. constricta</i> (2)	6 (0.33)
Dan	D21	260	N 36.55709 W 080.43560	Claudville	Patrick	<i>E. complanata</i> (100)	6 (16.7)
						<i>V. constricta</i> (2)	6 (0.33)
Dan	D22	259	N 36.55315 W 080.42788	Claudville	Patrick	<i>E. complanata</i> (69)	6 (11.5)
						<i>V. constricta</i> (10)	6 (1.7)
Smith	S193	16	N 36.80170 W 080.21607	Charity	Patrick	No mussels	6
Smith	S199	10	N 36.82330 W 080.17704	Charity	Patrick	<i>E. complanata</i> (24)	6 (4.0)
						<i>V. constricta</i> (28)	6 (4.7)
Smith	S202	7	N 36.83644 W 080.16632	Charity	Patrick	<i>E. complanata</i> (4)	6 (0.67)
						<i>V. constricta</i> (1)	6 (0.17)
Smith	S204	5	N 36.83996 W 080.15303	Charity	Patrick	<i>E. complanata</i> (7)	6 (1.2)

River	Random Site #	Rkm	Lat/Long	USGS Quad	County	Mussels Species Detected (<i>n</i>)	Person-Hours (CPUE)
Smith	S205	4	N 36.83706 W 080.14389	Charity	Patrick	No mussels	6
Smith	S207	2	N 36.85162 W 080.14030	Charity	Patrick	No mussels	6
Smith	S208	1	N 36.84810 W 080.13043	Charity	Patrick	No mussels	6
SF Mayo	SFM 209	47	N 36.63460 W 080.27099	Stuart	Patrick	No mussels	6
SF Mayo	SFM 212	44	N 36.63249 W 080.25492	Stuart	Patrick	No mussels	6
SF Mayo	SFM 216	40	N 36.62339 W 080.23228	Nettleridge	Patrick	No mussels	6
SF Mayo	SFM 217	39	N 36.61672 W 080.22747	Nettleridge	Patrick	No mussels	6
SF Mayo	SFM 219	37	N 36.60744 W 080.20987	Nettleridge	Patrick	No mussels	6
SF Mayo	SFM 226	30	N 36.59803 W 080.16484	Nettleridge	Patrick	<i>V. constricta</i> (1)	6 (0.17)
SF Mayo	SFM 229	27	N 36.57765 W 080.15487	Nettleridge	Patrick	<i>E. complanata</i> (2)	6 (0.33)
SF Mayo	SFM 230	26	N 36.57040 W 080.14950	Nettleridge	Patrick	<i>E. complanata</i> (57)	6 (9.5)
						<i>V. constricta</i> (4)	6 (0.67)
SF Mayo	SFM 232	24	N 36.56612 W 080.13322	Nettleridge	Patrick	<i>E. complanata</i> (17)	6 (2.8)
						<i>V. constricta</i> (3)	6 (0.5)
SF Mayo	SFM 235	21	N 36.56215 W 080.12231	Spencer	Patrick	<i>E. complanata</i> (60)	6 (10.0)
						<i>V. constricta</i> (12)	6 (2.0)
SF Mayo	SFM 236	20	N 36.55821 W 080.12061	Spencer	Patrick	<i>E. complanata</i> (20)	6 (3.3)
						<i>V. constricta</i> (5)	6 (0.83)
SF	SFM		N 36.56188	Spencer	Patrick	<i>E. complanata</i> (7)	6 (1.2)

River	Random Site #	Rkm	Lat/Long	USGS Quad	County	Mussels Species Detected (n)	Person-Hours (CPUE)
Mayo	237	19	W 080.11192			<i>V. constricta</i> (6)	6 (1.0)

Table B-3. Simple random survey sites (100 equal-area reaches, 10,000 m²) generated for the Roanoke River basin study area: Dan, South Mayo, Mayo, Smith, and Banister rivers, Virginia and North Carolina. Lat/Long coordinates reported in decimal degree format.

River	Site #	Rkm	Lat/Long	USGS Quad	State	County
Banister	B138	55	N 36.79423 W 079.33805	Spring Garden	VA	Pittsylvania
Banister	B144	49	N 36.81886 W 079.31305	Spring Garden	VA	Pittsylvania
Banister	B153	40	N 36.85859 W 079.25558	Spring Garden	VA	Pittsylvania
Banister	B154	39	N 36.86161 W 079.24556	Java	VA	Pittsylvania
Banister	B158	35	N 36.89183 W 079.22461	Mt. Airy	VA	Pittsylvania
Banister	B159	34	N 36.89738 W 079.22132	Mt. Airy	VA	Pittsylvania
Banister	B161	32	N 36.90101 W 079.20034	Mt. Airy	VA	Pittsylvania
Banister	B162	31	N 36.90508 W 079.19458	Mt. Airy	VA	Pittsylvania
Banister	B166	27	N 36.91277 W 079.17244	Mt. Airy	VA	Pittsylvania
Banister	B167	26	N 36.91498 W 079.16373	Mt. Airy	VA	Pittsylvania
Banister	B168	25	N 36.92064 W 079.16283	Mt. Airy	VA	Pittsylvania
Banister	B172	21	N 36.92519 W 079.13213	Mt. Airy	VA	Pittsylvania
Banister	B175	18	N 36.90340 W 079.11597	Republican Grove	VA	Halifax
Banister	B177	16	N 36.89640 W 079.09749	Republican Grove	VA	Halifax
Banister	B179	14	N 36.88367 W 079.07955	Republican Grove	VA	Halifax
Banister	B185	8	N 36.84706 W 079.03135	Vernon Hill	VA	Halifax
Banister	B186	7	N 36.84078 W 079.02724	Vernon Hill	VA	Halifax
Dan	D2	279	N 36.64608 W 080.45752	Meadows of Dan	VA	Patrick
Dan	D3	278	N 36.63880 W 080.45466	Meadows of Dan	VA	Patrick
Dan	D4	277	N 36.63052 W 080.45428	Meadows of Dan	VA	Patrick
Dan	D5	276	N 36.62479 W 080.44679	Claudville	VA	Patrick
Dan	D7	274	N 36.61611 W 080.44388	Claudville	VA	Patrick
Dan	D13	268	N 36.59313 W 080.44499	Claudville	VA	Patrick
Dan	D16	265	N 36.58237 W 080.44132	Claudville	VA	Patrick

River	Site #	Rkm	Lat/Long	USGS Quad	State	County
Dan	D17	264	N 36.57559 W 080.44021	Claudville	VA	Patrick
Dan	D19	262	N 36.56743 W 080.43432	Claudville	VA	Patrick
Dan	D21	260	N 36.55709 W 080.43560	Claudville	VA	Patrick
Dan	D22	259	N 36.55315 W 080.42788	Claudville	VA	Patrick
Dan	D24	257	N 36.54715 W 080.42148	Claudville	NC	Stokes
Dan	D26	198	N 36.8891 W 080.11408	Ayersville	NC	Stokes
Dan	D28	196	N 36.38080 W 080.12287	Ayersville	NC	Stokes
Dan	D29	195	N 36.37348 W 080.11876	Belews Lake	NC	Stokes
Dan	D31	193	N 36.37214 W 080.12967	Walnut Cove	NC	Stokes
Dan	D34	190	N 36.35180 W 080.11659	Belews Lake	NC	Stokes
Dan	D38	186	N 36.32246 W 080.09333	Belews Lake	NC	Stokes
Dan	D41	183	N 36.30198 W 080.08739	Belews Lake	NC	Stokes
Dan	D42	182	N 36.30713 W. 080.08043	Belews Lake	NC	Stokes
Dan	D45	179	N 36.31825 W 080.05319	Belews Lake	NC	Stokes
Dan	D46	178	N 36.32198 W 080.04293	Belews Lake	NC	Stokes
Dan	D50	174	N 3634475 W 080.02565	Belews Lake	NC	Rockingham
Dan	D55	169	N 36.36354 W 080.00473	Belews Lake	NC	Rockingham
Dan	D57	167	N 36.37416 W 079.99120	Ellisboro	NC	Rockingham
Dan	D58	166	N 36.36906 W 079.98337	Ellisboro	NC	Rockingham
Dan	D63	161	N 36.38598 W 079.94331	Mayodan	NC	Rockingham
Dan	D64	160	N 36.38708 W 079.90544	Mayodan	NC	Rockingham
Dan	D65	159	N 36.39050 W 079.92570	Mayodan	NC	Rockingham
Dan	D68	156	N 36.39126 W 079.89303	Mayodan	NC	Rockingham
Dan	D71	153	N 36.40278 W 079.86520	Southwest Eden	NC	Rockingham
Dan	D73	151	N 36.41146 W 080.84746	Southwest Eden	NC	Rockingham
Dan	D78	146	N 36.42941 W 079.81308	Southwest Eden	NC	Rockingham

River	Site #	Rkm	Lat/Long	USGS Quad	State	County
Dan	D81	143	N 36.43378 W 079.78948	Southwest Eden	NC	Rockingham
Dan	D84	140	N 36.44707 W 079.81345	Southwest Eden	NC	Rockingham
Dan	D85	139	N 36.45593 W 079.81507	Southwest Eden	NC	Rockingham
Dan	D87	137	N 36.46450 W 079.79704	Southwest Eden	NC	Rockingham
Dan	D88	136	N 36.47036 W 079.78862	Southwest Eden	NC	Rockingham
Dan	D92	132	N 36.48217 W 079.75272	Southwest Eden	NC	Rockingham
Dan	D95	129	N 36.47979 W 079.72875	Southeast Eden	NC	Rockingham
Dan	D97	127	N 36.49081 W 079.71219	Southeast Eden	NC	Rockingham
Dan	D98	126	N 36.49153 W 079.70095	Southeast Eden	NC	Rockingham
Dan	D103	121	N 36.50249 W 079.65202	Northeast Eden	NC	Rockingham
Dan	D104	120	N 36.51200 W 079.65202	Northeast Eden	NC	Rockingham
Dan	D105	119	N 36.52004 W 079.64666	Northeast Eden	NC	Rockingham
Dan	D108	116	N 36.52681 W 079.62102	Brosville	NC	Rockingham
Dan	D109	115	N 36.53542 W 079.61766	Brosville	NC	Rockingham
Dan	D110	114	N 36.54080 W 079.60940	Brosville	NC	Rockingham
Dan	D111	113	N 36.54513 W 079.60015	Brosville	VA	Pittsylvania
Dan	D112	112	N 36.54774 W 079.58980	Brosville	VA	Pittsylvania
Dan	D115	109	N 36.54699 W 079.55962	Brosville	VA	Pittsylvania
Dan	D117	107	N 36.55797 W 079.54398	Brosville	VA	Pittsylvania
Dan	D119	105	N 36.56113 W 079.52919	Brosville	VA	Pittsylvania
Dan	D121	103	N 36.54884 W 079.51832	Brosville	NC	Rockingham
Dan	D124	100	N 36.53960 W 079.49888	Danville	NC	Caswell
Dan	D132	92	N 36.57897 W 079.46236	Danville	VA	Pittsylvania
Dan	D134	90	N 36.57338 W 079.44190	Danville	VA	Pittsylvania
Mayo	M238	11	N 36.45587 W 079.93724	Mayodan	NC	Rockingham
Mayo	M239	10	N 36.45063 W 079.93950	Mayodan	NC	Rockingham
Mayo	M243	6	N 36.42874 W 079.94754	Mayodan	NC	Rockingham

River	Site #	Rkm	Lat/Long	USGS Quad	State	County
Mayo	M246	3	N 36.41420 W 079.96244	Mayodan	NC	Rockingham
Smith	S193	16	N 36.80170 W 080.21607	Charity	VA	Patrick
Smith	S199	10	N 36.82330 W 080.17704	Charity	VA	Patrick
Smith	S202	7	N 36.83644 W 080.16632	Charity	VA	Patrick
Smith	S204	5	N 36.83996 W 080.15303	Charity	VA	Patrick
Smith	S205	4	N 36.83706 W 080.14389	Charity	VA	Patrick
Smith	S207	2	N 36.85162 W 080.14030	Charity	VA	Patrick
Smith	S208	1	N 36.84810 W 080.13043	Charity	VA	Patrick
SF Mayo	SFM 209	47	N 36.63460 W 080.27099	Stuart	VA	Patrick
SF Mayo	SFM 212	44	N 36.63249 W 080.25492	Stuart	VA	Patrick
SF Mayo	SFM 215	41	N 36.62867 W 080.23580	Patrick Springs	VA	Patrick
SF Mayo	SFM 216	40	N 36.62339 W 080.23228	Nettleridge	VA	Patrick
SF Mayo	SFM 217	39	N 36.61672 W 080.22747	Nettleridge	VA	Patrick
SF Mayo	SFM 219	37	N 36.60744 W 080.20987	Nettleridge	VA	Patrick
SF Mayo	SFM 224	32	N 36.59794 W 080.18340	Nettleridge	VA	Patrick
SF Mayo	SFM 226	30	N 36.59803 W 080.16484	Nettleridge	VA	Patrick
SF Mayo	SFM 229	27	N 36.57765 W 080.15487	Nettleridge	VA	Patrick
SF Mayo	SFM 230	26	N 36.57040 W 080.14950	Nettleridge	VA	Patrick
SF Mayo	SFM 232	24	N 36.56612 W 080.13322	Nettleridge	VA	Patrick
SF Mayo	SFM 235	21	N 36.56215 W 080.12231	Spencer	VA	Patrick
SF Mayo	SFM 236	20	N 36.55821 W 080.12061	Spencer	VA	Patrick
SF Mayo	SFM 237	19	N 36.56188 W 080.11192	Spencer	VA	Patrick