Distribution, Genetic Characterization, and Life History of the James spinymussel, *Pleurobema collina* (Bivalvia: Unionidae), in Virginia and North Carolina

by

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(ABSTRACT)

Three spined, mussel species occur in the United States along the Atlantic slope; James spinymussel (*Pleurobema collina*), Tar spinymussel (*Elliptio steinstansana*), and Altamaha spinymussel (*E. spinosa*). The James spinymussel was listed as endangered in 1988, and was until recently considered to be endemic to the James River basin (Clarke and Neves 1984; USFWS 1990). Biologists with the North Carolina Department of Transportation (NCDOT) discovered spinymussel populations in the Dan and Mayo rivers in NC in 2000 and 2001, respectively. The U.S. Fish & Wildlife Service (USFWS) tentatively identified this species as *Pleurobema collina*.

My project proposed by the Virginia Cooperative Fish and Wildlife Research Unit to the USFWS and the Virginia Transportation Research Council, determined where *P. collina* resides in VA and what the extent of its range is within the state. An informal preliminary survey design for *P. collina* was used during the summer of 2002 and simple random sampling was deployed in 2003-2004 surveys to provide a good basis for comparison to gauge the efficiency of the informal sampling design.

In 2002, a total of 116 person-hours were spent surveying 39 localities on the Mayo, Dan, and Smith rivers. A total of 96 *P. collina* was observed in the South Fork of the Mayo River, Patrick and Henry counties, VA. A documented range of 24 rkm was established in the South Fork Mayo River. During the summers of 2003 and 2004, a total of 228 person-hours were spent surveying 38 equal-area river reaches (10, 000 m^2) on the mainstems of the Dan, Smith, South Mayo, and Banister rivers. No specimens of *P. collina* (live or relic shells) were detected. 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 only reported after 6 person-hours of searching within each randomly selected, equal-area river reach had been expended. *P. collina* was declared absent from the VA random sites surveyed in 2003-2004 with a confidence of ~90%.

A genetic characterization of four extant populations of *P. collina* was conducted to assess its taxonomic affinity and to resolve conservation issues related to recovery planning and management actions. The populations were examined for phenotypic variation, and were characterized phylogenetically using DNA sequences. A comprehensive analysis was performed for both separate and combined mitochondrial (357 bp of *cytochrome-b*, 916 bp of *ND-1*) and nuclear (502 bp of *ITS-1*) DNA sequences. Based on comprehensive molecular, morphological, and life history data, populations of *P. collina* sampled from the Dan River sub-drainage do not warrant separate species designation from *P. collina* sampled from the James River drainage.

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CHAPTER 1

Range of *Pleurobema collina* in the Roanoke River System

ABSTRACT

Three spined, mussel species occur in the United States along the Atlantic slope; James spinymussel (*Pleurobema collina*), Tar spinymussel (*Elliptio steinstansana*), and Altamaha spinymussel (*E. spinosa*). The James spinymussel was listed as endangered in 1988, and was until recently considered to be endemic to the James River basin (Clarke and Neves 1984; USFWS 1990). Biologists with the North Carolina Department of Transportation (NCDOT) discovered spinymussel populations in the Dan and Mayo rivers in NC 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, NC, have been proposed subsequent to over 380 person-hours spent conducting surveys. The species has been 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 (Savidge 2002).

The project proposed by the Virginia Cooperative Fish and Wildlife Research Unit to the USFWS and the Virginia Transportation Research Council was to determine where *P. collina* resides in VA and what is the extent of its range within the state. The USFWS requires surveys by VDOT for this species at all roadway projects on both rivers and tributaries in VA, 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 in order to improve the future survey design. Simple random sampling was deployed in 2003-2004 surveys to provide a good basis for comparison to gauge the efficiency of the informal sampling design used.

In 2002, a total of 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, VA. A total of 96 *P. collina* was observed; mean CPUE was high at 1.5/hr. A documented range of 24 rkm was established in the South Fork Mayo River at 12 localities. During the summers of 2003 and 2004, a total of 228 person-hours were spent surveying 38 equal-area river reaches (10, 000 m^2) 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* (mean CPUE=6.08) and *Villosa constricta* (mean CPUE=0.45), were detected at almost every site surveyed. 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). 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 only reported after 6 person-hours of searching within each randomly selected, equal-area river reach had been expended. *P. collina* was declared absent from the VA random sites surveyed in 2003-2004 with a confidence of ~90%.

INTRODUCTION

Three spined mussel species occur in the United States along the Atlantic slope: James spinymussel (*Pleurobema collina*), Tar spinymussel (*Elliptio steinstansana*), and Altamaha spinymussel (*E. spinosa*). Both the James spinymussel and the Tar spinymussel are federally endangered. The James spinymussel (*P. collina*) was listed as endangered in 1988, and was until recently considered to be endemic to the James River basin (Figure 1) (Clarke and Neves 1984; 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 (VDEQ 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 & Neves 1984; Hove 1990; USFWS 1990).

Newly-discovered distribution in the Dan River sub-basin

Biologists with the North Carolina Department of Transportation (NCDOT) discovered spinymussel populations in the Dan and Mayo rivers in NC in 2000 and 2001, respectively. The U.S. Fish & Wildlife Service (USFWS) tentatively identified this

species as the James spinymussel (*Pleurobema collina*). These streams are part of the Dan River sub-basin of the Roanoke River system, which flows into Albemarle Sound in northeastern NC.

The project was proposed by the Virginia Cooperative Fish and Wildlife Research Unit to the U.S. Fish and Wildlife Service and the Virginia Transportation Research Council. My thesis research objective was to determine where *P. collina* resides in VA and the extent of its range within the state. The VA 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 and State University (Virginia Tech) during the summer of 2002 revealed the presence of the same species of spinymussel in the South Fork Mayo River, Patrick and Henry counties, VA. The South Fork Mayo empties into the Mayo River, a major tributary of the Dan River.

The USFWS requires surveys by VDOT for this species at all roadway projects on both rivers and their tributaries in VA, 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. In the following two sections, I briefly review recent survey work conducted to better define the overall range of *P. collina* in VA and NC.

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) (Figure 2). 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 spinymussel (*P. collina*) in the Dan River in October 2000, NCDOT coordinated an extensive multi-agency survey of the Dan River sub-basin. Participants include the USFWS, Catena Consulting Group, North Carolina Wildlife Resources Commission (NCWRC), North Carolina State University, North Carolina Natural Heritage Program, and Virginia Tech. Survey efforts have concentrated in Stokes, Rockingham, and Caswell counties, NC. Over 380 person-hours were spent surveying the Dan River and its tributaries. In addition to the mainstem of the Dan River, the James spinymussel also 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, NC, have been proposed. The species

has been found in (1) a 57-km reach of the Dan River and (2) 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 three times before the species was detected. The current distribution in the Dan River extends from below the NC/VA border, near the first bridge crossing in NC (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 3). The species was not found in the reach between the SR 103 crossing in Patrick County, VA, and SR 1416 in NC (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 NC. Survey work will continue above and below the documented range (see above) in NC to establish the actual distribution of this species in this drainage (Savidge 2002).

In the upper part of the documented range, *P. collina* is extremely rare, and is known from only one individual. A small impoundment at Jessups Mill, located on the Dan River just above NC 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 & Warren 1998; Vaughn & Taylor 1999). Because of its small size (~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 & Taylor 1999; Kelner & 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 NC 704 and NC 89.

A distribution of 19 rkm in the Mayo River, NC, also has been established for *P. collina* (Figure 3), from the NC/VA border to just downstream of SR 770 in northwest Rockingham County, NC. 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 & Gasper 1996; Goudreau *et al.* 1998; Brown *et al.* 1998; Vaughn & Taylor 1999). The species has been found in a short reach (~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).

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 $CaCO_3$ 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*. 1996). 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).

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 & 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 area 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).

I conducted surveys in 2002-2004 to determine whether *P. collina* occurs in the VA portion of the Dan River sub-basin and what is the extent of its range within the sub-basin. VDOT requires surveys for this endangered species at all roadway projects on both rivers and tributaries in VA, 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, VA, a major tributary of the Dan River. Because good survey design is based on knowledge of the target population and site characteristics, my goal was to use the estimated mean CPUE (i.e., sampling effort defined as the encounter rate) from the informal survey design to improve 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 & 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 & 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 & 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 & 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 VA (i.e., 64% of the total watershed area). The VA 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 VA is the state line. The headwaters begin in the mountains of eastern Montgomery County and flow southeast to the VA/NC state line. In VA, 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 VA, 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 NC 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 4). Over 62% of the basin is forested, nearly 25% is in cropland and pasture, and approximately 10% is urban. The human population in the VA portion of the Roanoke River basin in 1994 was ~669,681 (VADEQ 2002).

The NC 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 NC in the eastern section. The Roanoke River mainstem enters Kerr and Gaston lakes in NC and then flows into Roanoke Rapids Lake before regaining its riverine form and flowing into Albermarle Sound. The entire Roanoke River watershed is approximately 25, 035 square kilometers, with about 7,770 square kilometers in NC (i.e., 16% of total watershed area). Flow in the Roanoke River in NC is regulated by the operation of Kerr Reservoir and Lake Gaston (NCDWQ 2001).

Based on 1990 census data, the NC 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).

Dan River sub-basin in the Roanoke River

The Dan River arises in the uplands of the Blue Ridge Province in Patrick County, VA and flows south through the Blue Ridge Escarpment before crossing into NC 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, NC. The river flows into southern Pittsylvania County, VA, back into Rockingham County, NC, east into Caswell County, NC, then north back into Pittsylvania County, VA. The river then flows east through the city of Danville, turns to the south and re-enters NC in north-central Caswell County. It flows east before turning back to the north, re-entering VA, 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 km^2 (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 21tons/0.4 hectare/yr have been documented for cultivated cropland in the upper Dan River (NRCS 1992). The upper Dan River is classified as trout waters, and part of the area is also designated a State Water Trail by the NC 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

I designed and conducted preliminary survey work in the mainstem and major tributaries of the Dan, Mayo, and Smith rivers in VA, 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 NC/VA border and progressing north in these rivers. Sites were qualitatively surveyed for presence of mussels in a minimum of 200 m or 3 person-hours (arbitrary threshold) by at least 3 experienced biologists. On average, one person

searched ~100 m^2 over one 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. 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 measured 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, VA

I designed and conducted survey work in the mainstem and major tributaries of the Dan, Mayo, Smith, and Banister rivers, VA, in 2003 and 2004. Survey efforts were focused in Patrick, Henry, Pittslyvania and Halifax counties, where these drainages occur. To more objectively define distribution (i.e., occurrence) and abundance, I followed a probability-based sampling design (Strayer and Smith 2003) to assess the

range of *P. collina* across the Roanoke drainage. I used a simple random survey design 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, I set the grain size as a 1 km river reach with 10, 000 m^2 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* person-hours 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),

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

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 person-hours), timed searches. Green and Young (1993) state that any species having true density > 0.1 per sample unit size is *not* rare. Therefore, I used Green and Young's (1993) probablility of detection formula and extremely conservative estimates of CPUE (i.e., 0.01, 0.05, and 0.1), 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 $1 - \beta$ 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 (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 VA and NC 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 VA and NC (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 m^2) by assigning each reach an identification number referenced by 1 km reaches (N = 249 total rkm). The 89 rkm in VA and NC with known 'presence' of *P. collina* were not included. Using results from the power curve analysis (see Figure 5), one hundred reaches (n = 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, I had to modify sample size (n). The VA random survey sites (n = 56 rkm) became the main focus of study with the probability of detection still high at 96%. I conducted 2-hr timed searches with at least 3 biologists (total of 6 person-hours) on each reach (10,000 m^2 per rkm).

CPUE data are sensitive to sampling conditions and workers' skill; therefore, I minimized these effects by sampling at low flow, when water was clear, and by deploying experienced field crews. At least 2 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 person-hours of searching (Metcalfe-Smith *et al.* 2000; Lellis 2001). Methods for detecting mussels were visual searches by observers wearing mask and snorkel or viewscopes.

Simple random sampling with 3 equi-distant starting points in relation to each river (based on 3 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. I included multiple equi-distant starts 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 'zig-zag' search pattern to maximize chances of finding mussels (Figure 6). 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 & Downing 1991; Balfour & 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 spinymussel and other species. I provided a geographic description of occurrence and range per tributary and mainstem 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 (CMI) at Virginia Tech.

RESULTS

Informal preliminary survey in the Roanoke River drainage, Virginia

In 2002, a total of 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, VA, confirming its occurrence near the NC/VA 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 1-2 individuals were found per every hour assuming that one person searched ~100 m^2 over one hour per site. Thus, by Green and Young's (1993) definition of rare (<0.1/m^2), *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 VA or NC. Consequently, if the distribution was restricted to habitat patches and clustered in the South Fork Mayo River, then *P. collina* may still be rare over the whole state of VA, and thus, the United States. A range of 24 km was documented in the South Fork Mayo River at 12 localities (Figure 7). Localities surveyed in VA, dates, and species composition are listed in Table 1.

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 (~50-200 m) in most reaches.

Simple random survey in the Roanoke River drainage, Virginia

During the summers of 2003 and 2004, 228 person-hours were spent surveying 38 equal-area river reaches (10, 000 m^2) on the mainstems of the Dan, Smith, South Mayo, and Banister rivers (Figure 8; red 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 2). 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) (Figures 9 and 10).

Due to unfavorable river conditions, n = 100 or 56 planned surveys were not completed. However, n = 38-km sites were completed with ~90% probability of detecting one mussel in 6 p-h of searching, assuming the most conservative estimate of

CPUE = 0.01/m^2 (Figure 5). 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, NC; South Mayo River near Nettleridge, VA; and Banister River at Halifax, VA; 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.

Of the 56-km sites that were randomly selected for survey in VA in the Dan, South Fork Mayo, Smith, and Banister rivers, *P. collina* was not detected despite 228 person-hours of sampling effort over 38-km sites. Negative results were reported only after 6 person-hours of searching within each randomly selected, equal-area river reach (10,000 m^2) had been expended. I can state with reasonable confidence (~90%) that *P. collina* is absent from the remaining 18 random sites in VA (Table 3, Dan and Banister rivers; Figure 5).

Informal sampling was most useful in preliminary surveys for determining the presence, but not absence, of *P. collina* in the VA 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 I 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.

DISCUSSION

The informal surveys conducted in 2002 indicated that *P. collina* has a wide and apparent patchy distribution in the Dan River sub-basin of VA. This species was detected throughout a 24-km reach of the South Fork Mayo River, VA, in summer 2002, after using informal sampling, based on the discovery of this species in the Dan and Mayo Rivers in NC, 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.

Visual searches for mussels, whether the surveyor is wading or snorkeling, can cover a large area (500—5000 m^2/hr) and result in high "catch" rates for exposed mussels (sometimes 100s to 1000s of mussels/hr—e.g., Huehner and Corr 1994; Strayer *et al*.

1997; Hoggarth *et al*. 2000). As a result, visual search using snorkeling was an effective method for detecting the presence of this rare species (Strayer and Smith 2003). Of course, all visual searching methods are nullified by poor visibility in turbid or deep water and will not be useful if a large part of the target population is completely burrowed.

The main shortcoming of the informal sampling method was the inability to assess sampling error, even though results were affected by substantial uncertainty. Thus, abundance was biased, and a standard error (or confidence interval) for an estimate of abundance could not be calculated. Also, there was no way to decide whether an observed difference was a result of sampling error, variation in the proportion detected, or a difference in true abundance when comparing counts across place, time, or taxa. Therefore, interpretation of the results should be limited to a list of species present and estimated mean CPUE, when the survey design involves informal sampling. The completeness of that list depends on spatial coverage and effort (Strayer and Smith 2003).

I estimated the presence-absence (~90% probability of detection) of the endangered *P. collina* using probability-based sampling in 2003-2004, in order 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, I can apply mean CPUE data to provide estimates of population densities of *E. complanata* and *V. constricta* within the rivers sampled in VA. 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).

The important advantage of using probability-based sampling was that sampling error was measured by the standard deviation (Table 2) and used to gauge the reliability of an estimated CPUE for *E. complanata* and *V. constricta*. *E. complanata* was more variable (SD=10.1) from one sample to the next when compared to *V. constricta* (SD=0.89). Accounting for sampling error was integral to rigorous statistical inference and allowed uncertainty to be incorporated into my mean CPUEs. My survey results applied only to mussels at the substrate surface, since excavation was not done, and the results were likely confounded by variability in the proportion at the surface (Strayer and Smith 2003).

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 we have confidence that timed searches can be used rigorously 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 & Smith 2003). Adaptive designs are described in detail by Thompson (1992) and Thompson & 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 we know where *P. collina* is present in the Dan River sub-basin, VA, adaptive sampling could be applied to delineate a complete distribution of this species.

Conclusion and management implications

Given that *P. collina* was not detected during the formal simple random surveys of 2003-2004, I can say with ~90% confidence that it was absent from the Dan, Smith, and Banister rivers in VA. Any additional surveys could include completion of the other 62 random sites originally planned for this study, especially focusing on the NC sites (n = 44). Based on the '6 p-h/site power analysis curve' (see Figure 5), the probability of detection then would increase to almost 100% (n = 100). If only the remaining 18 sites in VA are sampled, the probability of detection would be ~96% (n = 56). The range limits for *P. collina* have not been completely defined; therefore, it is recommended that surveys continue until the complete distribution is delineated. Adaptive sampling could be applied to help allocate sampling effort. Many of the sites that were surveyed possessed suitable habitat for most mussels and often had other unionids present.

The distribution of *P. collina* is more widespread than previously recognized (Clarke and Neves 1984; Hove 1990). Prior to my 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, VA and NC in 2000-2002.

The range extension of *P. collina* includes a 57-km reach of the Dan River, Stokes and Rockingham counties, NC, which is separated from the 19-km distribution in the Mayo River, Rockingham County, NC. There is approximately 40 rkm separating the downstream extent of *P. collina* in the Dan and Mayo rivers (Savidge 2002). The distribution I documented of *P. collina* in the Mayo River continues upstream into VA, 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 NC and VA. A large, extensive falls area (height ~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.

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, *P. collina* should still be considered a rare and endangered species unless and until we ascertain the true density. The Dan and Mayo river populations in NC 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. Molecular genetic data (i.e., DNA sequencing) needed to define populations as ESUs or MUs are described in detail at length in Chapter 2.

It is imperative that the recovery of the James spinymussel 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.

I can infer from my study that populations of *P. collina* in the South Fork Mayo River, VA, 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). At this time, I recommend that *P. collina* remain classified as endangered due to the patchiness of the distribution and uncertainty of the true population density in the Dan River sub-basin.

It is essential to outline in detail the management necessary to recover the species based on new research and insight into population viability. 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 & 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).

A James spinymussel 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.

LITERATURE CITED

Amyot, J.-P., and J.A. Downing. 1991. Endo- and epibenthic distribution of the unionid mollusk *Elliptio complanata*. Journal of the North American Benthological Society 10:280-285.

Balfour, D.L., and L.A. Smock. 1995. Distribution, age structure, and movements of the freshwater mussel *Elliptio complanata* (Mollusca: Unionidae) in a headwater stream. Journal of Freshwater Ecology 10:255-268.

Boss, K.J., and W.J. Clench. 1967. Notes on *Pleurobema collina* (Conrad) from the James River, VA. Occasional Papers on Mollusks, Museum of Comparative Zoology 3(37): 45-52.

Brown, A.V., M.M. Lyttle, and K.B. Brown. 1998. Impacts of gravel mining on gravel bed streams. Transactions of the American Fisheries Society 127:979-994.

Clarke, A.H., and R.J. Neves. 1984. Status survey of the James River spinymussel, *Canthyria collina*, in the James River, Virginia. Final Report, U.S. Fish and Wildlife Service, Newton Corner, MA. 73 pp.

Conrad, T.A. 1837. Monograph of the family Unionidae 8: 65.

Downing, J.A., J.-P. Amyot, M. Pérusse, and Y. Rochon. 1989. Visceral sex, hermaphroditism, and protandry in a population of the freshwater bivalve *Elliptio complanata*. Journal of the North American Benthological Society 8:92-99.

Downing, J.A., and W.L. Downing. 1992. Spatial aggregation, precision, and power in surveys of freshwater mussel populations. Canadian Journal of Fisheries and Aquatic Sciences 49:985-991.

Goudreau, S.E., R.J. Neves, and R.J. Sheehan. 1998. Effects of sewage treatment effluents on mollusks and fish of the Clinch River in Tazewell County, Virginia. Final Report, U.S. Fish and Wildlife Service. 128 pp.

Green, R.H., and R.C. Young. 1993. Sampling to detect rare species. Ecological Applications 3: 351-356.

Haag, W.R., and M.L. Warren. 1998. The role of ecological factors and reproductive strategies in structuring freshwater mussel communities. Canadian Journal of Fisheries and Aquatic Sciences 55:297-306.

Hoggarth, M.A., D.L. Rice, and T.L. Grove. 2000. The correlation of mussels with fish in the upper Blanchard River in Hardin and Hancock counties, Ohio, with special regard to the rayed bean (*Villosa fabalis*). Pages 19-26 *in*: R.A. Tankersley, D.I. Warmolts, G.T. Watters, and B.J. Armitage (editors). Proceedings of the Conservation, Captive Care, and Propagation of Freshwater Mussels Symposium, Ohio Biological Survey, Columbus, OH.

Hornbach, D.J., and T. Deneka. 1996. A comparison of a qualitative and a quantitative collection method for examining freshwater mussel assemblages. Journal of the North American Benthological Society 15:587-596.

Hove, M. 1990. Distribution and life history of the endangered James spinymussel, *Pleurobema collina* (Bivalvia: Unionidae). M.S. Thesis. Virginia Polytechnic Institution and State University, Blacksburg, Virginia. 113 pp.

Huehner, M.K., and C.L. Corr. 1994. The unionid mussel fauna of Pymatuning Creek in Ashtabula County, Ohio, including the federally endangered clubshell (*Pleurobema clava*). Ohio Journal of Science 94: 147-150.

Kelner, D.E., and B.E. Sietman. 2000. Relic populations of the ebony shell, *Fusconaia ebena* (Bivalvia: Unionidae), in the upper Mississippi River drainage. Journal of Freshwater Ecology 15:371-377.

Kondolf, G.M. 1997. Hungry water: effects of dams and gravel mining on river channels. Environmental Management 21:533-551.

Lellis, W.A. 2001. Freshwater mussel survey of the Upper Delaware Scenic and Recreational River: qualitative survey 2000. Final Report to the National Park Service.

Master, L.L., B.A. Stein, L.S. Kutner, and G.A. Hammerson. 2000. Vanishing assets: conservation status of U.S. species. Pages 93-118 *in* B.A. Stein, L.S. Kutner, and J.S. Adams, editors. Precious heritage: the status of biodiversity in the United States. Oxford University Press, NY.

Meffe, G.K., and R.C. Carroll. 1997. Genetics: conservation of diversity within species. Page 176 *in* G.K. Meffe and R.C. Carroll (eds.). *Principles of Conservation Biology*. Sinauer Associates, Inc., Sunderland, MA.

Metcalfe-Smith, J.L., J. Di Maio, S.K. Staton, and G.L. Mackie. 2000. Effect of sampling effort on the efficiency of the timed search method for sampling freshwater mussel communities. Journal of the North American Benthological Society 19: 725-732.

Moritz, C. 1994. Defining 'Evolutionary Significant Units' for conservation. Trends in Ecology and Evolution 9: 373-375.

Murphy, D.D., K.E. Freas and S.B. Weiss. 1990. An environment-metapopulation approach to population viability analysis for a threatened invertebrate. Conservation Biology 4:41-51.

North Carolina Division of Water Quality. 2001. Roanoke River basinwide water quality plan. Water Quality Section, Raleigh, NC. 181 pp.

Noss, R.F., and B. Csuti. 1997. Habitat fragmentation. Pages 269, 289-290 *in* G.K. Meffe and R.C. Carroll (eds.). *Principles of Conservation Biology*. Sinauer Associates, Inc., Sunderland, MA.

Obermeyer, B.K. 1998. A comparison of quadrats versus timed snorkel searches for assessing freshwater mussels. American Midland Naturalist 139:331-339.

Pulliam, H.R., and J.B. Dunning. 1997. Demographic processes: population dynamics on heterogeneous landscapes. Pages 215-220 *in* G.K. Meffe and R.C. Carroll (eds.). *Principles of Conservation Biology*. Sinauer Associates, Inc., Sunderland, MA.

Rohde, F.C., R.G. Arndt, and S.M. Smith. 2001. Longitudinal succession of fishes in the Dan River in Virginia and North Carolina (Blue Ridge/Piedmont Provinces). Southeastern Fishes Council Proceedings 42: 1-13.

SAS Institute. 2001. SAS user's guide: Statistics. Statistical Analysis System Institute, Cary, NC.

Savidge, T.W. 2002. Programmatic Section 7 consultation for the James spinymussel (*Pleurobema collina*) bridge replacement projects on the Dan River (B-2639 and B-3045). North Carolina Department of Transportation Environmental Analysis Branch for Federal Highway Administration, Raleigh, NC. 56 pp.

Shaffer, M.L. 1990. Population viability analysis. Conservation Biology 4:39-40.

Smith, D.R., R.F. Villella, and D.P. Lemarié. 2003. Application of adaptive cluster sampling to low-density populations of freshwater mussels. Environmental and Ecological Statistics 10:7-15.

Soulé, M.E. (ed.). 1987. Viable populations for conservation. Cambridge University Press, NY.

Starnes, L.B., and D.C. Gasper. 1996. Effects of surface mining on aquatic resources in North America. Fisheries 21(5):24-26.

Strayer, D.L. 1999. The statistical power of presence-absence data to detect population declines. Conservation Biology 13: 1034-1038

Strayer, D.L., S. Claypool, and S. Sprague. 1997. Assessing unionid populations with quadrats and timed searches. Pages 163-169 *in*: K.S. Cummings, A.C. Buchanan, C.A. Mayer, and T.J. Naimo (editors). Conservation and management of freshwater mussels II. Initiatives for the future. Upper Mississippi River Conservation Committee, Rock Island, IL.

Strayer, D.L., and D.R. Smith. 2003. A guide to sampling freshwater mussel populations. American Fisheries Society Monograph 8. American Fisheries Society, Bethesda, MD. 157 pp.

Strayer, D.L., S. Sprague, and S. Claypool. 1996. A range-wide assessment of populations of *Alasmidonta heterodon*, an endangered freshwater mussel (Bivalvia: Unionidae). Journal of the North American Benthological Society 15: 308-317.

Thompson, S.K. 1992. Sampling. Wiley, NY.

Thompson, S.K., and G.A.F. Seber. 1996. Adaptive sampling. Wiley, NY.

Turner, M.G., R.H. Gardner, and R.V. O'Neill. 2001. Landscape ecology in theory and practice: pattern and process. Springer-Verlag.

United States Fish and Wildlife Service. 1990. James spinymussel (*Pleurobema collina*) Recovery Plan. Newton Corner, MA. 38 pp.

Vaughn, C.C. 1997. Regional patterns of mussel species distributions in North American rivers. Ecography 20:107-115.

Vaughn, C.C, C.M. Taylor, and K.J. Eberhard. 1997. A comparison of the effectiveness of timed searches vs. quadrat sampling in mussel surveys. Pp. 157-162 *in* K.S. Cummings, A.C. Buchanan, C.A. Mayer, and T.J. Naimo, eds. Conservation and management of freshwater mussels II. Initiatives for the future. Upper Mississippi River Conservation Committee, Rock Island, IL.

Vaughn, C.C., and C.M. Taylor. 1999. Impoundments and the decline of freshwater mussels: a case study of an extinction gradient. Conservation Biology 13:912-920.

Virginia Department of Environmental Quality (VDEQ). 2002. Water quality assessment report 305(b) and report on impaired waters 303(d). Virginia Department of Environmental Quality, Richmond, VA.

Waples, R.S. 1991. Pacific salmon, *Oncorhynchus* spp., and the definition of "species" under the Endangered Species Act. U.S. National Marine Fisheries Service Marine Fisheries Review 53: 11-22.

Watters, G.T. 1995. Small dams as barriers to freshwater mussels (Bivalvia, Unionoida) and their hosts. Biological Conservation 75:79-85.

Watters, G.T. 1992. Unionids, fishes, and the species-area curve. Journal of Biogeography 19:481-490.

Williams, J.D., M.L. Warren, Jr., K.S. Cummings, J.L. Harris, and R.J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18:6-22.

Table 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 m^2 over one hour per site.

Waterway	County	State	Locality or Lat/Long	Species Observed	Number of *P. collina*	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	*E. complanata V. constricta*	0	5.0
N. Fork Mayo River	Henry	VA	N36.5563 W079.99171	*V. constricta*	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	*E. complanata V. constricta*	**5**	4.75
S. Fork Mayo River	Henry	VA	N36.5514 W080.020533	*E. complanata V. constricta*	**4**	3.5
S. Fork Mayo River	Henry	VA	N36.54073 W080.019583	*E. complanata V. constricta*	**2**	3.5

Table 1. Continuation.

Waterway	County	State	Locality or Lat/Long	Species Observed	Number of *P. collina*	Effort p-h
S. Fork Mayo River	Rockingham	NC	N36.54156 W079.99253	*E. complanata* *V. constricta* *L. subviridus*	**4**	2.33
S. Fork Mayo River	Patrick and Henry	VA	N36.56551 W080.0521	*E. complanata* *V. constricta*	**2**	1.0
S. Fork Mayo River	Henry	VA	N36.55446 W080.03946	*V. constricta*	**3**	0.5
S. Fork Mayo River	Henry	VA	N36.555616 W080.021316	*E. complanata* *V. constricta*	**11**	6.0
S. Fork Mayo River	Henry	VA	N36.56528 W080.1216	*E. complanata* *V. constricta*	0	2.0
S. Fork Mayo River	Patrick	VA	N36.57093 W080.130383	*E. complanata*	0	8.0
S. Fork Mayo River	Patrick	VA	N36.566 W080.0535	*E. complanata* *V. constricta*	**57**	6.0
S. Fork Mayo River	Patrick	VA	N36.5675 W080.054816	*E. complanata* *V. constricta*	**4**	1.0
S. Fork Mayo River	Patrick	VA	N36.56685 W080.0582	*E. complanata* *V. constricta*	**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	*E. complanata* *V. constricta*	**1**	3.0
S. Fork Mayo River	Patrick	VA	N36.56647 W080.11311	*E. complanata* *V. constricta*	**1**	3.0

Table 1. Continuation.

Waterway	County	State	Locality or Lat/Long	Species Observed	Number of *P. collina*	Effort p-h
Dan River	Patrick	VA	VA Hwy103 canoe float to Flippin Rd, NC	*E. complanata* *V. constricta* *L. subviridus*	0	*
Dan River	Patrick	VA	VA773 bridge, Ararat Hwy	*E. complanata* *V. constricta*	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 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, VA. 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	*E. complanata* (13)	6 (2.2)
						V. constricta (2 shells)	6 (0.33)
Banister	B158	35	N 36.89183 W 079.22461	Mt. Airy	Pittsylvania	*E. complanata* (204)	6 (34.0)
						V. constricta (2)	6 (0.33)
Banister	B159	34	N 36.89738 W 079.22132	Mt. Airy	Pittsylvania	*E. complanata* (156)	6 (26.0)
Banister	B161	32	N 36.90101 W 079.20034	Mt. Airy	Pittsylvania	*E. complanata* (18)	6 (3.0)
Banister	B162	31	N 36.90508 W 079.19458	Mt. Airy	Pittsylvania	*E. complanata* (23)	6 (3.8)
						V. constricta (1 shell)	6 (0.17)

Table 2. Continuation.

River	Random Site #	Rkm	Lat/Long	USGS Quad	County	Mussels Species Detected (*n*)	Person-Hours (CPUE)
Banister	B168	25	N 36.92064 W 079.16283	Mt. Airy	Pittsylvania	*E. complanata* (67)	6 (11.2)
						V. constricta (7)	6 (1.2)
Banister	B185	8	N 36.84706 W 079.03135	Vernon Hill	Halifax	*E. complanata* (4)	6 (0.67)
Banister	B186	7	N 36.84078 W 079.02724	Vernon Hill	Halifax	*E. complanata* (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
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	*E. complanata* (105)	6 (17.5)
						V. constricta (2)	6 (0.33)
Dan	D16	265	N 36.58237 W 080.44132	Claudville	Patrick	*E. complanata* (57)	6 (9.5)
						V. constricta (3)	6 (0.5)

Table 2. Continuation.

River	Random Site #	Rkm	Lat/Long	USGS Quad	County	Mussels Species Detected (*n*)	Person-Hours (CPUE)
Dan	D17	264	N 36.57559 W 080.44021	Claudville	Patrick	*E. complanata* (260)	6 (43.3)
						V. constricta (12)	6 (2.0)
Dan	D19	262	N 36.56743 W 080.43432	Claudville	Patrick	*E. complanata* (110)	6 (18.3)
						V. constricta (2)	6 (0.33)
Dan	D21	260	N 36.55709 W 080.43560	Claudville	Patrick	*E. complanata* (100)	6 (16.7)
						V. constricta (2)	6 (0.33)
Dan	D22	259	N 36.55315 W 080.42788	Claudville	Patrick	*E. complanata* (69)	6 (11.5)
						V. constricta (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	*E. complanata* (24)	6 (4.0)
						V. constricta (28)	6 (4.7)
Smith	S202	7	N 36.83644 W 080.16632	Charity	Patrick	*E. complanata* (4)	6 (0.67)
						V. constricta (1)	6 (0.17)
Smith	S204	5	N 36.83996 W 080.15303	Charity	Patrick	*E. complanata* (7)	6 (1.2)

Table 2. Continuation.

River	Random Site #	Rkm	Lat/Long	USGS Quad	County	Mussels Species Detected (*n*)	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	*V. constricta* (1)	6 (0.17)
SF Mayo	SFM 229	27	N 36.57765 W 080.15487	Nettleridge	Patrick	*E. complanata* (2)	6 (0.33)
SF Mayo	SFM 230	26	N 36.57040 W 080.14950	Nettleridge	Patrick	*E. complanata* (57)	6 (9.5)
						V. constricta (4)	6 (0.67)

Table 2. Continuation.

River	Random Site #	Rkm	Lat/Long	USGS Quad	County	Mussels Species Detected (*n*)	Person-Hours (CPUE)
SF Mayo	SFM 232	24	N 36.56612 W 080.13322	Nettleridge	Patrick	*E. complanata* (17)	6 (2.8)
						V. constricta (3)	6 (0.5)
SF Mayo	SFM 235	21	N 36.56215 W 080.12231	Spencer	Patrick	*E. complanata* (60)	6 (10.0)
						V. constricta (12)	6 (2.0)
SF Mayo	SFM 236	20	N 36.55821 W 080.12061	Spencer	Patrick	*E. complanata* (20)	6 (3.3)
						V. constricta (5)	6 (0.83)
SF Mayo	SFM 237	19	N 36.56188 W 080.11192	Spencer	Patrick	*E. complanata* (7)	6 (1.2)
						V. constricta (6)	6 (1.0)

Table 3. Simple random survey sites (100 equal-area reaches, 10,000 m^2) generated for the Roanoke River basin study area: Dan, South Mayo, Mayo, Smith, and Banister rivers, VA and NC. 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

Table 3. Continuation.

River	Site #	Rkm	Lat/Long	USGS Quad	State	County
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
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

Table 3. Continuation.

River	Site #	Rkm	Lat/Long	USGS Quad	State	County
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 07986520	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
Dan	D81	143	N 36.43378 W 07978948	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

Table 3. Continuation.

River	Site #	Rkm	Lat/Long	USGS Quad	State	County
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
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

Table 3. Continuation.

River	Site #	Rkm	Lat/Long	USGS Quad	State	County
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

Figure Headings

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

Figure 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).

Figure 3. Current distribution of *Pleurobema collina* in the Dan River sub-basin, Patrick and Henry counties, Virginia, and Stokes and Rockingham counties, North Carolina. Green line denotes North Carolina distribution, red dots denote Virginia.

Figure 4. Dan, South Mayo, Smith, and Banister rivers of the Roanoke River basin study area, Virginia.

Figure 5. 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).

Figure 6. Diagram of simple random sampling methodology used in 2003-2004.

Figure 7. Preliminary, informal surveys conducted for *Pleurobema collina* throughout the Dan River sub-basin, VA, 2002. Mussels present throughout 24 rkm of the South Mayo River, Patrick and Henry counties, Virginia (denoted by red dots).

Figure 8. Formal simple random surveys (present/not observed) for *Pleurobema collina* in the Roanoke River basin (Dan River sub-basin), Virginia, 2003-2004.

Figure 9. USGS hydrographs depicting flow conditions for the years 2002 and 2003 in the Dan and South Mayo rivers, particularly March-October, 2003.

Figure 10. USGS hydrographs depicting flow conditions for the Dan, Smith, South Mayo, and Banister rivers, Virginia, 2004

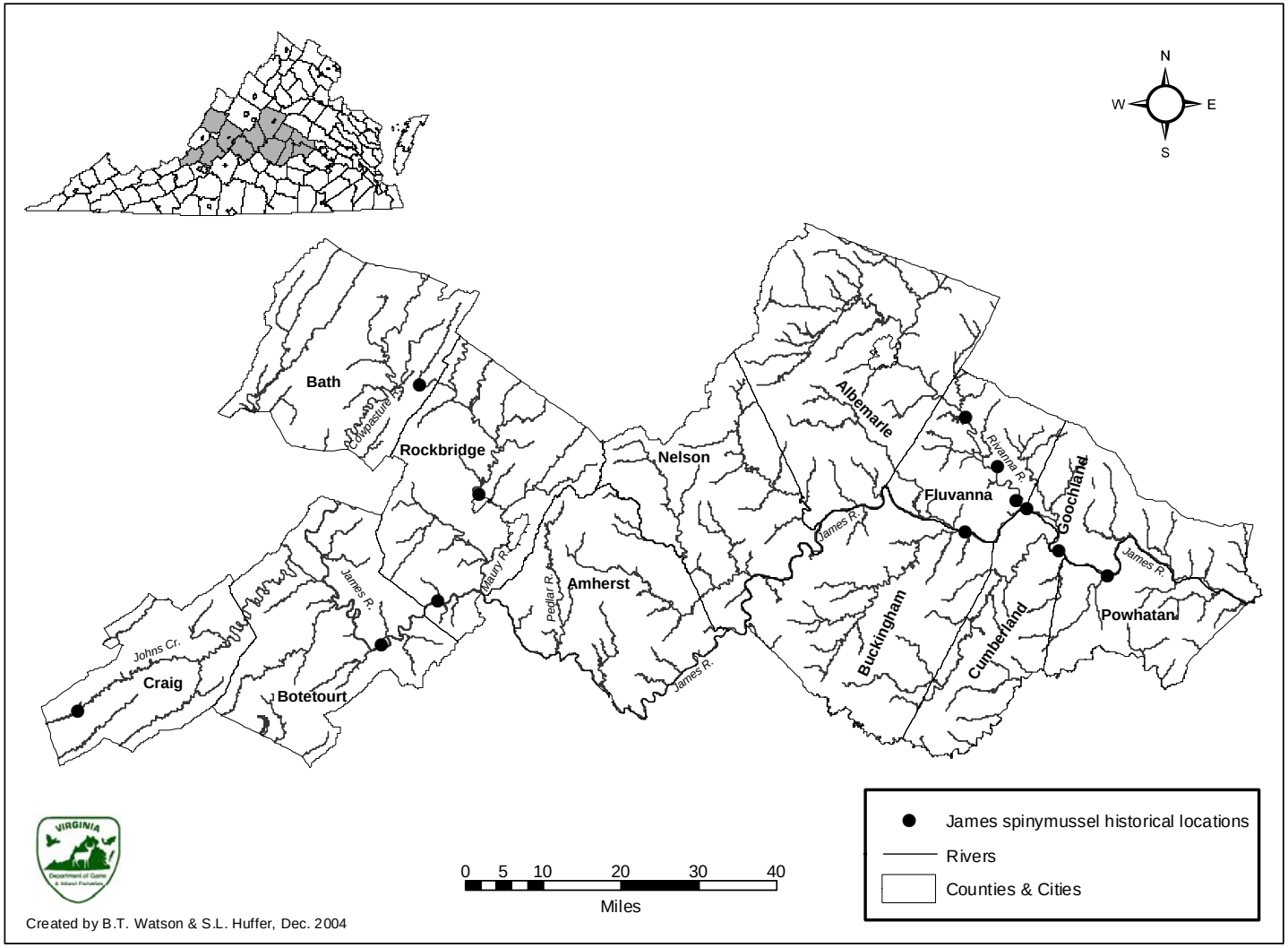

N
W
E
S
Bath
Rockbridge
Nelson
Albemarle
Fluvanna
Goochland
Powhatan
Amherst
Buckingham
Cumberland
Botetourt
Craig
Johns Cr.
James R.
Maury R.
Pedlar R.
Rivanna R.
Created by B.T. Watson & S.L. Huffer, Dec. 2004
0 5 10 20 30 40
Miles
James spinymussel historical locations
Rivers
Counties & Cities

Figure 1.

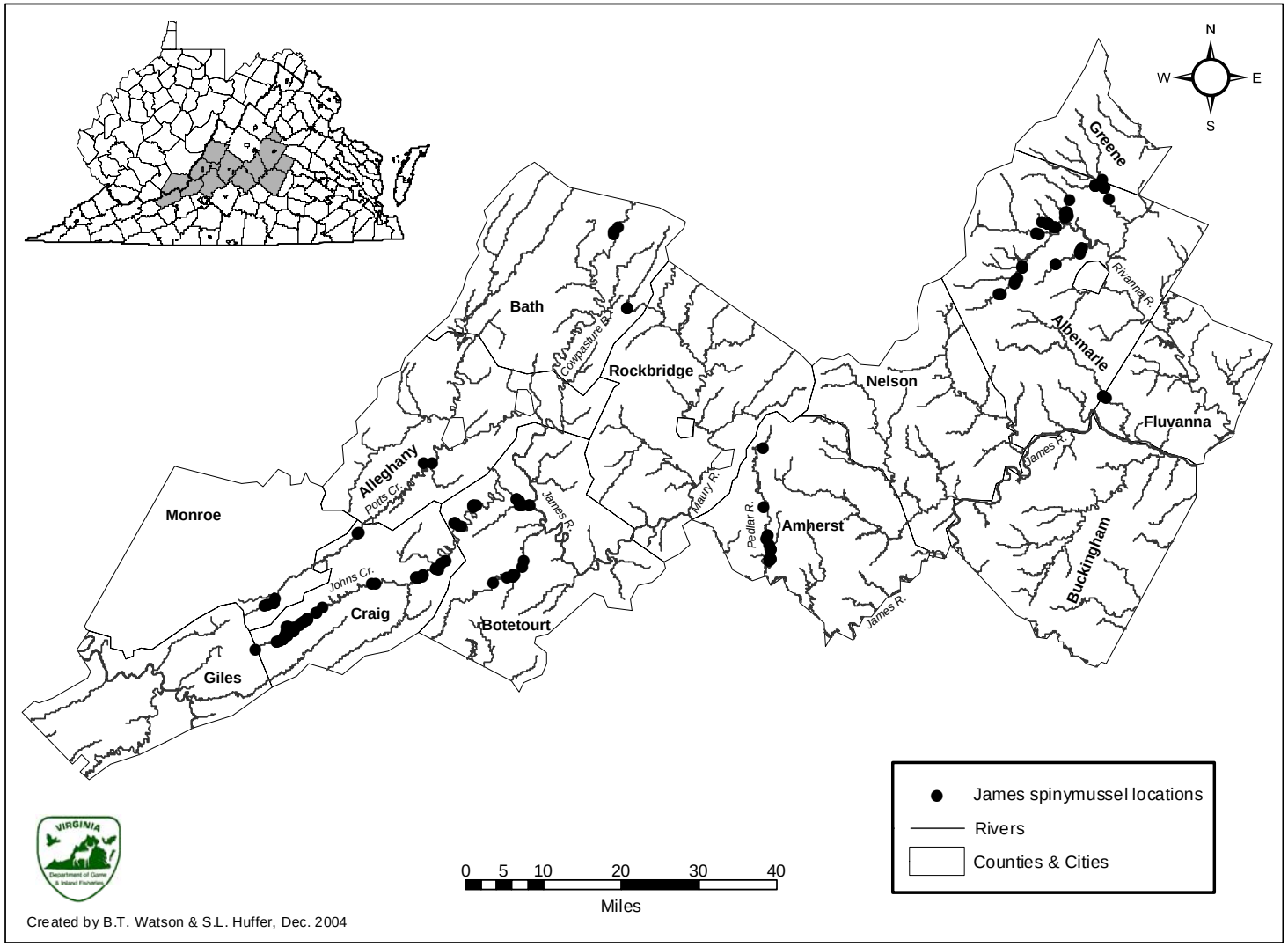

N
W
E
S
Greene
Albemarle
Rivanna R.
Fluvanna
Nelson
Bath
Rockbridge
Alleghany
Potts Cr.
Cowpasture R.
James R.
Maury R.
Pedlar R.
Amherst
Buckingham
Monroe
Craig
Botetourt
Giles
Johns Cr.
James R.
James R.
James spinymussel locations
Rivers
Counties & Cities
0 5 10 20 30 40
Miles
Created by B.T. Watson & S.L. Huffer, Dec. 2004

Figure 2.

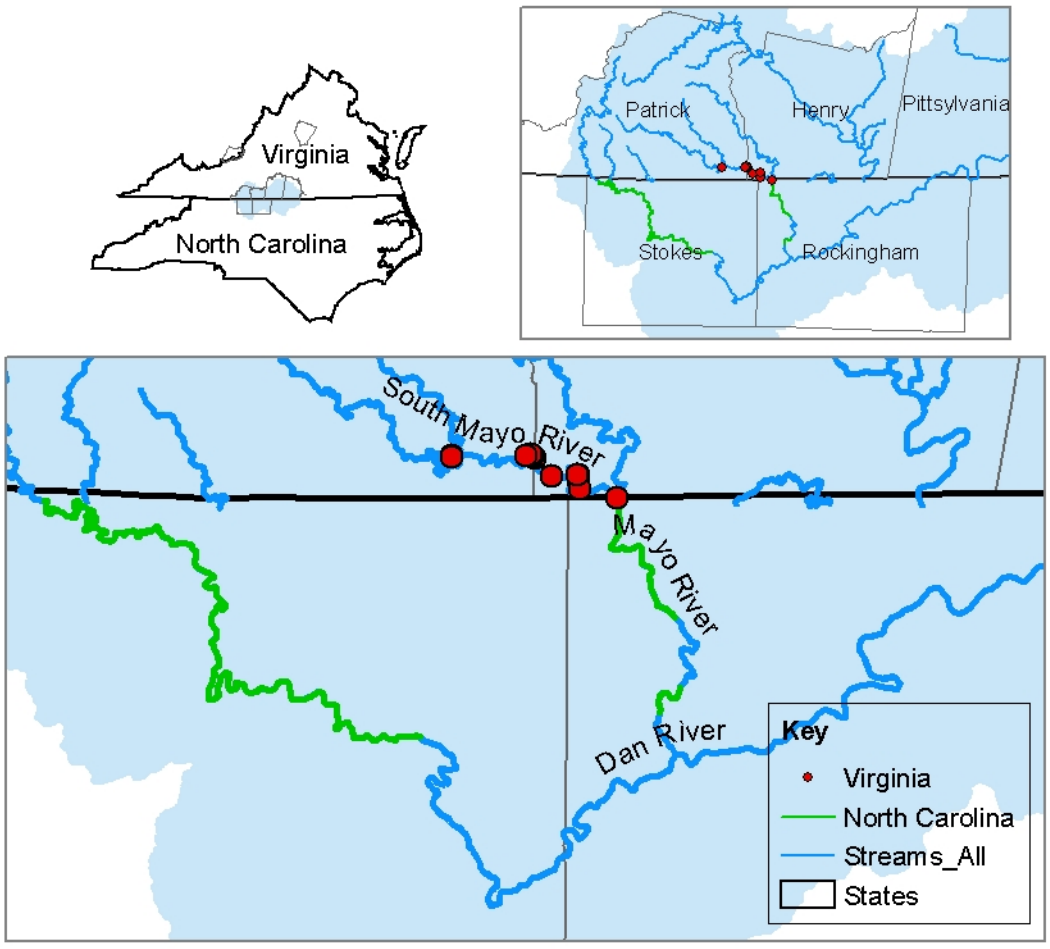

Virginia
North Carolina
Patrick
Henry
Pittsylvania
Stokes
Rockingham
South Mayo River
Mayo River
Dan River
Key
Virginia
North Carolina
Streams_All
States

Figure 3.

Figure 4.

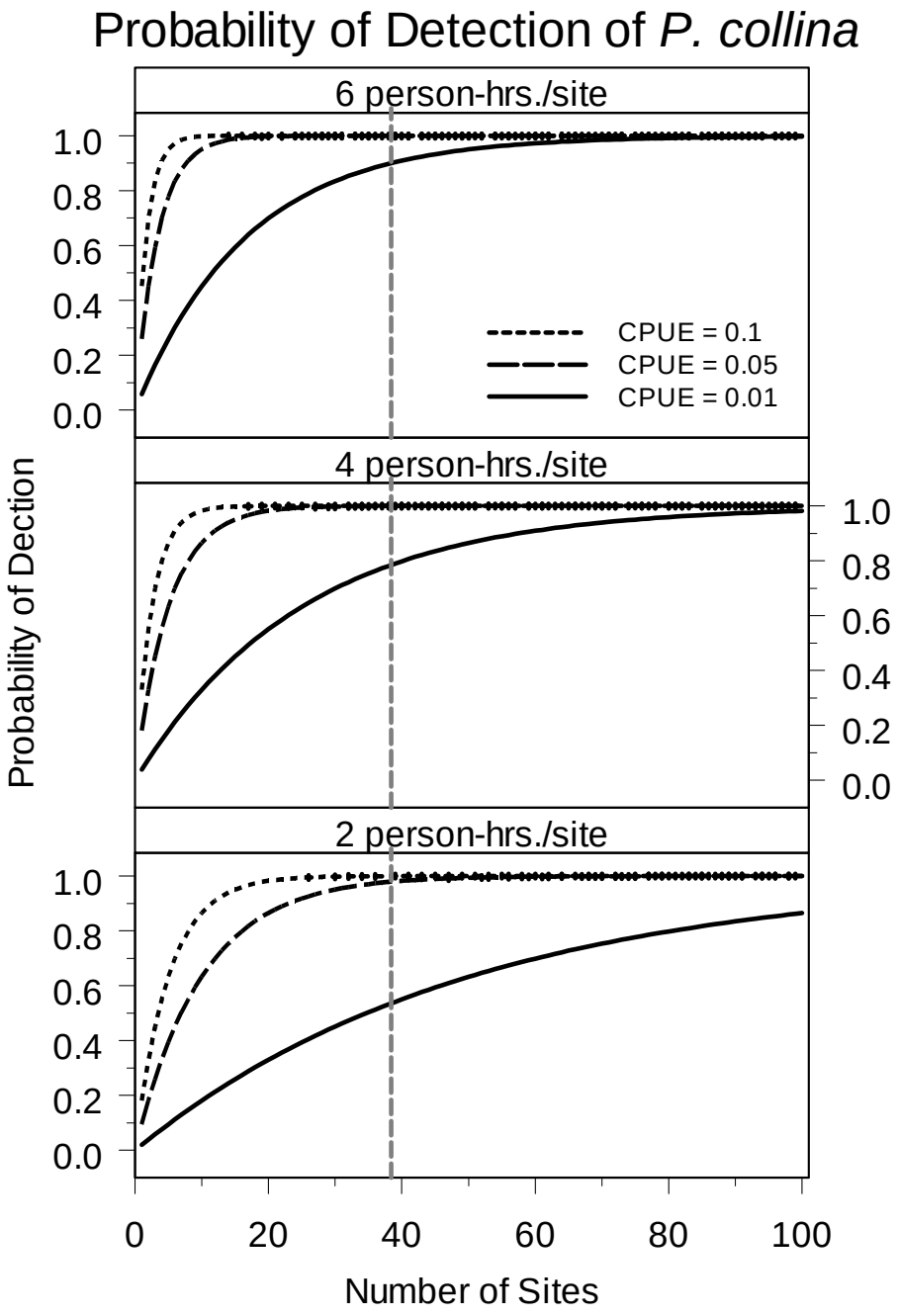

Probability of Detection of P. collina
6 person-hrs./site
4 person-hrs./site
2 person-hrs./site
CPUE = 0.1
CPUE = 0.05
CPUE = 0.01
Probability of Dection
Number of Sites
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100

Figure 5.

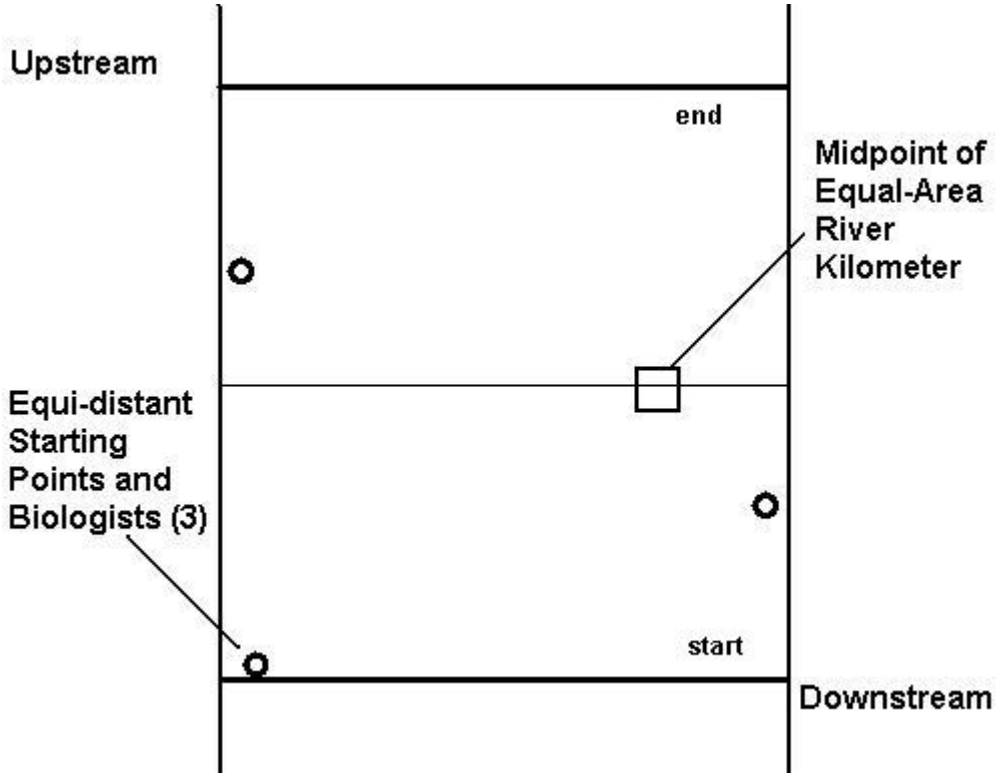

Upstream
end
Midpoint of
Equal-Area
River
Kilometer
Equi-distant
Starting
Points and
Biologists (3)
start
Downstream

Figure 6.

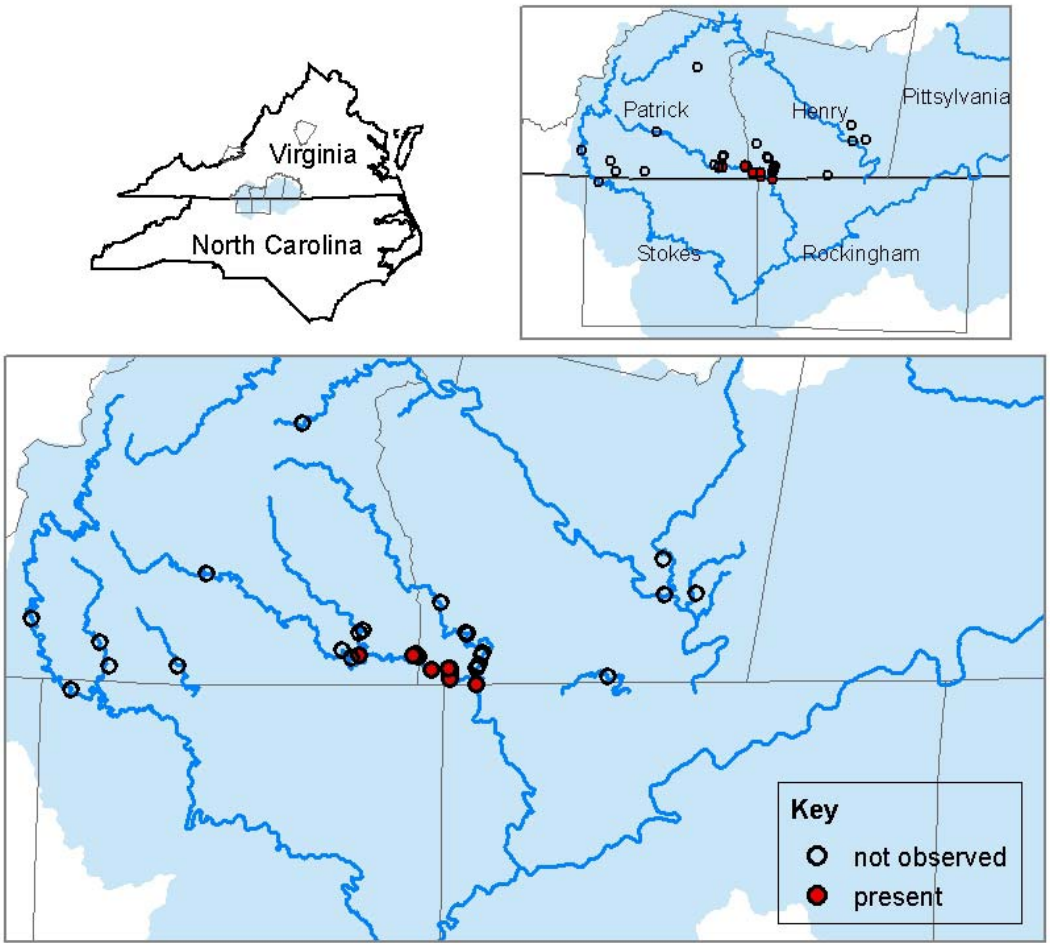

Virginia
North Carolina
Patrick
Henry
Pittsylvania
Stokes
Rockingham
Key
not observed
present

Figure 7.

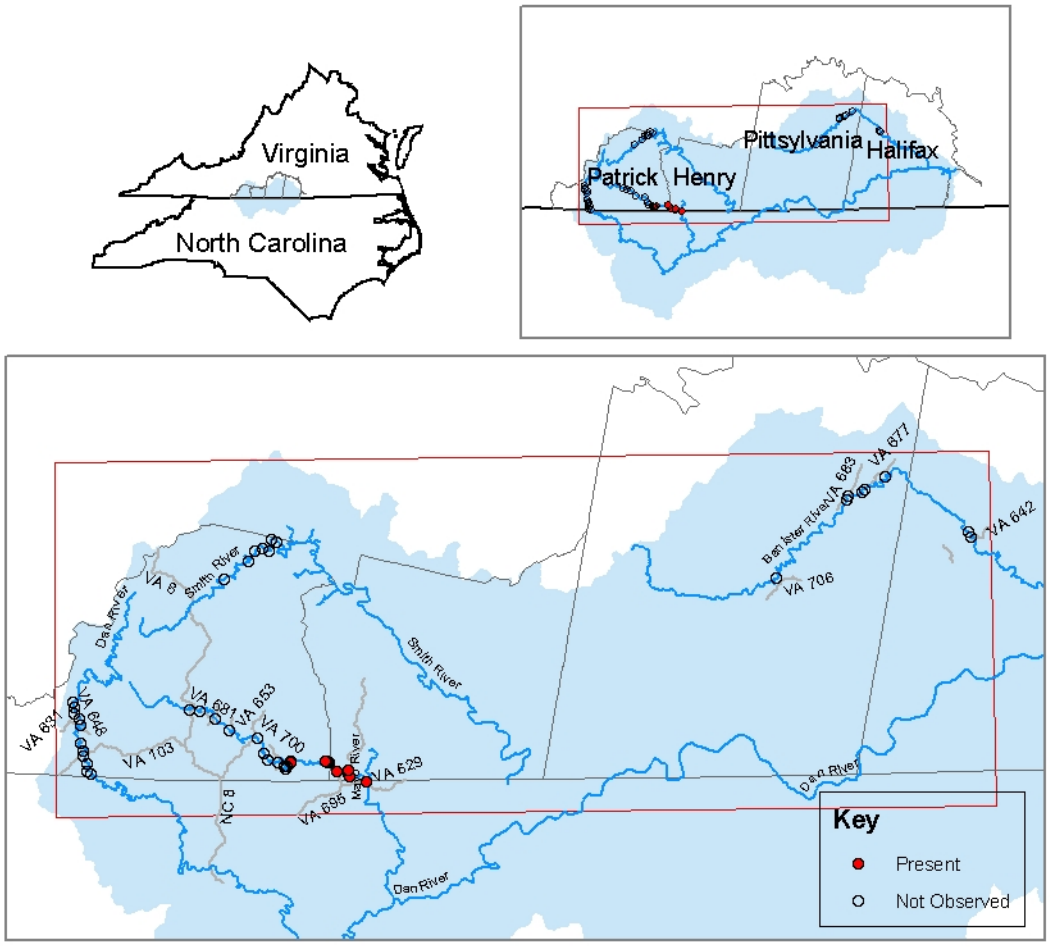

Virginia
North Carolina
Patrick
Henry
Pittsylvania
Halifax
Dan River
VA 8
Smith River
VA 631
VA 648
VA 703
VA 653
VA 700
VA 695
NC 8
Mayo River
VA 629
Smith River
Dan River
Banister River
VA 683
VA 677
VA 642
VA 706
Dan River
Key
Present
Not Observed

Figure 8.

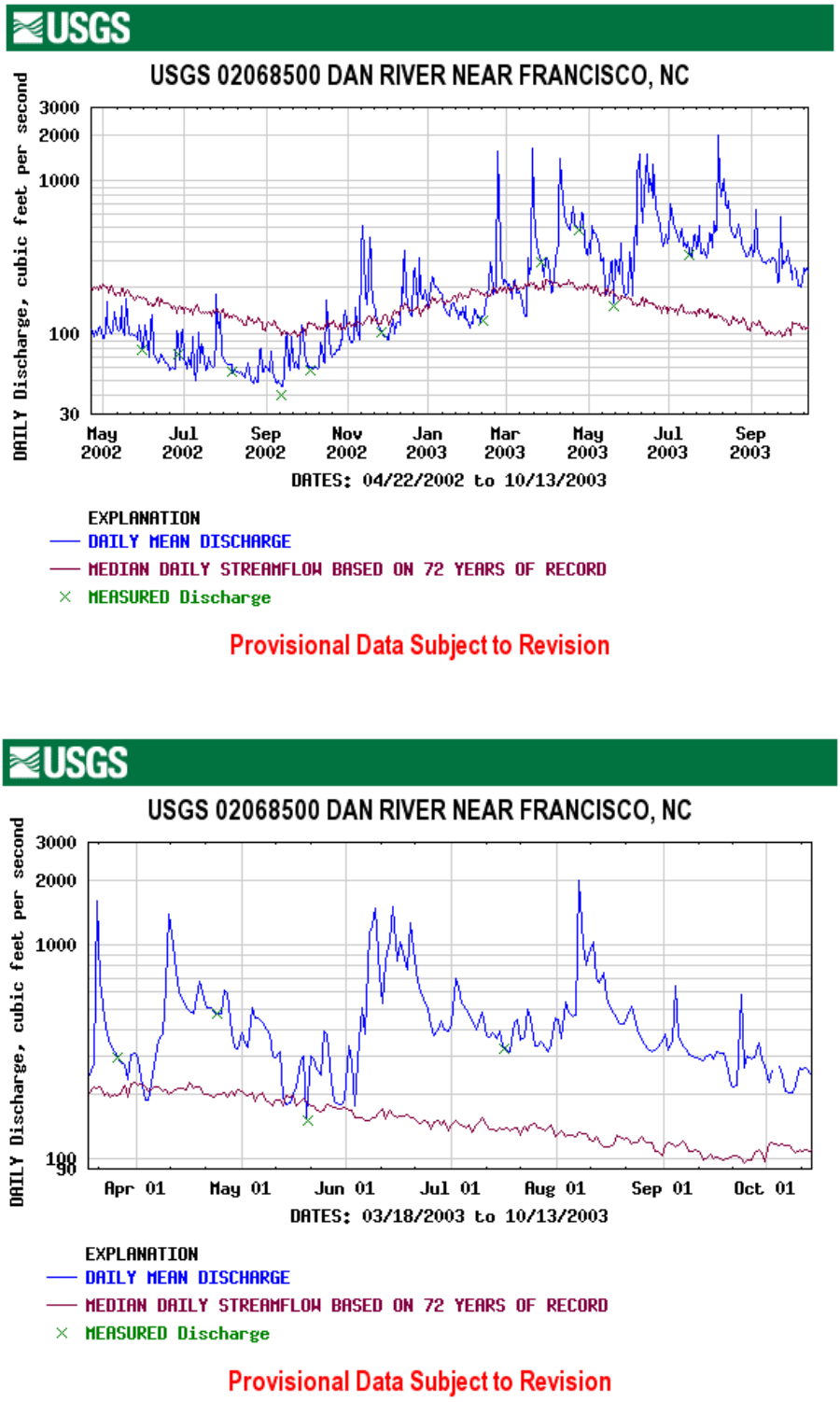
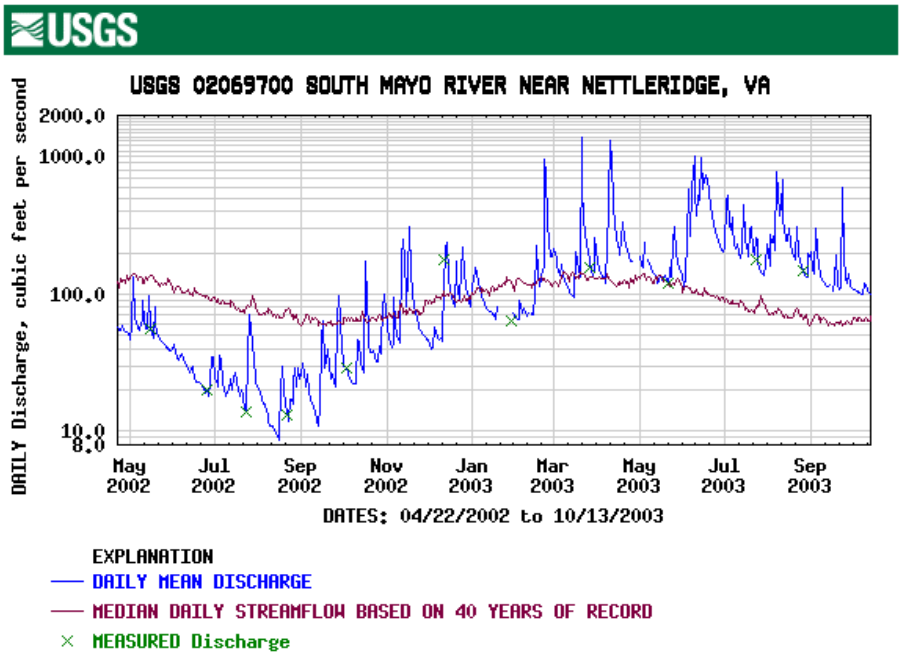

USGS
USGS 02068500 DAN RIVER NEAR FRANCISCO, NC
DAILY Discharge, cubic feet per second
3000
2000
1000
100
30
May 2002
Jul 2002
Sep 2002
Nov 2002
Jan 2003
Mar 2003
May 2003
Jul 2003
Sep 2003
DATES: 04/22/2002 to 10/13/2003
EXPLANATION
DAILY MEAN DISCHARGE
MEDIAN DAILY STREAMFLOW BASED ON 72 YEARS OF RECORD
MEASURED Discharge
Provisional Data Subject to Revision
USGS
USGS 02068500 DAN RIVER NEAR FRANCISCO, NC
DAILY Discharge, cubic feet per second
3000
2000
1000
100
90
Apr 01
May 01
Jun 01
Jul 01
Aug 01
Sep 01
Oct 01
DATES: 03/18/2003 to 10/13/2003
EXPLANATION
DAILY MEAN DISCHARGE
MEDIAN DAILY STREAMFLOW BASED ON 72 YEARS OF RECORD
MEASURED Discharge
Provisional Data Subject to Revision

Figure 9.



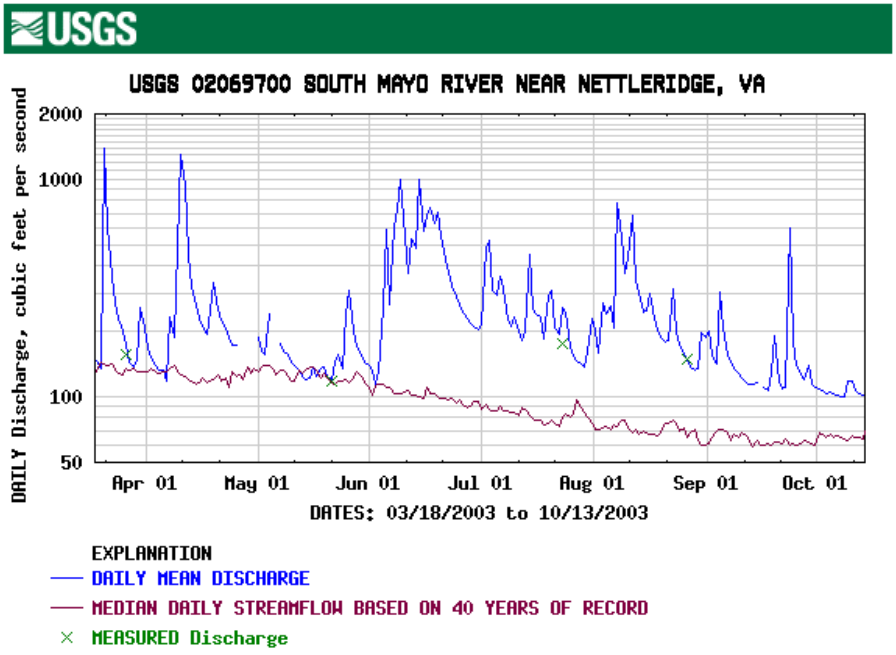
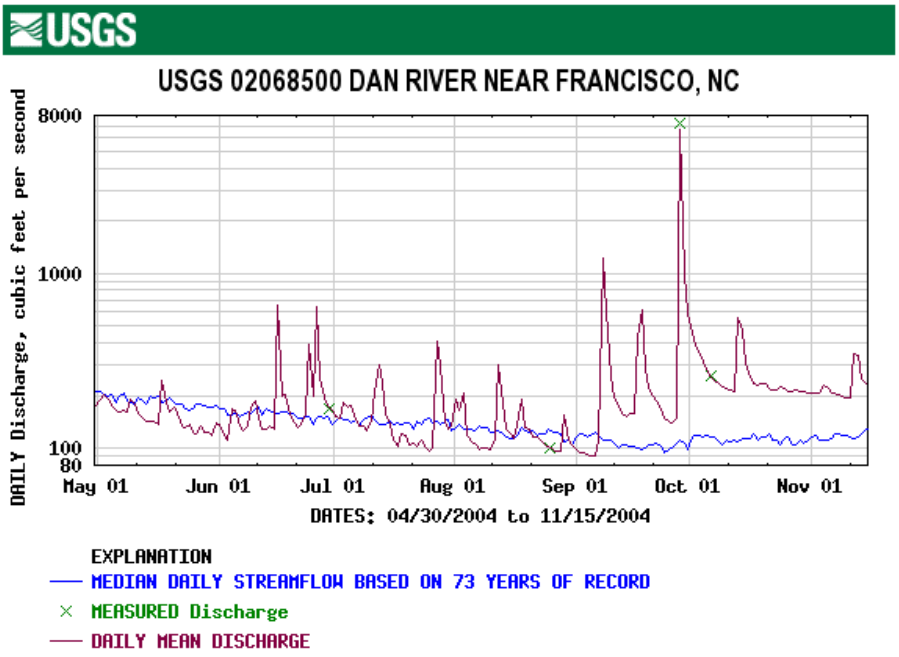


Figure 9. Continuation.


USGS
USGS 02068500 DAN RIVER NEAR FRANCISCO, NC
DAILY Discharge, cubic feet per second
8000
1000
100
80
May 01
Jun 01
Jul 01
Aug 01
Sep 01
Oct 01
Nov 01
DATES: 04/30/2004 to 11/15/2004
EXPLANATION
MEDIAN DAILY STREAMFLOW BASED ON 73 YEARS OF RECORD
MEASURED Discharge
DAILY MEAN DISCHARGE
Provisional Data Subject to Revision

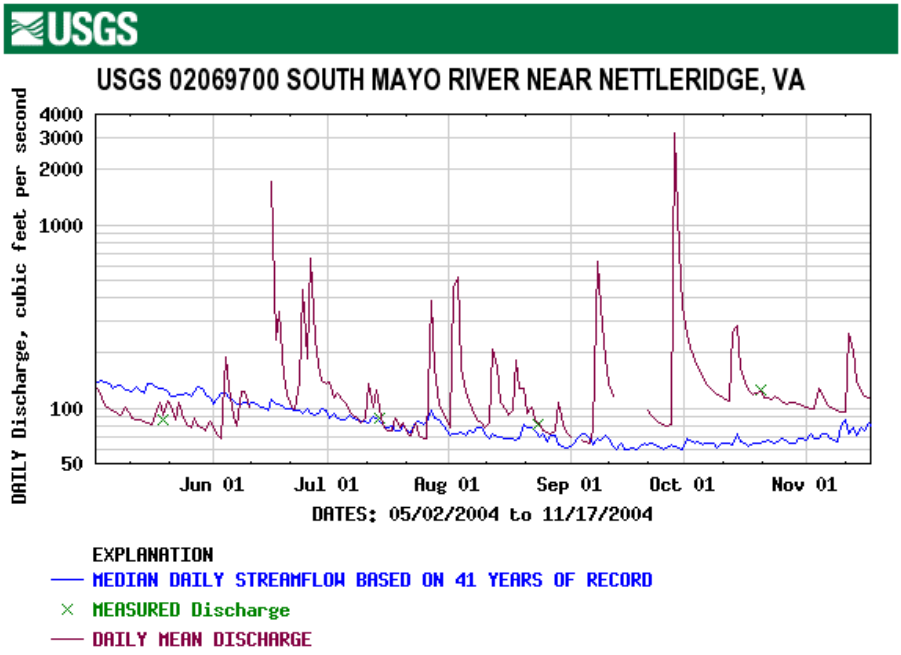

USGS
USGS 02069700 SOUTH MAYO RIVER NEAR NETTLERIDGE, VA
DAILY Discharge, cubic feet per second
4000
3000
2000
1000
100
50
Jun 01
Jul 01
Aug 01
Sep 01
Oct 01
Nov 01
DATES: 05/02/2004 to 11/17/2004
EXPLANATION
MEDIAN DAILY STREAMFLOW BASED ON 41 YEARS OF RECORD
MEASURED Discharge
DAILY MEAN DISCHARGE
Provisional Data Subject to Revision

Figure 10.

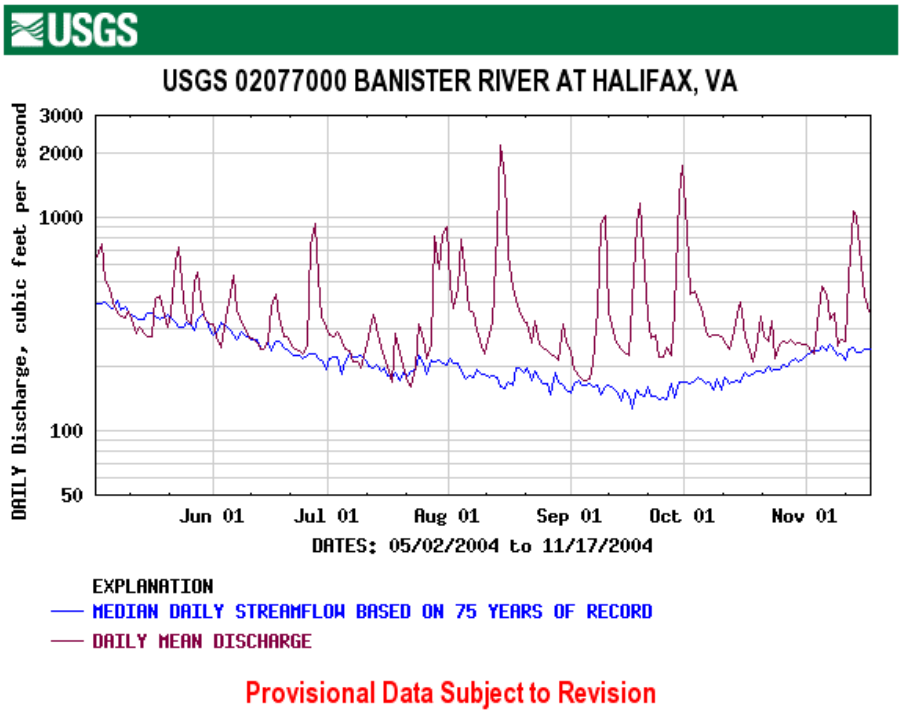


Figure 10. Continuation.

CHAPTER 2

Patterns of Genetic Differentiation and Life History of the Endangered James Spinymussel *Pleurobema collina* (Bivalvia: Unionidae)

ABSTRACT

Following the discovery of a spinymussel in the Dan River sub-basin of the Roanoke River basin, the U.S. Fish and Wildlife Service tentatively identified this species as the endangered James spinymussel (*Pleurobema collina*). A genetic characterization of four extant populations of *P. collina* was conducted to assess its taxonomic affinity and to resolve conservation issues related to recovery planning and management actions. The populations were examined for phenotypic variation, and were characterized phylogenetically using DNA sequences. A comprehensive analysis was performed for both separate and combined mitochondrial (357 bp of *cytochrome-b*, 916 bp of *ND-1*) and nuclear (502 bp of *ITS-1*) DNA sequences. Analyses of molecular variation (AMOVA) values for both *ND-1* and *ITS-1* suggested that almost equal variation in *P. collina* resided within (48.4-52.3%) and among (47.7-51.6%) populations. Clustering of haplotypes in minimum spanning networks did not conform to population boundaries, reflecting maintenance of ancestral haplotype frequencies with beginnings of evolutionary divergence. Significant divergence of F_{ST} values (range 0.19-0.87, $P<0.05$) were suggestive of random genetic drift effects following a prolonged or severe demographic bottleneck in a large ancestral population during historical times. No quantitative genetic variation was observed in fish host specificity ($P>0.05$), with transformation success of glochidia greatest on *Nocomis leptocephalus* (61%) both in this study and in Hove (1990). In addition, specimens in the populations had the same shape and color, but different-sized glochidia, comparable mean number of conglutinates per female, and similar fecundity estimates ($P>0.05$). A monophyletic lineage [congruent with lack of phenotypic, quantitative, or geographic genetic variation among populations]

was inferred. Based on comprehensive molecular, morphological, and life history data, populations of *P. collina* sampled from the Dan River sub-drainage do not warrant separate species designation from *P. collina* sampled from the James River drainage. However, because of the genetic distinctiveness of several haplotype frequencies and the isolated geographic ranges, I propose that populations in the Dan and populations in the James rivers be regarded as separate Management Units (MUs), unless and until analysis of allele frequency at microsatellite loci provides further insights into histories and adaptive characters of each extant population of *P. collina*.

INTRODUCTION

Conservation of freshwater mussel diversity is predicated on the accurate identification of those taxa requiring protection (e.g., *Pleurobema collina*). However, these animals present significant challenges to conservation biologists because they exhibit broad taxonomic uncertainty due to phenotypic plasticity and complex life histories (King *et al.* 1998). Poorly described ranges, high endemism, and convergence in conchological characters make field identifications of some species difficult, even by experienced malacologists. This is particularly the case with rare taxa because field biologists encounter them infrequently.

Definitive identification of freshwater mussel taxa is imperative for recovery and management planning and for compiling accurate range information. The development of extensive DNA sequence databases for freshwater mussels can provide conservation biologists with a valuable tool to confirm or determine the identity of rare or otherwise problematic species (Roe 1999). Though the value of genetic assays is beyond debate, Davis (1983), Roe & Lydeard (1998), and Jones (2004) recommend morphological, life history, and distributional studies as a necessary companion to molecular genetic studies.

Incomplete understanding of variation in morphology, anatomy, life history, and genetic characters has resulted in numerous disagreements in mussel taxonomy and phylogenetics, which persist today (Heard and Guckert 1971; Davis 1983; Stiven and Alderman 1992; Hoeh and Gordon 1996; Lydeard *et al*. 1996; Berg and Berg 2000). The relationships among many species and genera are controversial. Cryptic species may be common because of the difficulties in resolving mussel relationships with traditional taxonomy, which focuses on morphological characters associated with soft anatomy and

conchology (Lea 1834; Conrad 1853; Simpson 1896, 1900; Johnson 1970; Davis 1983; Hoeh *et al.* 2001). Given the difficulties with making phylogenetic inference from shell morphology, investigators increasingly rely on molecular evaluations to resolve controversial taxonomy (Bowen & Richardson 2000). However, genetic data must be coupled with knowledge of biogeography, life history, and ecological data to formulate management plans that meet conservation goals: to increase the likelihood of species survival, and to conserve ecological and evolutionary processes for the long term.

Managers can apply the Endangered Species Act (ESA) to counteract escalating bivalve declines. However, taxonomy forms the basis for legal protection under the ESA. In order for a species to be afforded protection under the ESA, taxonomic uncertainty that exists in endangered or threatened taxa must be resolved (Mulvey *et al.* 1998). Recovery of a species already under federal protection of the ESA demands identification and conservation of as much of their genetic variability as still exists (King *et al.* 1998). Genetics can inform and assist management decisions regarding whether or not a species should be listed under the ESA, or recover a species already under federal protection (King *et al*. 1998).

Biologists from North Carolina and Virginia in 2000-2002 discovered a spinymussel in the Dan River sub-basin of the Roanoke River. Based on a preliminary molecular genetic analysis by Dr. Chuck Lydeard, University of Alabama, the U.S. Fish and Wildlife Service (USFWS) tentatively identified this species as the James spinymussel (*P. collina*), with genotypic variation between the Dan River population and James River populations. The same genetic analysis also indicated that the James spinymussel and the Tar spinymussel (*Elliptio steinstansana*) are distinct but closely

related species (sister groups), and that the placement of these two species in separate genera is dubious. The analysis also supported the placement of the Altamaha spinymussel (*E. spinosa*) in the genus *Elliptio*, and not in the same genus as the other two spinymussels. This suggests that the presence of spines is a convergent character, as proposed by Johnson (1970).

My overall research objective was to collect information on the distribution, life history, and genetics of this newly-found population to resolve whether it is the federally endangered James spinymussel. Confirmation requires that studies of genetically determined characters be congruent with other such studies on different sets of characters (i.e., life history characters) of *P. collina* from the Dan River sub-basin and the James River basin. Part of my overall objective was to characterize the genetic structure and diversity of the James spinymussel as a basis for implementing conservation and management actions. Because of difficulty in characterizing closely related mussel taxa with current genetic technology, I used a multi-dimensional approach. A comprehensive analysis of morphology, anatomy, life history, and genetics was conducted to assess taxonomic validity. Taxa can be wrongly classified as a result of studies that do not use such a holistic approach (Davis 1983, 1994; Mulvey *et al*. 1998). Ambiguous or misleading interpretation of molecular genetic analysis can affect a species' status, and management actions taken on its behalf. Although this 'new' taxon was designated as endangered, without a valid taxonomic name, it could not be evaluated for protection under the ESA (Mulvey *et al*. 1998). In subsequent sections, I describe morphological, anatomical, and other ecological characteristics of populations of *P. collina* from the James River basin as described by Clarke and Neves (1984) and Hove (1990), which

contributed to the development of my overall objective: definitive identification of the newly-found population in the Dan River sub-basin.

Historical classification and characteristics of the James spinymussel

The James spinymussel was discovered by T.A. Conrad in the Calfpasture River of the James River basin, and subsequently described as *Unio collinus* (Conrad 1837). Since its description, this species has been placed in five genera. The species was moved from *Unio* to *Alasmidonta* by Simpson (1900), to *Pleurobema* by Boss and Clench (1967), then to *Fusconaia* by Johnson and Clarke (1983), to *Canthyria* by Clarke and Neves (1984), and back to *Pleurobema* by Turgeon *et al*. (1988). The taxonomic history of this species is described more fully by Clarke and Neves (1984). Observations made during Hove's (1990) study revealed that the James spinymussel shares some characteristics with each of these genera, but does not fit readily into any one genus. Traits shared with *Pleurobema* spp. include similar gravidity period, glochidial release period, glochidial shape and size, number of marsupial gills, and fish hosts. Differences between these two taxa include conglutinate morphology and color, and glochidial arrangement within the conglutinate (Hove 1990).

The James spinymussel is a small mussel that reaches a maximum length of approximately 70 mm. A specimen collected in the Dan River was 74 mm in length (Savidge 2002). Valves are irregularly sub-rhomboidal, and rounded anteriorly. The valves of young individuals (<40 mm) are sub-rhomboidal in shape, with an obliquely sub-truncated posterior with widely spaced concentric annuli. The periostracum is yellow to dark-brown (juvenile to adult), and nacre color is light-orange to pink antero-

ventrally and iridescently bluish posteriorly. The spines of this mussel, if present, are usually arranged symmetrically, and one to four spines may occur between the first and sixth annuli. Spines are normally one to three millimeters high. Individuals four years or older frequently have umbos eroded to varying degrees. With age, the shell becomes more ovate or even arcuate, the periostracum becomes brownish to black, and any spines that were once present become lost. However, it appears that some spinymussels from the James River basin never had spines (Clarke and Neves 1984; Hove 1990). Other identifying characteristics include an orange mantle and foot, and well-developed hinge teeth (Johnson 1970).

The James spinymussel and the Tar spinymussel share many morphological traits, but are clearly distinct species (Clarke and Neves 1984). The Tar spinymussel has characteristics intermediate between the small, short-spined James spinymussel and the large, long-spined Altamaha spinymussel (USFWS 1990; USFWS 1992). Internal anatomical differences between the two species were described by Clarke and Neves (1984). Tar spinymussels can have up to 12 spines (USFWS 1992) and tend to have spines more consistently than the James spinymussel. Clarke and Neves (1984) stated that most specimens of James spinymussel "never develop spines". However, the James spinymussels observed from the Dan and Mayo rivers (164 individuals) (personal observations 2002; Savidge 2002) generally have spines, and as many as 8 spines have been observed on one individual. This difference between the Dan River and James River populations suggest that spine number may be related to environmental rather than genetic factors, but this hypothesis warrants testing.

Knowledge of the reproductive biology of the James spinymussel in the James River basin is limited to that of Hove and Neves (1989) and Hove (1990). Based upon laboratory infestation experiments, Hove (1990) identified seven fish species, all in the family Cyprinidae (minnows), as suitable fish hosts for the James spinymussel. Most of the fish species that serve as hosts for *P. collina* are common, such that the extirpation of this mussel due solely to limited host abundance or distribution is unlikely, and all of these fish species occur in the Dan River basin (Rohde *et al.* 2001). I tested the null hypothesis that there are no differences in James spinymussel life history characteristics, morphology, or genetics when comparing the Dan River sub-basin population to the James River basin populations. I compared life history characteristics on the Dan and Mayo river populations to Hove and Neves' (1989) and Hove's (1990) *Pleurobema collina* life history studies conducted on the James River basin populations.

Identification of freshwater mussel conservation units

Historically, *P. collina* populations were interconnected with continuous suitable habitat (Johnson 1970). There was often an exchange of immature individuals among tributaries with suitable (i.e., not degraded or fragmented) habitat within the James and Roanoke river systems. That is, movement of glochidia via dispersing fish hosts linked populations in the various tributaries. A reduction in the total amount of suitable *P. collina* habitat in the James and Roanoke river systems, and fragmentation of the remaining habitat into smaller, more isolated patches occurred as a result of habitat degradation. The longitudinal continuum of the river ecosystem was disrupted by the interaction of many factors: severe pollution, impoundments, erosion, and nutrient runoff

resulting from land use changes (Hove 1990; USFWS 1990). The impact of fragmentation has produced small populations where large populations once existed.

Unionid mussels in the United States present unique conservation challenges because the once-extensive ranges of many species, including the James spinymussel, have become highly fragmented (Neves *et al*. 1997). The relatively recent and drastic decline in freshwater mussels can be attributed primarily to rapid and widespread anthropogenic degradation and loss of habitat. River systems can be considered habitat "islands" on the larger landscape; each river is separated from other rivers by habitat unsuitable for bivalves (Mulvey *et al* 1998).

Habitat fragmentation is common for freshwater species, causing disjunct gene pools. Population fragmentation is likely associated with loss or redistribution of genetic variability and is a concern for conservation biologists attempting to manage the genetic legacy of freshwater bivalve species. Genetically differentiated, geographically isolated populations are especially problematic because it is difficult to determine whether they represent variation within a species or distinct species (Mulvey *et al*. 1998).

Because of the current allopatric distributions of the in-group taxon, the Phylogenetic Species Concept, defined as the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent (Cracraft 1983), was primarily used to define species. In addition, the 'Biological Species Concept' of Mayr and Ashlock (1991), defined as a group of interbreeding natural populations that is reproductively isolated from other such groups, was applied indirectly. In populations that were recently sympatric, evidence for lack of gene flow (exchange of genes) was evaluated using molecular genetic data. Populations were considered

'Evolutionarily Significant Units' if they met the criteria of Waples (1991); namely, a population (or group of populations) that: (1) is substantially reproductively isolated from other conspecific population units (i.e., reproductive incompatibility), and (2) represents an important component in the evolutionary legacy of the species. Populations that were differentiated, but not as highly divergent as ESUs, were evaluated as possible Management Units (MUs). MUs were recognized as populations with significant divergence of genetic material in the mitochondrial or nuclear genome, even if there was not complete divergence (Moritz 1994). MUs are defined by population-level distinctions, and ESUs are defined primarily by phylogenetic distinctions.

According to Moritz (1994), one of the criteria for distinguishing ESUs is the occurrence of different forms of mitochondrial DNA in different populations, with no haplotypes shared between populations. According to Waples (1991), ESUs also can show significant divergence of genetic material in the nuclear genome or significant divergence in ecological and morphological traits. Populations that are different, but not as highly divergent as ESUs, may warrant conservation status as MUs. MUs are recognized as populations with significant divergence of genetic material in the mitochondrial or nuclear genome, even if there is not complete divergence (Moritz 1994). The investigation of variation at mitochondrial and nuclear DNA markers provided the opportunity to identify possible management units for the James spinymussel, should significant differences among populations be found in the mitochondrial and nuclear DNA, regardless of the phylogenetic distinctiveness of the alleles. Few studies have been conducted using mitochondrial DNA analysis in a species of freshwater mussel (Lydeard *et al*. 1996; Mulvey *et al*. 1997; King *et al*. 1999), but it holds great promise for

identifying MUs (Mulvey *et al*. 1998). The USFWS (2002) defined Management Units for the endangered red-cockaded woodpecker (*Picoides borealis)* as geographic or otherwise identifiable subunits of the listed entity that individually are necessary to conserve genetic robustness, demographic robustness, important life stages, or some other feature necessary for long-term sustainability of the overall listed entity. MUs are valuable in conservation because they describe the fundamental units of wildlife management, reproductively isolated populations (Bowen and Richardson 2000).

The taxonomic issues with *P. collina* might be best resolved when molecular techniques are used in conjunction with ecological, morphological, and distributional data, an approach rarely applied to unionid mussels (Davis 1994). Mitochondrial DNA might be especially appropriate for the study of this endangered species because the mitochondrial genome of populations differentiates more rapidly than does the nuclear genome (Moritz 1994).

Designation of conservation units

Population-level designations were based on the presence of multiple diagnostic or unique characters that were fixed in *P.collina*. Designation of taxonomic status was based on examination of a suite of characters from the following data sets: (1) molecular genetics, (2) shell and mantle-pad morphology, (3) length of glochidia, (4) degree of fish host specificity, (5) population distribution, and (6) other relevant ecological and life history information. If concordance among multiple independent characters occurred within and between populations of each putative species, the case for species-level designations was supported (Avise 2000).

METHODS

Shell material

Shell material for *P. collina* was collected from the Dan River sub-basin, VA and NC ($N = 15$) and qualitatively compared to shell material from the James River system ($N = 100$). The latter material was collected by Hove (1989).

Tissue collection

Samples of mantle tissue from live *P. collina* were collected from various river locations throughout the range of the species (Figure 1): (1) Dan River (DR) at the NC Rte. 89 bridge crossing (DRKM 225 and 226), Stokes Co., NC; (2) South Fork Mayo River at (SFMRKM 6 and 11), Henry and Patrick counties, VA; (3) Wards Creek (at Millington Rd., VA Rte. 665 crossing), Albemarle Co., VA; (4) South Fork Potts Creek at John Furrow private property (off of VA Rte. 631), Monroe Co., WV. Sample sizes were limited because of the endangered status of the species. A small piece of mantle tissue (20-30 mg) was collected non-lethally (Naimo et al. 1998) from 7-34 live mussels from each population (Table 1). To provide outgroup taxa, a tissue sample of *Elliptio steinstansana* was received from the University of Alabama tissue repository for freshwater mollusks (UAUC #3037), and comparable DNA sequences of *Epioblasma brevidens* were provided by Jess Jones in 2004. Tissue was preserved in 95% ethanol and stored at -20˚C prior to DNA extraction.

Total genomic DNA was isolated from ~20 mg of mantle tissue using the Purgene DNA extraction kit (Gentra Systems, Inc., Minneapolis, MN, USA). DNA concentration

was determined by fluorescence assay (Hoefer TKO 1000 Flourometer, Hoefer Scientific Instruments, San Francisco, CA), and its quality visually inspected on a 0.8% agarose gel. Diluted DNA stock samples (25 ng/μL) were stored long-term at -60°C.

DNA sequences

Sequences of two regions of mitochondrial DNA (mtDNA) and one region of nuclear DNA (nDNA) were amplified by polymerase chain reaction (PCR) in a PTC-200 Thermal Cycler (MJ Research) using primers and conditions reported by the following authors: (1) *cytochrome-b* (Merritt *et al*. 1998; Bowen and Richardson 2000), (2) *ND1*, the first subunit of NADH dehydrogenase (Buhay et al. 2002; Serb et al. 2003), and (3) *ITS-1* (nDNA), the first internal transcribed spacer region between the *5.8S* and *18S* ribosomal DNA genes (King et al. 1999). Primer sequences are reported in Table 2.

The PCR reaction mixtures for *cytochrome-b* consisted of 25 ng of genomic DNA, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 μM each primer, and 1.5 U Ampli*Taq* DNA polymerase (GeneAmp, Applied Biosystems) in a total volume of 20 μL. PCR thermal cycling conditions were 94 °C for 2 min, followed by 40 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min; a final extension at 72 °C for 6 min and a final hold at 4 °C.

The PCR reaction mixtures for *ND1* consisted of 100 ng of genomic DNA, 1x PCR buffer, 2.5 mM $MgCl_2$, 0.4 mM dNTPs, 1.0 μM each primer, and 1.5 U Ampli*Taq* DNA polymerase (GeneAmp, Applied Biosystems) in a total volume of 50 μL. PCR thermal cycling conditions were 95 °C for 7 min, followed by 35 cycles of 94 °C for 40

sec, 50 °C for 60 sec, and 72 °C for 90 sec; a final extension at 72 °C for 2 min and a final hold at 4 °C.

The PCR reaction mixtures for *ITS-1* consisted of 50 ng of genomic DNA, 1x PCR buffer, 2.0 mM $MgCl_2$, 0.25 dNTPs, 0.5 μM each primer, and 1.0 U Ampli*Taq* DNA polymerase (GeneAmp, Applied Biosystems) in a total volume of 20 μL. PCR thermal cycling conditions were 95 °C for 7 min, followed by 35 cycles of 94 °C for 30 sec, 64 °C for 30 sec, and 72 °C for 90 sec; and a final extension at 72 °C for 5 min and a final hold at 4 °C.

A similar sequencing protocol was followed for *cytochrome-b*, *ND-1*, and *ITS-1*. PCR products first were purified using a Qiagen DNA Purification kit (Qiagen, Carlsbad, CA). All PCR products then were sequenced with a Big Dye Terminator Cycle Sequencing kit with Ampli*Taq* DNA Polymerase (Applied Biosystems, Foster City, CA). Products of sequencing reactions were resolved on an Applied Biosystems (ABI3100) automated sequencer.

Length of glochidia and fecundity

Conglutinates and glochidia of *Pleurobema collina* were examined from the Dan River sub-basin, VA. The lengths of 80 glochidia of 4 female mussels from the South Mayo River, VA, were measured using a dissecting microscope with an ocular micrometer. The mean number of conglutinates per female was obtained by counting the number of conglutinates of the same 4 females. Fecundity was estimated by counting the number of glochidia from 34 conglutinates from 4 females (10 conglutinates/female

except for one female) from the South Mayo River, VA. Results were compared to those for *P. collina* from the James River system as observed by Hove (1989).

Because this research was conducted on a federally endangered freshwater mussel species, life history characteristics were verified from a low abundance of gravid females ($n = 4$). Therefore it should be mentioned that low sample sizes could decrease the statistical power needed to detect differences. However, studies by Watson (1999) and Rogers (1999) on federally endangered species have produced significant data derived from limited numbers of gravid mussels (i.e., $n \leq 4$ gravid females *Epioblasma florentina walkeri*, *Villosa perpurpurea*, and *Dromus dromas*).

Anatomy (mantle and papillae)

Underwater photographs of the mantle and papillae of live, siphoning mussels in the South Mayo River were taken by Jess Jones using a Nikonos V underwater camera using macro-lenses and Kodak 200 Ektachrome film. Mussels also were held in temperature-controlled, recirculating artificial streams with gravel-filled bottoms. When mussels displayed their mantle and papillae, photographs were taken using a Nikon CoolPix 995 digital camera using macro-lenses. Observations of coloration and texture of the mantle and papillae of *P. collina* ($N > 25$) from SFMR were compared with those of Hove (1989) for *P. collina* from the James River system.

Fish host specificity

Laboratory experiments were conducted to identify potential fish hosts for *P. collina*. Gravid females were collected from the South Fork Mayo River in the above-

mentioned reaches. No gravid females of *P. collina* from the Dan River, NC were used for fish host analyses because of endangered species permit restrictions by North Carolina Wildlife Resources Commission. Fish host specificity was assessed using 7 species of cyprinids; bluehead chub *Nocomis leptocephalus*, rosyside dace *Clinostomus funduloides*, satinfin shiner *Notropis analostanus*, rosefin shiner *Lythrurus ardens*, central stoneroller *Campostoma anomalum*, blacknose dace *Rhinichthys atratulus*, and mountain redbelly dace *Phoxinus oreas*, which previously had been identified as natural hosts for *P. collina* (Hove 1990). Common and scientific names follow Robins et al. (1991) for fishes, and Turgeon et al. (1988) for mussels.

Methods for infesting fish with mussel glochidia followed those of Zale and Neves (1982). A plastic container 29 cm long, 19 cm wide, and 12 cm deep was filled with 1500 mL of water to hold fish during 1 hr infestations; water was aerated and agitated with an airstone. From 1-25 fish of each species were infested with glochidia from one mussel which were added to the container. Four trial infestations were conducted, each trial with glochidia from one of 4 female mussels. Not every potential host was used in every trial. After infestation, fish were separated by species and placed in 38 L aquaria at densities of 5-10 per aquarium to allow transformation of glochidia to juveniles. Contents from the bottoms of aquaria were siphoned every 2 to 3 d until juvenile mussels were collected; then siphoning occurred every 1 to 2 d until juveniles completed excystment from fish, or until it was confirmed that no juveniles had transformed.

DATA ANALYSIS

DNA sequences

Mitochondrial and nuclearDNA markers were examined to investigate genetic variation of populations within and among rivers where James spinymussel currently occurs. All analyses were done separately for results of the mtDNA markers (*cytochrome-b* and *ND-1*) and nDNA marker (*ITS-1*). DNA sequences were edited and aligned using the program SEQUENCHER (version 3.0, Gene Codes Corporation, Ann Arbor, MI). DNA sequence variation was summarized by identifying haplotypes and assigning each individual to a specific haplotype. I also calculated basic indices of population diversity; i.e., number of polymorphisms, number of haplotypes, and nucleotide diversity (π), using the ARLEQUIN software (Schneider *et al*. 2000). The spatial distribution of genetic variation was assessed using an analysis of molecular variance (AMOVA) as implemented in ARLEQUIN, with sequence variation partitioned into within- and among-population components. One thousand permutations were used to provide boot-strapped significance tests for each of the variance components. For more specific measures of pair-wise population differentiation, I estimated conventional F_{ST} indices from haplotype frequency data (with related *P* values) and Kimura two-parameter (K2P) genetic distances among haplotypes for *ND-1* (Kimura 1980). I used the Jukes-Cantor model of nucleotide substitution for estimating *ITS-1* genetic distances (Swofford 1998). Minimum spanning networks were constructed based on the minimum number of nucleotide mutations between different mitochondrial and nuclear haplotypes. The networks were constructed using NETWORK, version 6.1.0.0 software

(www.fluxus-engineering.com), employing the median joining approach (Bandelt *et al*. 1999).

Phylogenetic analyses were performed using PAUP*, version 4.0b2 (Sinauer Associates, Sunderland, MA). Phylogenetic trees were constructed by maximum parsimony (MP) and minimum evolution (ME) approaches (Nei and Kumar 2000). The MP tree was constructed using a full heuristic search with ACCTRAN and TBR options; insertions and deletions were treated as missing data. The ME tree was constructed using K2P and Jukes-Cantor genetic distances and the neighbour-joining algorithm, followed by tree-bisection-reconnection branch swapping (Nei and Kumar 2000). Because in-group taxa were closely related, characters were treated as unordered and of equal weight (Nei and Kumar 2000). Bootstrap analyses (1000 replicates) were conducted using the FAST step-wise addition option of PAUP* to assess support for the individual nodes of each phylogenetic tree (Felsenstein 1985). Separate analyses of each gene sequence were conducted.

Sequences from mtDNA and nDNA then were combined for analysis in a total evidence approach (Kluge 1989). This approach combined the sequence data from all three genes to enhance resolution of phylogenetic relationships. The in-group taxa were *P. collina* (DR), *P. collina* (SFMR), *P. collina* (Wards Crk, WDCK), and *P. collina* (South Potts Crk, SPCK). The Tar spinymussel *Elliptio steinstansana* from Swift Creek, NC, and the Cumberlandian combshell *Epioblasma brevidens* from the Clinch River, VA, were designated as the out-groups (Table 1).

Length of glochidia and fecundity

Mean lengths of glochidia from females of the Dan River sub-basin were compared to Hove's (1990) estimate of mean lengths of glochidia from the James River system using a two-sample *t*-test (Minitab Inc. 2000). Estimated fecundities from population samples were compared using analysis of variance (ANOVA), with number of glochidia per conglutinate replicates nested within female mussels (SAS Institute 2001). Log transformations were performed on data prior to analysis.

Fish host specificity

The degree of fish host specificity among populations was quantified as the mean number of juvenile mussels transformed per fish for each cyprinid species. Results were adjusted so as not to be biased by the unbalanced number of fish per tank per infestation, and then compared using ANOVA (SAS Institute 2001). To compare fish species separately for each river system, I tested the interaction of fish species separately by rivers in a post-ANOVA procedure termed 'simple effects'. Log transformations were performed on data prior to analysis.

RESULTS

Valve characters

A total of 115 *P. collina* valves was qualitatively examined. The shell characters of *P. collina* from the Dan River drainage was nearly identical to that examined from the James River drainage; no difference was observed (Figure 2). Valves were sub-

rhomboidal in shape, and rounded anteriorly, with an obliquely sub-truncated posterior with widely spaced concentric annuli. The background color of the periostracum was yellow to dark-brown (juvenile to adult), and the nacre color was light-orange to pink antero-ventrally and iridescently bluish posteriorly. The spines, when present, usually were arranged symmetrically. James spinymussels observed from the Dan and Mayo rivers (164 individuals) (personal observations 2002; Savidge 2002) generally have spines, and as many as 8 spines have been observed on one individual (Figure 3). Spines were normally one to three millimeters high. With age, the shell became even more ovate or even arcuate, the periostracum became dark brown, and any spines that were once present became lost. Other similar characteristics included a light orange mantle and foot, and well-developed hinge teeth. Shells varied in color and size throughout their ranges in the Dan and James drainages. Hence, no consistent differences were observed among collected shell material and live specimens for *P. collina* between these two major drainages.

Phylogenetic analysis of DNA sequences

Mitochondrial DNA (*cytochrome-b*)

I was able to consistently amplify 357 base-pairs (bp) for the mitochondrial *cytochrome-b* gene for 86 *P. collina* specimens. Results from *cytochrome-b* sequence data revealed no nucleotide variation within or among all four populations.

Mitochondrial DNA (*ND-1*)

I was able to consistently amplify a total of 916 bp for the mitochondrial *ND-1* gene for 81 specimens of *P. collina*. Five polymorphisms and 5 distinct haplotypes were identified. Haplotypes resolved, frequency of each haplotype, and indication of haplotypes shared among populations are presented in Table 3. No indels occurred in *ND-1*, which provided additional evidence for phylogenetic homology among DNA haplotypes, even though indels were not included in any phylogenetic analysis. Observed nucleotide site variation defined only 5 haplotypes in the *P. collina* examined. The greatest number of DNA haplotypes observed was 4 in the South Fork Potts Creek population, with haplotypes 3, 4, and 5 the most distinct. The least number of haplotypes observed was 1 (haplotype 1) in the Wards and S. Mayo populations. Haplotype 1 was identical in 62 individuals out of 80 sampled in all populations, indicating a low level of nucleotide variation at this region. The *ND-1* sequence region showed the *P. collina* populations sampled were monophyletic with little to no population structure.

Inspection of haplotype frequencies in Table 3 reveals haplotype frequency differences between the Dan River population and all other populations, with a large percentage (68.2%) of haplotype '2' unique to the Dan River population. Haplotypes '3' (5.9%), '4' (2.9%), and '5' (2.9%) were unique to the South Fork Potts Creek population at small frequencies. Haplotypes 3, 4, and 5 were unique not only among populations but also within the South Fork Potts Creek population (i.e., with a frequency of one or two individuals). Haplotype '1' was shared among all populations, with 100% frequency (fixed for all individuals) in both the Wards and South Mayo populations. Results from AMOVA showed high levels of both intra- and among-population variability, with 48.4% of variation in *P. collina* within populations and 51.6% among populations.

Since haplotypes at a low frequency could by chance be sampled in one population but not another, interpretation was repeated using a more conservative analysis, focusing on haplotypes observed in more than one individual. Applying this approach, there was considerable overlap between all populations and individuals sampled. Haplotype "1" occurred in 100% of the Wards and South Mayo individuals, 88.2% of the South Fork Potts Creek individuals, and 31.8% of the Dan mussels. This result suggests relatively uninterrupted gene-flow historically, with subsequent isolation and random genetic drift among these four populations.

Pairwise F_{ST} values indicative of significant (P<0.05) differentiation were observed between the Dan River population and all other populations (Table 4). No F_{ST} values among the Wards, South Fork Potts Creek, and South Mayo populations suggested significant differentiation (P=0.324-0.991). Since only one haplotype was observed in these populations, the relative uniqueness of the Wards (James system) and South Mayo (Roanoke system) populations may reflect loss of ancestral genetic diversity rather than an indication of adaptive differentiation. By contrast, the apparent distinctiveness of the Dan River population reflects the presence of the unique haplotype '2' at a high frequency (68.2%), suggesting that differentiation due to a localized mutation at high frequency or genetic drift between this population the other three populations. The presence of three unique haplotypes in the South Potts population is suggestive of a stable population currently having a large, long-term effective population size. However, all four populations of *P. collina* may have experienced a prolonged or severe demographic bottleneck through the decline of a large ancestral population in recent historical times.

The K2P genetic distance among individuals *within* populations ranged from 0-0.91, with an average of 0.29 (Table 4); and *between* populations ranged from 0-0.91, and averaged 0.46. A physical stream distance of 39 river kilometers (rkm) separates the South Mayo and Dan populations (Roanoke drainages); a distance of 459 rkm separates the South Potts and Wards populations (James River drainages). Hence, these four populations are geographically isolated not only between the two major drainages, but also from other populations within the same watersheds. Patterns of genetic distance and stream distance did not reflect isolation-by-distance, apart from the Dan River population. Inspection of genetic distances in Table 4 suggests a significant difference in K2P values between the Dan River populations and all other populations ($P<0.05$).

The minimum spanning network constructed from *ND-1* haplotypes (Figure 4) showed that haplotype '1' appears to be the ancestral haplotype from which the other four haplotypes derived. This network did not group *P. collina* individuals along unambiguous population boundaries. However, there is a grouping of Dan River individuals, with 15 of 22 individuals sampled within 2 mutational steps from a single haplotype (haplotype '1'), and 3 individuals of 34 sampled from South Fork Potts Creek within 1 mutational step from haplotype '1'. This grouping indicates some genetic distinctiveness for the Dan River population, which suggests the beginnings of evolutionary divergence from the common ancestral haplotype '1'. The South Fork Potts Creek population showed some degree of relatedness, with one mutational step between haplotypes '3, 4, and 5' and haplotype '1'. In constrast, the *ND-1* haplotypes from the South Mayo and Wards populations were identical and fixed for haplotype '1'.

Indices of nucleotide diversity within populations (Table 3) show no diversity in the South Mayo River and Wards Creek populations. Values for the other populations were 6.2% for South Fork Potts Creek and the most diversity, 14.6%, in the Dan River population.

The phylogenetic analysis of haplotypes using MP and ME produced nearly identical tree topologies (Figures 5 and 6). Only one ME tree was retained, which scored 0.31. The MP analysis resulted in equally parsimonious trees of 239 length (Consistency Index = 1.0, Retention Index = 1.0). The in-groups and out-groups were recovered as monophyletic lineages in both the MP and ME trees and were well supported by bootstrap values.

Moritz (2002) established criteria for the recognition of ESUs that require reciprocal monophyly for mitochondrial loci. Observation of shared haplotype '1' in Table 1 does not demonstrate unambiguous fixation for different sets of haplotypes between most pairs of populations.

Nuclear DNA (ITS-1)

A total of 502 bp was amplified for this nuclear gene for 69 *P. collina* specimens, with 15 polymorphisms and 13 distinct haplotypes identified. Haplotypes resolved, frequency of each haplotype, and indication of haplotypes shared among populations are presented in Table 5. The greatest number of observed DNA haplotypes was 8 in the South Fork Potts Creek population, with haplotypes 8, 9, and 10 the most distinct from other haplotypes. The smallest number of haplotypes observed was 1 (haplotype 1) in the Wards population, suggestive of loss of ancestral genetic diversity. This nuclear region

showed *P. collina* to be monophyletic when analyzed alone. Given the overlap in haplotype frequencies, *ITS-1* DNA sequences did not differentiate the four populations sampled.

Haplotype 4 was shared among South Fork Potts Creek, Dan, and South Mayo populations of *P. collina* (at frequencies of 10.7%, 4.4%, and 45.5%, respectively). Haplotype 2 was shared among the Dan and South Fork Potts Creek populations at frequencies of 91.3% and 10.7%, respectively. Of haplotypes occurring at lower frequencies, the South Fork Potts Creek population had the highest number (6) of unique haplotypes (Table 5). Results from AMOVA showed that most variation for *ITS-1* in *P. collina* resided within populations, with 52.3% variation within populations and 47.7% among populations.

Pairwise F_{ST} values indicative of significant ($P<0.05$) differentiation were observed among all four populations (Table 4). This may be due to the high number of unique haplotypes occurring at low frequencies in the South Mayo and South Fork Potts Creek populations. Average Jukes-Cantor genetic distances within populations ranged from 0-2.17 and averaged 0.72 (Table 4). Jukes-Cantor average distances between populations ranged from 0.23-1.28 and averaged 0.79. No pattern of isolation-by-distance was evident when comparing Jukes-Cantor values and stream distances.

The minimum spanning network from *ITS-1* data did not follow population boundaries (Figure 7). All haplotypes scored, with the exception of haplotypes 8 and 9, could be linked by three or fewer mutational steps to haplotypes 2 and 4. Haplotype 1 appears to be 'fixed' among the 7 individuals sampled from the Wards population, suggesting genetic drift following prolonged or severe population bottleneck events.

For gene diversity within populations, results from *ITS-1* showed the greatest nucleotide diversity (15.7%) in the Dan River population (Table 5), with lower values for the South Mayo and South Fork Potts Creek populations (10.5-4.0%) and no diversity (0%) in the Wards Creek mussels.

The phylogenetic analysis of haplotypes using MP and ME produced nearly identical tree topologies (Figures 8 and 9). Only one ME tree was retained, which scored 0.31. The MP analysis resulted in equally parsimonious trees of 149 length (CI = 0.94, RI = 0.59). The in-groups and out-groups were recovered as monophyletic lineages in both the MP and ME trees and were well supported by bootstrap values.

As with *ND-1*, examination of shared haplotypes show no fixation for different sets of haplotypes between any pair of populations, and thus no population meets the molecular criteria of Moritz (2002) for unambiguous classification as a unique ESU.

Combined mitochondrial and nuclear DNA sequences

DNA sequence data from combined mitochondrial DNA regions of *cytochrome-b* (357 bp), *ND-1* (916 bp), and from the nuclear DNA region *ITS-1* (502 bp), revealed 18 variable sites, which were phylogenetically informative under maximum parsimony analysis. Observed nucleotide site variation defined 18 haplotypes among 65 specimens of *P. collina* examined. The greatest number of observed DNA haplotypes was 11 in the South Fork Potts Creek population, with haplotypes SP9-12 and SP14-18 the most distinct. The smallest number of haplotypes observed was 1 in the Wards population (haplotype WD13), which was unique to this population, probably due to a locally

common mutant haplotype or a decrease in genetic diversity, rather than to adaptive genetic differentiation.

Haplotype 4 was shared among the Dan and South Fork Potts Creek populations; haplotype 5 was shared among the South Mayo and South Fork Potts Creek populations. Therefore, all haplotypes of combined sequences were not unique to each population. Shared haplotypes 4 and 5 indicated a low level of nucleotide variation. Furthermore, combining the mt-and nDNA sequence regions showed *P. collina* to be a globally monophyletic lineage. The out-group taxa *Epioblasma brevidens* and *Elliptio steinstansana* were clearly differentiated from the in-group taxon, *P. collina*.

The phylogenetic analysis of haplotypes using ME and MP produced nearly identical tree topologies (Figures 10 and 11). Only one ME tree was retained, which scored 0.33. The MP analyses of the combined sequence data resulted in 14 equally parsimonious trees of 491 length (CI = 0.97, RI = 0.84). The putative species group *P. collina* was recovered as a monophyletic lineage in both the ME and MP trees, which were well supported by bootstrap values.

Length of glochidia and fecundity

Mean lengths of glochidia were significantly different ($p<0.05$) between the Dan and James river populations (Table 6). Glochidia of Dan River *P. collina* were longer, averaging 196.5 μm, while those of James River *P. collina* averaged 190 μm. This difference (6.5 μm) may be an effect of female size (i.e., length mm) on fecundity. Significant differences also were observed in the variances (SD). For example, length varied from 153 to 243 μm (SD = 21.4) for glochidia of Dan River *P. collina*. Valves of

glochidia were subovate and symmetrical; exterior surface was smooth with very few "pits". Glochidia were transparent and colorless, and the dorsal hinge was slightly convex. No discernible difference in glochidia shape or color was observed between populations in drainages.

No significant difference was observed in fecundity between the Dan and James river populations of *P. collina* ($p>0.05$) (Table 7). Similarly, no significant difference was observed in mean number of conglutinates per female between the river drainages ($p>0.001$) (Table 8), and no discernible difference was observed in conglutinates released by female mussels between river drainages. Conglutinates released were sub-cylindrical, thin, and compressed. A tan-colored ribbon of pigmentation ran down the center of the conglutinate, and the glochidia were arranged in two staggered rows around its perimeter. The tips of conglutinates were generally rounded (Figure 12). There were varying degrees of pigmentation in any group of conglutinates released by a gravid female, but no consistent difference in conglutinates between these two major drainages was observed. Conglutinates were not pigmented medially, and the only color present was a white outline around the conglutinate, from the translucent glochidia arranged around the perimeter of the conglutinate (Hove 1990; this study).

Anatomy (mantle and papillae)

The mantle and papillae of *P. collina* from the Dan River drainage were nearly identical to those examined from the James River drainage; no discernible difference was observed (Figure 13). As described by Neves and Clarke (1984), the foot and mantle were conspicuously light orange, and the mantle was darkly pigmented in a narrow band

around and within the edges of the branchial and anal openings. I examined one fresh dead individual from the South Fork Mayo, VA, 2003. The branchial opening was surrounded at the edge and within the edge by many (>50) simple, large (up to 1.5 mm long) and small papillae, arranged principally in a double row. The anal opening was surrounded by a single row of approximately 50 short (< 0.5 mm), triangular papillae, which were little more than crenulations (Figure 14). Clarke and Neves (1984) provided a complete description of anatomy.

Fish host specificity

Little difference was observed (p>0.05) in fish host specificity between Dan and James river populations of *P. collina* (Table 9). *Clinostomus funduloides* and *Lythrurus ardens* were the only cyprinids tested that exhibited significant differences in numbers of transformed juveniles between drainages (p = 0.0008 and 0.0202, respectively). The differences may be attributed to the high fish mortality in Hove's (1990) fish host tests. Glochidia of Dan River *P. collina* transformed in greatest numbers on the bluehead chub *Nocomis leptocephalus*, which produced an average of 61% of the juveniles obtained from the seven host fish species. These findings corroborate those of Hove (1990), who also found *N. leptocephalus* to be the most suitable host for *P. collina* in the James River system.

DISCUSSION

Valve characters

Phenotypic characters of shell morphology appear fixed within and among populations of *P. collina*. That is, there was an overall lack of geographic variation in periostracum and nacre color in shells of *P. collina*, an observation supporting the view that these characters are heritable and biologically meaningful. It is unlikely that such characters could be maintained over a wide geographic range, of varying environmental conditions, without a genetic basis (see Ch. 12 in Hallerman 2003).

Phylogenetic analysis of DNA sequences

Phylogenetic analysis of the combined mtDNA and nDNA sequences did not lead to my rejecting the null hypothesis that the James River and Dan River populations are genetically similar. Phylogenetic analysis of sequence data showed the in-group taxon to be one distinct species, *P. collina*. From the set of diagnostic molecular and phenotypic characters, it was apparent that the DNA sequences were not unique to their respective source populations. This finding suggests the existence of a monophyletic lineage with a single common ancestor that presumably traces back to the Pleistocene with a stream capture event that took place between the headwaters of the Roanoke and James river systems (Campbell 1896; Johnson 1970). This is concordant with the observed lack of phenotypic variation in morphology, anatomy, and life history characteristics. Random genetic drift appears to have played a role in causing low levels of genetic variation for all molecular markers used in this study. Convergence of tree topologies in both

molecular markers, *ND-1* and *ITS-1*, suggested agreement between a species-tree and gene-tree, which could be uncommon for closely related species that have been reproductively isolated for a long time (Avise 2000, Hartl 2000, Nei and Kumar 2000). The phylogenetic relationship implied by tree topologies (Figures 9 and 10) is likely correct, aligned with the observed lack of phenotypic variation.

For inter-specific comparisons in unionids, DNA sequence divergence of 3-6% is typical (Lydeard *et al.* 1996; Roe and Lydeard 1998, Roe *et al.* 2001, Serb *et al.* 2003). Estimates of inter-and intra-specific genetic distances were less than 3% for populations of *P. collina* (Table 4). Genetic distance estimates are dependent on the amount of time elapsed since reproductive or geographic isolation of populations (Nei and Kumar 2000). If an average rate of mtDNA evolution of 2% per million years is assumed, then 330 base pair (bp) substitutions would be expected per million years in a 16,500 bp mtDNA molecule. If the entire molecule is surveyed, this corresponds to 3.3 substitutions per 10,000 yr. Therefore, even with complete isolation, populations that have colonized habitats since the end of the Pleistocene will show little divergence (Billington 2003; Jones 2004). The limited amount of genetic divergence observed in populations of *P. collina* is consistent with the theory that populations may have colonized habitats since the end of the Pleistocene. For example, the endangered Higgins' eye pearlymussel (*Lampsilis higginsii*) in the Upper Mississippi River and a few large tributaries from Minnesota and Wisconsin in the north, to Iowa and Illinois in the south, exhibited high levels of genetic variation at mtDNA markers within the populations, but no genetic differentiation among the populations (Bowen and Richardson 2000).

Another excellent example of closely related species that have been difficult to characterize genetically include *Epioblasma capsaeformis* found in the Duck and Clinch rivers, separate tributaries of the Upper Tennessee River. These were clearly two morphologically and behaviorally (phenotypically) distinct species, but contained nearly identical haplotypes when mt- and nDNA regions were analyzed separately (Jones 2004). Other examples include African cichlids in Lake Malawi (Stauffer *et al*. 1995), finches in the Galapagos Islands (Nei 1987), pupfishes in Death Valley (Echelle and Dowling 1992; Duvernnell 1998), and sturgeons in the Mobile River basin (Avise 2000).

Among recently diverged taxa, such as certain groups of freshwater fishes and mussels, a limited molecular survey of the mtDNA genome may not contain sufficient genetic variation to discriminate "species- or population-level differences" (Jones 2004). According to Jones (2004), coalescence of the *Epioblasma* taxa into their respective monophyletic lineages only was achieved by sequencing ~1900 bp of DNA sequences. Use of only one gene's DNA sequence was insufficient to discriminate among *Epioblasma* species with high statistical support, which can result in unresolved paraphyletic trees, such as reported in Jones (2004) and Buhay *et al.* (2002). This assertion relates to my phylogenetic inference. First, the separate analysis of two mtDNA sequence genes and one nDNA gene was sufficient to characterize the populations of *P. collina* as one discrete species and into its respective monophyletic lineage. Second, the DNA sequence data were congruent with morphological, anatomical, and life history characters. Thus, sufficient evidence from the convergence of independent and discrete phenotypic and genotypic characters supported and enabled the phylogenetic inference among populations of *P. collina* to be made.

In this study, certain DNA sequence regions contained more genetic variation than others, i.e., *ND-1* and *ITS-1*, while *cytochrome-b* revealed no variation. This highlights the need for a better understanding of DNA sequence variation among unionids, especially in the mtDNA genome (see Jones 2004). In the future, analysis of the complete mtDNA sequence regions of *cytochrome-b* and *ND-1* and other regions with potentially higher rates of nucleotide substitution, such as the control region, should be targeted (Serb and Lydeard 2003).

With sufficient molecular markers, modern DNA techniques have the power to discriminate between individuals, populations, species, and higher taxonomic groups. Thus, groups of individuals within populations and many geographically and demographically independent populations potentially can be diagnosed as monophyletic. Monophyly is currently used to define phylogenetic species, and since monophyly or diagnosable units are relative concepts, caution should be used when interpreting sequence data. For closely-related species, sequence data are useful for evaluating evolutionary states of populations and delineating phylogenetic species relationships (Zhang and Hewitt 2003). However, additional analyses of multi-locus nuclear markers, such as microsatellites, are usually essential for taxonomic questions involving closely-related species or populations (Jones 2004). Further genetic analyses using large sample sizes of all of the remaining extant subpopulations of *P. collina* with a suite of co-dominant nuclear markers to assess levels of gene-flow between populations are strongly recommended. Data obtained from hyper-variable DNA microsatellites may provide additional evidence to assess whether populations of *P. collina* are genetically distinct from one another. However, these populations are morphologically indistinguishable and

share many important biological traits. Complex morphological and life history traits were maintained by once-sympatric populations throughout the James and Roanoke river systems, which supports the inference that historically these populations were large and relatively undisturbed until bottlenecked by first glacial and then anthropogenic factors. Study populations are demographically separated by 39 to several hundred river kilometers. Therefore, reproductive isolation does not adequately explain the low levels of variation at mtDNA and nDNA markers maintained by these populations. Thus, reduction of haplotype frequency diversity through random genetic drift caused by glacial and anthropogenic impacts may help to explain the overlap in haplotypes and high F_{ST} values among some of the four populations.

Length of glochidia and fecundity

The greater length of glochidia from females collected in the Dan River appears to be the only significant quantitative difference between this population and that of *P. collina* in the James River system. The variation in mean lengths of glochidia is a statistically significant difference of only 6.5 μm (Table 6). Phenotypic and quantitative genetic characters can vary in response to environmental conditions, or due to other genes (alleles) that individuals carry (Ch. 12 in Hallerman 2003). An investigator must decide whether a difference of 3% is biologically meaningful or simply due to environmental effects. Furthermore, this difference (6.5 μm) may be due to a bias in size (i.e., length in mm) of females collected between rivers. Hove (1990) measured lengths of 80 glochidia from 8 female mussels of varying lengths (10 glochidia per female), whereas I measured

lengths of 80 glochidia from 4 female mussels of varying length (20 glochidia per female).

No significant variation in fecundity estimates of female *P. collina* from the Dan and James river systems suggests no quantitative genetic difference between populations for this life history trait (Table 7). Fecundity estimates may prove to be the most significant piece of data derived from the limited life history information. Typical estimates of unionid fecundity range from 100,000 to 3.5 million (Yeager & Neves 1986; Neves & Widlak 1988), and 200,000 to 17 million (McMahon 1991), depending on the species and size of the female. However, fecundity estimates of *P. collina* were low (i.e., approximately 9,000 to 11,000) when compared to other mussel species (Hove 1990; see Rogers 1999; Watson 1999; and Jones 2004). This estimate is significantly less than the lowest fecundity values typically reported for unionids. Thus, if high population densities are required for successful reproduction the "Allee effect" (inability to reproduce if too few individuals are present) may impact breeding if the population drops below the required density (Allee *et al*. 1949). Low fecundity, combined with host specificity and environmental perturbations most likely has contributed to the federal endangered status for the James spinymussel.

Anatomy (mantle and papillae)

The lack of discrete phenotypic variation in mantle color and papillae color, number or size likely represents classical Mendelian trait congruence between populations of *P. collina* in the Dan and James river drainages. There was an overall lack of geographic variation, an observation supporting the view that these characters are

heritable. It is unlikely that these characters could be maintained or fixed over a wide geographic range, of varying environmental conditions, without a genetic basis. Additional observations on mantle and papillae for this species are reported in Johnson (1970) and Clarke and Neves (1984).

Fish host specificity

The morphology and coloration of mantle, papillae, and conglutinates of *P. collina* may be adaptively significant and may indicate how species persist in certain environments and attract fish hosts. The cryptically colored mantle and papillae appear adapted to a range of habitats where displaying females are camouflaged in shallow-to-medium depth, in small-to-medium width streams, which can provide protection against predation.

Conglutinates of *P. collina* may be effective for attracting cyprinid fish hosts, such as *Nocomis leptocephalus*, that feed upon insects. This minnow, as well as other closely related fish species belonging to the family Cyprinidae, co-occurs in abundance with *P. collina* in the Dan and James river drainages. Female *P. collina* released wormlike conglutinates, which mimicked prey items that attracted host fish and elicited feeding responses from fishes in the laboratory, both in this study and in Hove (1990). Fertile eggs were located on the surface and ruptured to release the glochidia when a host fish bit the conglutinate. Conglutinates were durable, adhesive, and stuck to the substratum.

In many conglutinate-producing species (e.g. *Fusconaia*, *Pleurobema*, *Plethobasus*, and *Cyprogenia*), a large fraction of the eggs are not fertilized or normally

do not develop. These sterile (structural) eggs appear to increase the durability and visibility of conglutinates. Improved host fish infestation by conglutinates bearing sterile eggs presumably offsets the consequent reduction in the number of larvae that are produced (Haag & Warren 2003). A high percentage of eggs of *P. collina* were undeveloped or unfertilized, although this observation may not indicate poor fertilization. In this and many other conglutinate-producing species, the structural matrix of conglutinates is formed by cohesion between unfertilized eggs (Haag & Warren 2003). Thus, the prevalence of undeveloped eggs is characteristic of these and other species that produce this type of conglutinate (e.g. Layzer *et al.* 2003).

Fish host specificity may be a major quantitative genetic trait for freshwater mussels, and variation is likely to be fitness-related and may serve to isolate mussel populations geographically, ecologically, and ultimately, reproductively. The fish host specificity data produced in this study support the inference that cyprinids, notably *Nocomis leptocephalus*, are quantitatively better hosts for populations of *P. collina* in the Dan and James drainages (Table 9). Though populations of *P. collina* are geographically and reproductively isolated, similarity in fish host specificity among populations of *P. collina* may be due to the cyprinids that co-occur in abundance with *P. collina* in the Dan and James river drainages.

Designation of conservation units and management implications

My results indicate that *P. collina* from the Dan River sub-drainage are not separate species because they shared the following traits with *P. collina* from the James River drainage: (1) yellow to dark-brown periostracum (juvenile to adult), (2) light

orange nacre, mantle, and foot, (3) number and arrangement of spines (if present) and papillae, (4) similar fish host specificity, (5) mean number of conglutinates per female, (6) similar fecundity estimate, and (7) many shared mitochondrial and nuclear DNA sequences. The populations had similar, but different-sized glochidia and were a monophyletic lineage based on the molecular phylogeny. However, because of the genetic distinctiveness of several haplotype frequencies between populations of *P. collina* in the Dan and James drainages and isolated geographic ranges, I propose that populations in the Dan and James rivers be regarded as separate Management Units (MUs), unless and until analysis of allele frequency at microsatellite loci or measurement of other traits can provide evidence of adaptive genetic differentiation as an ESU.

Moritz (2002) set specific molecular genetic criteria for recognition of ESUs. Populations sampled in the current study were not *reciprocally monophyletic* for mtDNA and did not show *significant divergence of allele frequencies* at nuclear loci. Vogler and DeSalle (1994) considered a biological unit an ESU only if all individuals in the unit shared at least one heritable trait not found in any individuals from any other units. One population examined met this criterion. There was a unique haplotype frequency in all individuals in the Wards population (nDNA), but this unique haplotype frequency was most likely a localized variant due to loss of ancestral genetic diversity. At this time, substantial genetic variation was not documented in *P. collina* across their range to justify designating any population sampled as an ESU.

Waples (1991) proposed that a population must be *reproductively isolated* from other conspecific units and represent an important component of the *evolutionary legacy* of the species to qualify as an ESU. To meet the latter criterion, the population must: (1)

be genetically distinct, (2) occupy unique habitat, (3) exhibit unique adaptation to its environment, or (4) pose a significant loss to the ecological or genetic diversity of the species if it became extinct. Because the populations of *P. collina* sampled in this study appeared geographically and demographically independent, but not genetically independent, the criterion of reproductive isolation was met, but the second criterion was not met. The level of historical connectedness among these related populations supports this conclusion. Due to the current level of disjunct, fragmented populations and the complex modes of reproduction of unionids, direct tests of reproductive isolation are unlikely in the near future. The best scientific data were used in this study to reasonably identify *P. collina* as a biological species with multiple MUs. The lack of direct data on reproductive isolation should not prevent this diagnosis. I recommend that management agencies recognize the proposed MUs when implementing the revised recovery plan of *P. collina* and manage the species based on appropriate geographic and genetic data.

The James and Dan river drainages harbor various sizes of localized populations of *P. collina*, isolated from conspecific populations by pollution barriers and impoundments, as well as absolute geographic distance. The pattern of differentiation among populations does not reflect a simple pattern of isolation-by-distance. It is difficult to determine whether the genetic uniqueness of the Wards population reflects historical evolutionary processes or recent fragmentation. According to Liu *et al. (*2003), lack of contemporary gene flow among hydrographically separated populations may result in pronounced geographical structuring in freshwater mussels. Considering that barriers to gene flow may have existed since the Pleistocene (the time period that spanned from 1.8 million to 11,000 years ago), it seems likely that substantial molecular

population differentiation should have developed as a result of this influence on gene flow. Yet, it appears that populations within the same drainage separated by impoundments that have existed for less than a century are more differentiated than populations among drainages that have been geographically isolated for a longer time.

Because of *P. collina*'s current isolated ranges within the Dan and James river systems, which are fragmented by degraded habitat (urbanization and other human land use), the populations are subject to reduced gene flow and the loss of genetic variation. There is no potential for genetic exchange among populations, if fish hosts do not disperse between/among tributaries because of pollution or distance barriers. The continuity in the life history process has been disrupted (Noss and Csuti 1997). Without immigration through fish host dispersal of glochidia, some *P. collina* populations may not be large enough to avoid future loss of genetic variability through genetic drift. This loss of genetic variation may reduce a species' 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 (via captive propagation and translocation). Designation of populations as MUs can provide the means to ensure that natural and artificial immigration can occur and be managed.

At this time, genetic haplotype frequency data indicate no reason to restrict reciprocal exchanges of *P. collina* from the South Mayo and Dan river MUs, should any of these populations become candidate source or recipient populations for re-establishment or augmentation. The James spinymussel population within the South Fork Potts Creek MU should become a candidate source population if any population of *P.*

collina within the James River drainage requires population augmentation or re-establishment. However, use of *P. collina* from the James River-Wards Creek MU for re-establishments should be done only after due consideration of some apparent loss of genetic diversity. Should re-stocking become vitally important, analysis of allele frequency at microsatellite loci is recommended before reciprocal exchanges among populations among the drainages (e.g., South Mayo, Dan, and South Fork Potts) is implemented. At this time, the mixing of individuals among drainages is not recommended.

In some species such as *P. collina* where fecundity is low compared to other freshwater mussel species (Hove 1990), high population densities may be required to allow reproduction to occur. This phenomenon, the "Allee effect," may affect breeding if the population drops below the required density (Allee *et al*. 1949). According to recent surveys on population densities from the James River system, this may already be a major factor in the continuing decline of *P. collina* populations. It is imperative to prevent mussel populations from becoming too small, or recruitment may not be successful (Franklin 1980). When populations have become small or recruitment is unsuccessful, juveniles can be produced in the laboratory at a higher rate than what is experienced in the wild (Neves 1997). The recovery plan for the James spinymussel identifies improved water quality, habitat enhancement, and re-establishment of mussels into historically inhabited reaches as means for promoting recovery (USFWS 1990). Today, mussel conservationists are using artificial propagation for population augmentation and establishment for species recovery in habitats that have improved (Neves 1997).

Once again, promoting natural and artificial immigration may help to remedy the Allee effect by increasing and maintaining viable population densities over the long term. Captive culture of freshwater mussels in artificial conditions for the augmentation of natural populations may help to increase the size of declining mussel populations to a level where they can reproduce and survive on their own, given that required fish hosts are present and habitat is suitable. Also, it may be possible to re-establish historical populations of the James spinymussel if the cause of extirpation has been identified and remedied. The success of relocations in the past has been inconsistent (Cope and Waller 1995). The more that is known of mussel survival, growth, and reproduction, the better the chance of success of future conservation efforts (Neves 1997). Assessing the feasibility of captive culture of the James spinymussel in artificial conditions may prove instrumental in achieving these goals.

Concluding remarks on molecular genetic studies

Most molecular markers are selectively neutral and typically do not measure variation at loci that are adaptively significant (Hard 1995; Hallerman 2003). In this study, no variation at phenotypic and virtually no variation at quantitative markers among populations of *Pleurobema collina* was congruent with the low level of variation observed at DNA sequences. Indeed, it was the convergence of phenotypic and quantitative characters that allowed for DNA sequences to be put into perspective, and to conclude that the populations sampled were valid MUs within the species *P. collina*. Molecular genetic markers appeared to provide an adequate measure for variation, but whether the variation was at fitness-related loci is not known (Kimura 1983; Ohta 1992;

Hard 1995). The relationship between molecular genetic diversity and an animal's fitness is poorly understood (Hansson and Westerberg 2002).

When possible, taxonomic and phylogenetic studies should combine comparable information from molecular markers, morphology, life history, and biogeography (see Jones 2004). Comprehensive analyses allow biologists to seek concordance among multiple independent data sets, and to reduce errors interpreting indistinguishable or ambiguous characters (Avise 2000). Such holistic approaches are justified for endangered species, especially when study results could jeopardize or revise the status of a species (Jones 2004). For delineating freshwater mussel species, it is strongly recommended that molecular genetic studies be augmented by biologically and ecologically meaningful data from an animal's distribution, phenotype, life history traits, and important functional protein markers.

LITERATURE CITED

Allee, W.C., A.E. Emerson, O. Park, T. Park, and K.P. Schmidt. 1949. *Principles of Animal Ecology*. Sander, Philadelphia, PA.

Avise, J.C. 2000. Phylogeography: the history and formation of species. Harvard University Press, Cambridge, MA.

Bandelt, H-J., P. Forster, and A. Röhl. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16:37-48.

Berg, D.J., and P.H. Berg. 2000. Conservation genetics of freshwater mussels: comments on Mulvey *et al.* Conservation Biology 14:1920-1923.

Billington, N. 2003. Mitochondrial DNA. Pages 59-100 *in* Hallerman, E.M., (ed). 2003. *Population Genetics: Principles and Applications for Fisheries Scientists*. American Fisheries Society, Bethesda, MD.

Boss, K.J., and W.J. Clench. 1967. Notes on *Pleurobema collina* (Conrad) from the James River, Virginia. Occasional Papers on Mollusks, Museum of Comparative Zoology 3(37): 45-52.

Bowen, B.S., and W. Richardson. 2000. Genetic characterization of *Lampsilis higginsii.* Final Report to U.S. Fish and Wildlife Service, Bloomington, MN.

Buhay, J.E., J.M. Serb, C.R. Dean, Q. Parham, and C. Lydeard. 2002. Conservation genetics of two endangered unionid bivalve species, *Epioblasma florentina walkeri* and *E. capsaeformis* (Unionidae: Lampsilini). Journal of Molluscan Studies 68: 385-391.

Campbell, M.R. 1896. Drainage modifications and their interpretation. Journal of Geology 4:567-581.

Clarke, A.H., and R.J. Neves. 1984. Status survey of the James River spinymussel, *Canthyria collina*, in the James River, Virginia. Final Report, U.S. Fish and Wildlife Service, Newton Corner, MA. 73 pp.

Conrad, T.A. 1837. Monograph of the family Unionidae 8: 65.

Conrad, T.A. 1853. A synopsis of the family of naiads of North America, with notes, and a table of some of the genera and sub-genera of the family, according to their geographical distribution, and descriptions of genera and sub-genera. Proceedings of the Academy of Natural Sciences of Philadelphia 6:243-269.

Cope, W.G., and D.L. Waller. 1995. Evaluation of mussel relocation as a conservation and management strategy. Regulated Rivers Research and Management 11: 147-155.

Cracraft, J. 1983. Species concepts and speciation analysis. Pages 159-187 *in* R.F. Johnson, editor. Current Ornithology, volume 1. Plenum Press, NY.

Davis, G.M. 1983. Relative roles of molecular genetics, anatomy, morphometrics and ecology in assessing relationships among North American Unionidae (Bivalvia). Pages 193-221 *in* G.S. Oxford and D. Rollinson, (eds). *Protein Polymorphism: Adaptive and Taxonomic Significance*. Systematics Association Special Volume No. 24, Academic Press, NY.

Davis, G.M. 1994. Molecular genetics and taxonomic discrimination. Nautilus 2: 3-23.

Duvernell, D.D. 1998. Population genetics of Death Valley pupfishes (Cyprinodontidae: *Cyprinodon* Spp.) and the identification of a new retrotransposable element

family. PhD. Dissertation. Virginia Polytechnic Institute and State University, Blacksburg, Virginia. 107 pp.

Echelle, A.A., and T.E. Dowling. 1992. Mitochondrial DNA variation and evolution of the Death Valley pupfishes (Cyprinodon, Cyprinodontidae). Evolution 46:193-206.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783-791.

Franklin, I.R. 1980. Evolutionary change in small populations. Pages 135-149 *in* M.E. Soulé and B.A. Wilcox (eds.). *Conservation Biology: An Evolutionary-Ecological Perspective*. Sinauer Associates, Sutherland, MA. 395 pp.

Haag, W.R., and L.J. Staton. 2003. Variation in fecundity and other reproductive traits in freshwater mussels. Freshwater Biology 48:2118-2130.

Hallerman, E.M., Editor. 2003. Population genetics: principles and applications for fisheries scientists. American Fisheries Society, Bethesda, MD.

Hansson, B., and L. Westerberg. 2002. On the correlation between heterozygosity and fitness in natural populations. Molecular Ecology 11:2467-2474.

Hard, J.J. 1995. A quantitative genetic perspective on the conservation of intraspecific diversity. American Fisheries Society Symposium 17:304-326.

Hartl, D.L. 2000. A primer of population genetics, 3rd edition. Sinauer Associates, Inc., Sunderland, MA.

Heard, W.H., and R.H. Guckert. 1971. A re-evaluation of the recent Unionacea (Pelecypoda) of North America. Malacologia 10:333-355.

Hedrick, P.W., and K.J. Kim. 2000. Genetics of complex polymorphisms: Parasites and maintenance of the major histocompatibility complex variation. Pages 204-234 *in* R.S. Singh and C.B. Krimbas (eds). *Evolutionary Genetics: from Molecules to Morphology*. Cambridge University Press, Cambridge, U.K.

Hedrick, P.W., K.M. Parker, and R.N. Lee. 2001. Using microsatellite and MHC variation to identify species, ESUs, and MUs in the endangered Sonoran topminnow. Molecular Ecology 10:1399-1412.

Hoeh, W.R., and M.E. Gordon. 1996. Criteria for the determination of taxonomic boundaries in freshwater unionids (Bivalvia: Unionoida): comments on Stiven and Alderman (1992). Malacologia 38: 223-227.

Hoeh, W.R., A.E. Bogan, and W.H. Heard. 2001. A phylogenetic perspective on the evolution of morphological and reproductive characteristics in the Unionoida *in* G. Bauer and K. Wachtler (eds). *Ecology and Evolution of the Freshwater Mussels Unionoida*. Ecological Studies, Vol. 145. Springer-Verlag, Berlin and Heidelberg 2001.

Hove, M. 1990. Distribution and life history of the endangered James spinymussel, *Pleurobema collina* (Bivalvia: Unionidae). M.S. Thesis. Virginia Polytechnic Institute and State University, Blacksburg, Virginia. 113 pp.

Hove, M., and R. J. Neves. 1989. Life history of the James spinymussel. *In* North Carolina Wildlife Resources Commission. 1989. Population status, distribution, and biology of the Tar River spinymussel (*Elliptio* (*Canthyria) steinstansana* Johnson and Clarke), in North Carolina. Final Report to U.S. Fish and Wildlife Service.

Hove, M. C. and R. J. Neves. 1994. Life history of the endangered James spinymussel *Pleurobema collina* (Conrad, 1837) (Mollusca: Unionidae). American Malacological Bulletin 11(1): 29-40.

Johnson, R.I. 1970. The systematics and zoogeography of the Unionidae (Mollusca: Bivalvia) of the southern Atlantic Slope region. Bulletin of the Museum of Comparative Zoology Volume 140. 49 pp.

Johnson, R.I., and A. H. Clarke. 1983. A new spiny mussel, *Elliptio* (*Canthyria*) *steinstansana* (Bivalvia: Unionidae), from the Tar River, North Carolina. Occasional Papers on Mollusks, Museum of Comparative Zoology 4: 289-298.

Jones, J.W. 2004. A holistic approach to taxonomic evaluation of two closely related endangered freshwater mussel species, the oyster mussel (*Epioblasma capsaeformis*) and tan riffleshell (*Epioblasma florentina walkeri*) (Bivalvia: Unionidae). M.S. Thesis. Virginia Polytechnic Institute and State University, Blacksburg, Virginia. 177 pp.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111-120.

Kimura, M. 1983. The neutral theory of molecular evolution. Cambridge University Press, Cambridge, U.K.

King, T.L, E.C. Pendleton, and R.F. Villella. 1998. Gene conservation: management and evolutionary units in freshwater bivalve management—introduction to the proceedings. Journal of Shellfish Research 17: 1351-1353.

King, T.L., M.S. Eackles, B. Gjetvaj, and W.R. Hoeh. 1999. Intraspecific phylogeography of *Lasmigona subviridis* (Bivalvia: Unionidae): conservation implications of range discontinuity. Molecular Ecology 9: S65-S78.

Kluge, A.G. 1989. A concern for evidence and a phylogenetic hypothesis of relationships among Epicrates (Boidae, Serpentes). Systematic Zoology 38:7-25.

Layzer, J.B., B. Adair, S. Saha, and L.M. Woods. 2003. Glochidial hosts and other aspects of the life history of the Cumberland pigtoe (*Pleurobema gibberum*). Southeastern Naturalist 2:73-84.

Lea, I. 1857. Descriptions of six new species of unionids from Alabama. Proceedings of the Academy of Natural Sciences of Philadelphia 9:83.

Lefevre, G., and W.C. Curtis. 1910. Experiments in the artificial propagation of freshwater mussels. Bulletin of the Bureau of Fisheries 28:615-626.

Liu, H.P., R. Hershler, and K. Clift. 2003. Mitochondrial DNA sequences reveal extensive cryptic diversity within a western American springsnail. Molecular Ecology 12:2771-2782.

Lydeard, C., M. Mulvey, and G.M. Davis. 1996. Molecular systematics and evolution of reproductive traits of North American freshwater unionacean mussels (Mollusca: Bivalvia) as inferred from 16S rRNA gene sequences. Proceedings of the Royal Society of London Series B, 351: 1593-1603.

Mayr, E., and P.D. Ashlock. 1991. Principles of systematic zoology. McGraw-Hill, New York.

McMahon, R.F. 1991. Mollusca. Pp. 315-399 *in* J.H. Thorp and A.C. Covich, editors. Ecology and Classification of North American Freshwater Invertebrates. Academic Press, NY.

Meffe, G.K., and R.C. Carroll. 1997. Genetics: conservation of diversity within species. Page 176 *in*: G.K. Meffe and R.C. Carroll (editors). *Principles of Conservation Biology*. Sinauer Associates, Inc., Sunderland, MA.

Merritt, T.J.S., Shi, L., Chase, M.C., Rex, M.A., Etter, R.J., and J.M. Quattro. 1998. Universal cytochrome b primers facilitate intraspecific studies in molluscan taxa. Molecular Marine Biology and Biotechnology 7: 7-11.

Minitab Inc. 2000. MINITAB Reference Manual for Windows. Release 13. Minitab Inc. State College, PA.

Moritz, C. 1994. Defining 'Evolutionary Significant Units' for conservation. Trends in Ecology and Evolution 9: 373-375.

Moritz, C. 2002. Strategies to protect biological diversity and the evolutionary processes that sustain it. Systematic Biology 51:238-254.

Mulvey, M., C. Lydeard, D.L. Pyer, K.M. Hicks, J. Brim-Box, J.D. Williams, and R.S. Butler. 1997. Conservation genetics of North American freshwater mussels *Amblema* and *Megalonaias*. Conservation Biology 11: 868-878.

Mulvey, M., H.P. Liu, and K.L. Kandl. 1998. Application of molecular genetic markers to conservation of freshwater mussels. Journal of Shellfish Research 17: 1395-1405.

Naimo, T.S., E.D. Damschen, R.G. Rada, and E.M. Monroe. 1998. Nonlethal evaluations of the physiological health of unionid mussels: methods for biopsy and glycogen analysis. Journal of the North American Benthological Society 17: 121-128.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, NY.

Nei, M. and S. Kumar. 2000. Molecular evolution and phylogenetics. Oxford, University Press. U.K.

Neves, R.J. 1997. A national strategy for the conservation of native freshwater mussels. Pages 1-11 *in* K.S. Cummings, A.C. Buchanan, C.A. Mayer, and T.J. Naimo, (eds.). Conservation and management of freshwater mussels II: initiative for the future. Proceedings of a UMRCC symposium, St. Louis, MO. Upper Mississippi River Conservation Committee, Rock Island, IL.

Neves, R.J. and J.C. Widlak. 1988. Occurrence of glochidia in stream drift and on fishes of the upper North Fork Holston River, Virginia. American Midland Naturalist 119:111-120.

Neves, R.J., A.E. Bogan, J.D. Williams, S.A. Ahlstedt, and P.W. Hartfield. 1997. Status of aquatic mollusks in the southeastern United States: downward spiral of diversity. Pages 43-85 *in* G.W. Benz, and D.E. Collins (eds.). *Aquatic Fauna in Peril: the Southeastern Perspective*. Southeast Aquatic Research Institute, Chattanooga, TN.

Noss, R.F., and B. Csuti. 1997. Habitat fragmentation. Pages 269, 289-290 *in*: G.K. Meffe and R.C. Carroll (editors). *Principles of Conservation Biology*. Sinauer Associates, Inc., Sunderland, MA.

Ohta, T. 1992. The near-neutral theory of molecular evolution. Annual Reviews in Ecology and Systematics 23:263-286.

Robins, C.R., Bailey, R.M., Bond, C.E., Brooker, J.R., Lachner, E.A., Lea, R.N., and W.B. Scott. 1991. Common and scientific names of fishes from the United States and Canada, 5th edition. AFS Special Publication 20. American Fisheries Society, Bethesda, MD.

Roe, K.J. 1999. The utility of DNA sequences to aid the identification of rare or problematic species of freshwater mussels. Proceedings of the First Freshwater Mollusk Conservation Society Symposium: 197-202.

Roe, K.J, and C. Lydeard. 1998. Species delineation and the identification of evolutionarily significant units: lessons from the freshwater mussel genus *Potamilus* (Bivalvia: Unionidae). Journal of Shellfish Research 17: 1359-1363.

Roe, K.J., P.D. Hartfield, and C. Lydeard. 2001. Phylogeographic analysis of the threatened and endangered superconglutinate-producing mussels of the genus *Lampsilis* (Bivalvia: Unionidae). Molecular Ecology 10:2225-2234.

Rogers, S.O. 1999. Population biology of the tan riffleshell (*Epioblasma florentina walkeri*) and the effects of substratum and light on juvenile mussel propagation. M.S. Thesis. Virginia Polytechnic Institute and State University, Blacksburg, Virginia. 103 pp.

Rohde, F.C., R.G. Arndt, and S.M. Smith. 2001. Longitudinal succession of fishes in the Dan River in Virginia and North Carolina (Blue Ridge/Piedmont Provinces). Southeastern Fishes Council Proceedings 42: 1-13.

SAS Institute. 2001. SAS user's guide: Statistics. Statistical Analysis System Institute, Cary, NC.

Savidge, T.W. 2002. Programmatic Section 7 consultation for the James spinymussel (*Pleurobema collina*) bridge replacement projects on the Dan River (B-2639 and B-3045). North Carolina Department of Transportation Environmental Analysis Branch for the Federal Highway Administration. Raleigh, NC. 56 pp.

Schneider, S., D. Roessli, and L. Excoffier. 2000. ARLEQUIN, Version 2.000: A software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Serb, J.M., J.E. Buhay, and C. Lydeard. 2003. Molecular systematics of the North American freshwater bivalve genus *Quadrula* (Unionidae: Ambleminae) based on mitochondrial *ND-1* sequences. Molecular Phylogenetics and Evolution 28:1-11.

Serb, J.M., and C. Lydeard. 2003. Complete mtDNA sequence of the North American freshwater mussel, *Lampsilis ornata* (Unionidae): an examination of the evolution and phylogenetic utility of the mitochondrial genome organization in Bivalvia (Mollusca). Molecular Biology and Evolution 20:1854-1866.

Simpson, C.T. 1896. The classification and geographical distribution of the pearly freshwater mussels. Proceedings of the United States National Museum 18:295-343.

Simpson, C.T. 1900. Synopsis of the naiades, or pearly freshwater mussels. Proceedings of the United States National Museum 22: 501-1044.

Stauffer, J.R., N.J. Bowers, K.R. McKaye, and T.D. Kocher. 1995. Evolutionarily significant units among cichlid fishes: the role of behavioral studies. American Fisheries Society Symposium 17:227-244.

Stiven, A.E., and J. Alderman. 1992. Genetic similarities among certain freshwater mussel populations of the *Lampsilis* genus in North Carolina. Malacologia 34:355-369.

Swofford, D.L. 1998. PAUP* Phylogenetic analysis using parsimony and other methods, computer program. Sinauer Associates, Sunderland, MA.

Turgeon, D.D., A.E. Bogan, E.V. Coan, W.K. Emerson, W.G. Lyons, W.L. Pratt, C.F.E. Roper, A. Scheltema, F.G. Thompson, and J.D. Williams. 1998. Common and scientific names of aquatic invertebrates from the United States and Canada: mollusks. American Fisheries Society Special Publication. 16, Bethesda, MD. 227 pp.

United States Fish and Wildlife Service. 1990. James spinymussel (*Pleurobema collina*) Recovery Plan. Newton Corner, MA. 38 pp.

United States Fish and Wildlife Service. 1992. Endangered and threatened species of the Southeast United States (The red book). Prepared by Ecological Services, Division of Endangered Species, Southeast Region. U.S. Government Printing Office, Washington, D.C. 1070 pp.

United States Fish and Wildlife Service. 2002. Reopening of public comment period for the technical/agency draft revised recovery plan for the red-cockaded woodpecker (*Picoides borealis*). Department of the Interior, Federal Register 67: 70237-70239.

Vogler, A.P., and R. DeSalle. 1994. Diagnosing units of conservation management. Conservation Biology 8:354-363.

Waples, R.S. 1991. Pacific salmon, *Oncorhynchus* spp., and the definition of "species" under the Endangered Species Act. U.S. National Marine Fisheries Service Marine Fisheries Review 53: 11-22.

Watson, B.T. 1999. Population biology and fish hosts of several federally endangered freshwater mussels (Bivalvia: Unionidae) of the upper Tennessee River drainage, Virginia and Tennessee. M.S. Thesis. Virginia Polytechnic Institute and State University, Blacksburg, Virginia. 134 pp.

Yeager, B.L. and R.J. Neves. 1986. Reproductive cycle and fish hosts of the rabbitsfoot mussel, *Quadrula cylindrical strigillata* (Mollusca: Unionidae), in the upper Tennessee River Drainage. American Midland Naturalist 116:329-340.

Zale, A.V., and R.J. Neves. 1982. Fish hosts of four species of lampsiline mussels (Mollusca: Unionidae) in Big Moccasin Creek, Virginia. Canadian Journal of Zoology 60: 2535-2542.

Zhang, D., and G.M. Hewitt. 2003. Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. Molecular Ecology 12:563-584.

Table 1. Sample locations and sample sizes for DNA sequences investigated for four populations of *Pleurobema collina*. *Specific sites are described in methods section.

Taxa	Collection* location	Total sample size	MtDNA *cytochrome-b*	MtDNA *ND-1*	nDNA *ITS-1*
In-group taxon *P. collina*	Dan River	25	25	22	23
	S. Mayo River	20	18	18	11
	S.F. Potts Creek	34	34	34	28
	Wards Creek	7	7	7	7
Out-group taxa					
Elliptio steinstansana	Swift Creek	1	1	1	1
Epioblasma brevidens	Clinch River	1	1	1	1

Table 2. Primer sequences used to amplify mussel mitochondrial and nuclear DNA sequences using polymerase chain reaction.

	Primers	
Locus	Forward	Reverse
cyt-b	5'-TGTGGRGCNACYGTWATYACTAA-3'	5'-AANAGGAARTAYCAYTCNGGYTG-3'
ND-1	5'-TGGCAGAAAAGTGCATCAGATTAAAGC-3'	5'-CCTGCTTGGAAGGCAAGTGTACT-3'
ITS-1	5'-AAAAAGCTTCCGTAGGTGAAC-3'	5'-TTCATCGACCCACGAGCCGAG-3'

Table 3. Haplotypes (with indication of polymorphic sites), haplotype frequencies, shared haplotypes, and indices of population diversity for *ND-1* in four populations of *Pleurobema collina*.

Haplotypes and polymorphic nucleotide sites:						**Population:**			
						James River:		Dan River:	
		3	4	8	9	Wards	South	Dan	South
	1	7	6	0	1		Potts		Mayo
	2	5	9	8	5	(*n*= 7)	(*n*= 34)	(*n*= 22)	(*n*= 18)
1	T	T	T	T	G	1.00	0.882	0.318	1.00
2	T	T	**C**	**C**	G			0.682	
3	**C**	T	T	T	G		0.059		
4	T	T	T	T	**C**		0.029		
5	T	**C**	T	T	G		0.029		
Number of haplotypes:						1	4	2	1
Polymorphic sites:						0	3	2	0
Nucleotide diversity per site within population (%)						0.00 ±0.00	0.062 ±0.046	0.146 ±0.181	0.00 ±0.00

Table 4. Pairwise F_{ST}, Kimura 2-parameter (**K2P**) and Jukes-Cantor (**JC**) distance values (%) among four populations of *P. collina*, from *ND-1* (below diagonal) and *ITS-1* (above diagonal) loci. Those F_{ST} values shown in bold denote significant ($P < 0.05$) differentiation. The **K2P** and **JC** values below locality names indicate average genetic distances among individuals within populations.

	Wards JC=0.00	**South Potts** JC=2.17	**Dan** JC=0.17	**South Mayo** JC=0.55
Wards K2P=0.00		**F_{ST}= 0.45** (*P*= 0001) JC=1.15	**F_{ST}= 0.87** (P= 0.001) JC=1.00	**F_{ST}= 0.59** (*P*= 0.001) JC=1.28
South Potts K2P=0.23	F_{ST}= -0.04 (*P*= 0.820) K2P=0.002		**F_{ST}= 0.4323** (P= 0.001) JC=0.67	**F_{ST}= 0.19** (P= 0.001) JC=0.44
Dan K2P=0.91	**F_{ST}= 0.55** (*P*= 0.009) K2P=0.91	**F_{ST}= 0.55** (*P*= 0.001) K2P=0.91		**F_{ST}= 0.62** (P= 0.001) JC=0.23
South Mayo K2P=0.00	F_{ST}= 0.00 (*P*= 0.991) K2P=0.00	F_{ST}= 0.02 (*P*= 0.324) K2P=0.002	**F_{ST}= 0.64** (*P*= 0.001) K2P=0.91	

Table 5. Haplotypes (with indication of polymorphic sites), haplotype frequencies, shared haplotypes, and indices of population diversity for *IT-S* in four populations of *Pleurobema collina* (":" denotes deletions).

Haplotypes and polymorphic nucleotide sites:												**Population:**			
												James River:		Dan River:	
				3	4	4	4	4	4	4	4	Wards	South	Dan	South
				3	1	6	7	8	8	9	9		Potts		Mayo
	4	5	6	0	3	4	1	4	8	3	7	(*n*= 7)	(*n*= 28)	(*n*= 23)	(*n*= 11)
1	A	A	G	G	T	:	:	T	T	G	G	1.00			
2	A	A	G	G	T	**T**	:	T	T	G	**T**		0.107	0.913	
3	A	A	G	G	T	**T**	:	**:**	**C**	G	**T**			0.044	
4	A	A	G	**C**	T	:	:	T	T	G	**T**		0.107	0.044	0.455
5	A	A	G	G	**A**	:	:	T	T	G	G		0.107		
6	A	A	G	**C**	**A**	:	:	T	T	G	**T**		0.393		
7	A	A	G	G	**A**	:	:	T	T	G	**T**		0.107		
8	A	A	G	G	T	**T**	:	T	T	**C**	G		0.071		
9	**C**	**G**	**A**	G	T	**T**	:	T	T	**C**	G		0.071		
10	A	A	G	G	T	:	:	T	T	**C**	G		0.036		
11	A	A	G	G	T	**T**	**G**	T	T	G	**T**				0.364
12	A	A	G	**C**	T	**T**	:	T	T	G	**T**				0.091
13	A	A	G	G	T	:	:	T	T	G	**T**				0.091
Number of haplotypes:												1	3	8	4
Polymorphic sites:												0	4	8	3
Nucleotide diversity per site within population (%)												0.00 ±0.000	0.040 ±0.035	0.157 ±0.254	0.105 ±0.146

Table 6. Mean lengths of glochidia measured from female mussels of *P. collina* collected in the James and Dan Rivers, Virginia, 2004.

River	Number Females	*n* (sample size)	Mean Length of Glochidia (μm)	SD (μm)	*t* value	*P* value
Dan	4	80	196.5	21.4	2.45	0.015
James	8	80	190	10		

Table 7. Fecundity estimates for female mussels of *P. collina* collected in the James and Dan Rivers, Virginia, 2004. Counts of glochidia were log-transformed prior to analysis.

River	Number Females	Number Conglutinates	Mean No. Glochidia per Female	SD standard deviation	MS mean squares	$F_{1,10}$ statistic	*P* value
Dan	4	34	9073	6433	0.3313	2.14	0.1744
James	8	16	11291	2733			

Table 8. Number of conglutinates from female mussels of *P. collina* collected in the James and Dan Rivers, Virginia, 2004. Counts of conglutinates were log-transformed prior to analysis.

River	Number Females	Number Conglutinates	Mean No. Conglutinates per Female	SD standard deviation	MS mean squares	$F_{1,12}$ statistic	*P* value
Dan	4	399	99.8	51.6	0.2519	2.63	0.1310
James	10	1229	122.9	23.1			

Table 9. Fish host specificity comparison of *P. collina* collected in the (M) South Mayo River (2004), and the (J) James River (Hove and Neves 1990), Virginia. Counts of juveniles per fish were log-transformed prior to analysis.

Fish Species	River	LS Least Squares	SE Standard Error	MS Mean Squares	$F_{1,21}$ Statistic	P Value
Nocomis leptocephalus	M	4.38	0.36	0.0200	0.0227	0.8817
	J	4.54	0.94			
Rhinichthys atratulus	M	1.70	0.38	0.0275	0.0312	0.8616
	J	1.56	0.66			
Campostoma anomalum	M	1.84	0.54	0.2788	0.3158	0.5801
	J	2.45	0.94			
Phoxinus oreas	M	3.21	0.66	0.2000	0.2267	0.6389
	J	2.76	0.66			
Notropis analostanus	M	4.80	0.66	0.7971	0.9029	0.3528
	J	3.70	0.94			
*Clinostomus funduloides**	M	4.81	0.66	13.412	15.194	0.0008
	J	1.15	0.66			
*Lythrurus ardens**	M	4.49	0.66	5.5729	6.3131	0.0202
	J	2.13	0.66			

* Significance of comparison possibly due to high fish mortality reported from Hove's (1990) fish host tests.

Figure Headings

Figure 1. Mantle tissue collection sites for *Pleurobema collina*, Monroe County, WV; Albemarle, Henry, and Patrick counties, VA; and Stokes County, NC, 2003.

Figure 2. Valves of *P. collina* from (A) James River basin (Hove 1990) and (B) Dan River sub-basin.

Figure 3. Numbers of valves examined and percentage of live and dead valves with spines in streams surveyed in the James River basin (solid bars) and the Dan River sub-basin, 2002-2004 (cross-hatched bars). James River basin data from Hove (1990).

Figure 4. Minimum spanning network between 5 *ND-1* haplotypes observed in *Pleurobema collina*. Each node represents a haplotype, and the size of the node indicates the number of individuals sharing that haplotype. Colors: Wards (W) = red, South Potts (P) = green, Dan (D) = black, South Mayo (M) = purple. Notation of nodes: number = haplotype number (following Table 3); letter = population(s) possessing the haplotype. The numbers in red indicate the specific base where a mutation occurred in order to move from one haplotype to the next.

Figure 5. Inferred phylogenetic relationships among the *Pleurobema collina* examined. DNA haplotypes were described using minimum evolution (ME) analysis. The numbers above the branches (ME) represent bootstrap support (10000 replicates); only values >50% are shown. The tree was generated using DNA sequences from the mitochondrial

DNA region *ND-1* (916 bp). The out-group taxon is *Epioblasma brevidens*; *Elliptio steinstansana* was included as a closely related sister-group.

Figure 6. Inferred phylogenetic relationships among the *Pleurobema collina* examined. DNA haplotypes were described using maximum parsimony (MP) analysis (tree length = 239, CI = 1.000, RI = 1.000). The numbers above the branches (MP) represent bootstrap support (10000 replicates); only values >50% are shown. The tree was generated using DNA sequences from the mitochondrial DNA region *ND-1* (916 bp). The out-group taxon is *Epioblasma brevidens*; *Elliptio steinstansana* was included as a closely related sister-group.

Figure 7. Minimum spanning network between 13 *ITS-1* haplotypes observed in *Pleurobema collina*. Each node represents a haplotype; and the size of the node indicates the number of individuals sharing that haplotype. Colors: Wards (W) = red, South Potts (P) = green, Dan (D) = black, South Mayo (M) = purple, yellow = median vector (hypothetical intermediate haplotype). Notation of nodes: number = haplotype number (following Table 5); letter = population(s) possessing the haplotype. The numbers in red indicate the specific base where a mutation occurred in order to move from one haplotype to the next.

Figure 8. Inferred phylogenetic relationships among the *Pleurobema collina* examined. DNA haplotypes were described using minimum evolution (ME) analysis. The numbers above the branches (ME) represent bootstrap support (10000 replicates); only values

>50% are shown. The tree was generated using DNA sequences from the nuclear DNA region *ITS-1* (502 bp). The out-group taxon is *Epioblasma brevidens*; *Elliptio steinstansana* was included as a closely related sister-group.

Figure 9. Inferred phylogenetic relationships among the *Pleurobema collina* examined. DNA haplotypes were described using maximum parsimony (MP) analysis (tree length = 149, CI = 0.9396, RI = 0.5909). The numbers above the branches (MP) represent bootstrap support (10000 replicates); only values >50% are shown. The tree was generated using DNA sequences from the nuclear DNA region *ITS-1* (502 bp). The out-group taxon is *Epioblasma brevidens*; *Elliptio steinstansana* was included as a closely related sister-group.

Figure 10. Inferred phylogenetic relationships among the *Pleurobema collina* examined. DNA haplotypes were described using minimum evolution (ME) analysis. The numbers above the branches (ME) represent bootstrap support (10000 replicates); only values >50% are shown. The tree was generated using DNA sequences from the combined mitochondrial DNA regions of *cytochrome-b* (357 bp), *ND-1* (916 bp), and the nuclear DNA region *ITS-1* (502 bp). The out-group taxon is *Epioblasma brevidens*; *Elliptio steinstansana* was included as a closely related sister-group.

Figure 11. . Inferred phylogenetic relationships among the *Pleurobema collina* examined. DNA haplotypes were described using maximum parsimony (MP) analysis (tree length = 491, CI = 0.9715, RI = 0.8427). The numbers above the branches (MP)

represent bootstrap support (10000 replicates); only values >50% are shown. The tree was generated using DNA sequences from the combined mitochondrial DNA regions of *cytochrome-b* (357 bp), *ND-1* (916 bp), and the nuclear DNA region *ITS-1* (502 bp). The out-group taxon is *Epioblasma brevidens*; *Elliptio steinstansana* was included as a closely related sister-group.

Figure 12. Conglutinates of *P. collina* released in the laboratory, courtesy of Hove (1990).

Figure 13. Aperatures of live *P. collina* from (A) James River system (Hove 1990), and (B) Dan River sub-basin ($N = 4$).

Figure 14. Fresh-dead *P. collina* from the South Mayo River, Dan River sub-basin, VA, 2003. Note mantle, papillae, and foot color.