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Water Quality, Sediment Characteristics, Aquatic Habitat, Geomorphology, and Mussel Population Status of the Clinch River, Virginia and Tennessee, 2009–2011

Data Series 802

U.S. Department of the Interior
U.S. Geological Survey

Cover: Clinch River mussel shoal at Kyles Ford, Tennessee. View looking downstream.

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U.S. Department of the Interior
SALLY JEWELL, Secretary

U.S. Geological Survey
Suzette M. Kimball, Acting Director

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Contents

Figure

Tables

Appendix Tables (Excel files available for download at *http://pubs.usgs.gov/ds/0802/*)

Conversion Factors

Inch/Pound to SI

Multiply	By	To obtain
	Length	
inch (in.)	2.54	centimeter (cm)
inch (in.)	25.4	millimeter (mm)
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)
	Area	
acre	4,047	square meter (m^2)
square foot (ft^2)	0.09290	square meter (m^2)
square mile (mi^2)	259.0	hectare (ha)
square mile (mi^2)	2.590	square kilometer (km^2)
	Volume	
million gallons (Mgal)	3,785	cubic meter (m^3)
cubic foot (ft^3)	0.02832	cubic meter (m^3)
cubic yard (yd^3)	0.7646	cubic meter (m^3)
	Flow rate	
foot per second (ft/s)	0.3048	meter per second (m/s)
cubic foot per second (ft^3/s)	0.02832	cubic meter per second (m^3/s)
million gallons per day (Mgal/d)	0.04381	cubic meter per second (m^3/s)
	Mass	
ounce, avoirdupois (oz)	28.35	gram (g)
	Pressure	
atmosphere, standard (atm)	101.3	kilopascal (kPa)
bar	100	kilopascal (kPa)

SI to Inch/Pound

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
	Flow rate	
meter per second (m/s)	3.281	foot per second (ft/s)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

°F=(1.8×°C)+32

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows:

°C=(°F–32)/1.8

Horizontal coordinate information is referenced to the North American Datum of 1983 (NAD 83).

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25 °C).

Concentrations of chemical constituents in water are given in milligrams per liter (mg/L) or micrograms per liter (µg/L); concentrations of chemical constituents in sediment are given in micrograms per gram (µg/g), micrograms per liter (µg/L), or micrograms per kilogram (µg/Kg); concentrations of chemical constituents in biological tissue are given in parts per million dry weight (ppm).

Water Quality, Sediment Characteristics, Aquatic Habitat, Geomorphology, and Mussel Population Status of the Clinch River, Virginia and Tennessee, 2009–2011

By Jennifer L. Krstolic, Gregory C. Johnson, Brett J.K. Ostby

Abstract

Chemical, physical, and biological data were collected during 2009–2011 as part of a study of the Clinch River in Virginia and Tennessee. The data from this study, data-collection methods, and laboratory analytical methods used in the study are documented in this report. The study was conducted to describe the conditions of the Clinch River and to determine if there are measurable differences in chemical, physical, or biological characteristics in a segment of the river where freshwater mussel populations are in decline, have low density, richness, little to no recruitment, and lack endangered species (low-quality reach) compared to a segment of the river where mussel assemblages have relatively high density, richness, evidence of recruitment, and support endangered species (high-quality reach). Five continuous water-quality monitors were installed and operated on the mainstem of the Clinch River and two tributaries. Discrete water-quality sample sets were collected during base-flow and stormflow conditions at two sites on the Clinch River and on the Guest River, a tributary to the Clinch River predominantly in the Appalachian Plateaus Physiographic Province. Base-flow water-quality samples were collected in July and August 2011 at 15 sites along the mainstem of the Clinch River. Other analyses included longitudinal sampling along the mainstem of the Clinch River at 10 sites to evaluate bed-sediment chemistry, habitat condition, and mollusk community status. In situ freshwater mussel growth and mortality experiments were conducted with hatchery propogated *Villosa iris* (rainbow mussels). Tissue from the *V. iris* as well as tissue from 16 *Actinonaias pectorosa* mussels were analyzed for trace metals, and *V. iris* mussel tissue was analyzed for organic compounds. Data collected during this investigation were analyzed by various U.S. Geological Survey or U.S. Fish and Wildlife Service laboratories.

Introduction

The upper Clinch River supports nationally noteable populations of rich, diverse, endemic, and endangered freshwater mussels as well as other unique aquatic fauna. Many of the riffles and shoals selected for this study have been extensively documented as important mussel habitats in the Clinch River. Freshwater mussel shoals in Virginia and Tennessee, including Dungannon, Semones Island, Pendleton Island, Speers Ferry, Kyles Ford, Frost Ford, and Swan Island (site numbers 15, 16, 19, 25, 28, 29, and 30; figure 1 and table 1), have been sampled by the Tennessee Valley Authority (TVA), U.S. Geological Survey (USGS), U.S. Fish and Wildlife Service (FWS), and Virginia Tech throughout the last 30 years (Salyor and Ahlstedt, 1990; Ahlstedt, 1991; Kerans and Karr, 1994; Ahlstedt and others, 2005). Mussel assemblage structure (richness, population density, and recruitment of juveniles) monitored at these sites since 1979 has shown a pattern of decline in an approximately 50-mile segment of the upper Clinch River from Carbo, Va., to Clinchport, Va. (fig. 1) denoted as reaches with low-quality mussel assemblages (low-quality reaches) (Ahlstedt, 1991; Ahlstedt and Tuberville, 1997; Ahlstedt and others, 2005; Eckert and others, 2007, 2008a, 2008b). Long-term monitoring and periodic surveys, however, have shown a stable, dense, and diverse mussel assemblage structure downstream from Clinchport, Va., through the Tennessee segment of the Clinch River upstream from Norris Reservoir denoted as reaches with high-quality mussel assemblages (high-quality reaches) (Ahlstedt, 1991; Ahlstedt and Tuberville, 1997; Ahlstedt and others, 2005; Eckert and others, 2007, 2008a, 2008b). Upstream from the low-quality reach, the mussel assemblages have maintained stable, low-density populations since the mid-1990s between Cleveland, Va., and Blackford, Va., including observations of juveniles of many species and federally listed species (Van Hassel, 2007; Eckert and others, 2010). Given the extent and duration of the mussel declines, an interdisciplinary study was designed incorporating continuous water-quality monitoring, discrete water-quality sampling, dissolved constituent transport, bed-sediment sampling, aquatic habitat assessments, mussel population status surveys, and mussel growth, survival, and bioaccumulation experiments. The goal of the study was to collect information towards better understanding the complexity of the mussel assemblage decline and identify the environmental factors likely associated with the low-quality reaches of the Clinch River.

Purpose and Scope

The purpose of this report is to present the findings of an investigation conducted by the USGS, in cooperation with the Tennessee Wildlife Resources Agency (TWRA), Tennessee Department of Environment and Conservation (TDEC), and the FWS, to describe conditions of the Clinch River in Virginia and Tennessee in 2009–2011. A primary objective of the investigation was to determine if there are measurable differences in biological, chemical, and physical characteristics in low-quality reaches of the Clinch River compared to high-quality reaches. This report provides a single source for data collected during this investigation and facilitates dissemination of data not stored in the USGS National Water Information System (NWIS) database. This report describes the study area, documents data-collection methods, and presents data tables that can be downloaded as Microsoft Excel files. Most of the chemical data are also accessible through the NWIS Web interface at *http://waterdata.usgs.gov/tn/nwis/qw* and *http://waterdata.usgs.gov/va/nwis/qw*. Samples collected during this investigation were analyzed at various USGS or FWS laboratories, and analytical results are presented in this report.

Approach

Low-quality and high-quality mussel assemblages in sections of the Clinch River have been defined by previous biological studies. The approach used in this investigation was to collect a broad suite of data intensively at two monitoring sites representative of the reach in which they were located and to collect data longitudinally upstream and downstream along the mainstem to describe the spatial distribution of water chemistry and bed-sediment conditions. The two primary monitoring sites were located at Dungannon, Va., in the low-quality reach and Horton Ford, Tenn., in the high-quality reach (table 1; fig. 1). A total of 30 monitoring sites in the Clinch River Basin—27 along the mainstem Clinch River and three on major tributaries—were sampled during the study (table 1 in the body of the text, and available for download as appendix table A1). Information obtained from continuous water-quality monitoring and sampling during base-flow and stormflow conditions was used to build an understanding about the acute or chronic nature of environmental factors that may be linked to decline. A time of travel dye study was conducted to determine transport velocities and dispersion of dissolved constituents during base flow. The depositional environment and substrate inhabited by mussels was examined with bed-sediment quality sampling, pebble-count particle size distribution, embeddedness, and depth-of-silt measurements. Physical aquatic habitat assessments were conducted along with mussel-population status surveys to provide a current understanding of health and decline, and whether habitat quality was associated with declines. Juvenile mussel growth and survival studies and adult mussel tissue chemistry analyses were conducted as a first step toward understanding the response of an organism with a complex life history to environmental factors that may be linked to decline.

Description of the Study Area

The Clinch River is a headwater tributary of the Tennessee River located mostly in the Valley and Ridge Physiographic Province with the western edge in the Appalachian Plateaus Physiographic Province (Fenneman and Johnson, 1946). The upper Clinch River begins in Tazewell County, Va., flows freely for approximately 200 miles above Norris Lake in northeast Tennessee, and has a drainage area of 1,474 square miles (mi^2) near the city of Tazewell, Tenn. The course of the Clinch River trends southwest through valleys controlled by folded and faulted sedimentary rocks of Paleozoic age. The coal-bearing portions of the Clinch River Basin are Pennsylvanian-age sedimentary rocks within the Lee, Wise, and Norton Formations in the Appalachian Plateaus (Hufschmidt and others, 1981). This free-flowing section of the Clinch River has historically maintained one of the most diverse mussel assemblages in all of North America (Ortmann, 1918; Neves and others, 1997; Parmalee and Bogan, 1998), but mussel fauna in portions of the upper Clinch River are in decline (Diamond and others, 2002; Ahlstedt and others, 2005). Point-source discharges within the Clinch River Basin are few, and the primary stressors on the aquatic biota are thought to be nonpoint-source discharges possibly associated with agriculture, coal mining, and urban development (Locke and others, 2006).

Data Collection

The data collected in the Clinch River Basin included continuous water-quality data, discrete water-quality samples, bed-sediment chemistry and size class, and time of travel and dispersion at base-flow conditions. At many of the same locations aquatic habitat and geomorphology characteristics were documented along with qualitative and quantative mollusk assemblage characterization.

Continuous Water-Quality Monitoring

Continuous water-quality data were collected at five USGS monitoring stations between September 18, 2007, and October 31, 2011. Various combinations of selected properties, including temperature, specific conductance, pH, and turbidity, were collected, using standard USGS methods as described in Sauer (2002) and Wagner and others (2006), and are summarized in appendix table A2. Continuous water-quality data (logged at 15-minute intervals) were tabulated for a common period from March 27, 2009, to October 31, 2011, for publication in this report (table A3). These data are also accessible through the USGS NWIS Web interface (National Water Information System, 2012).

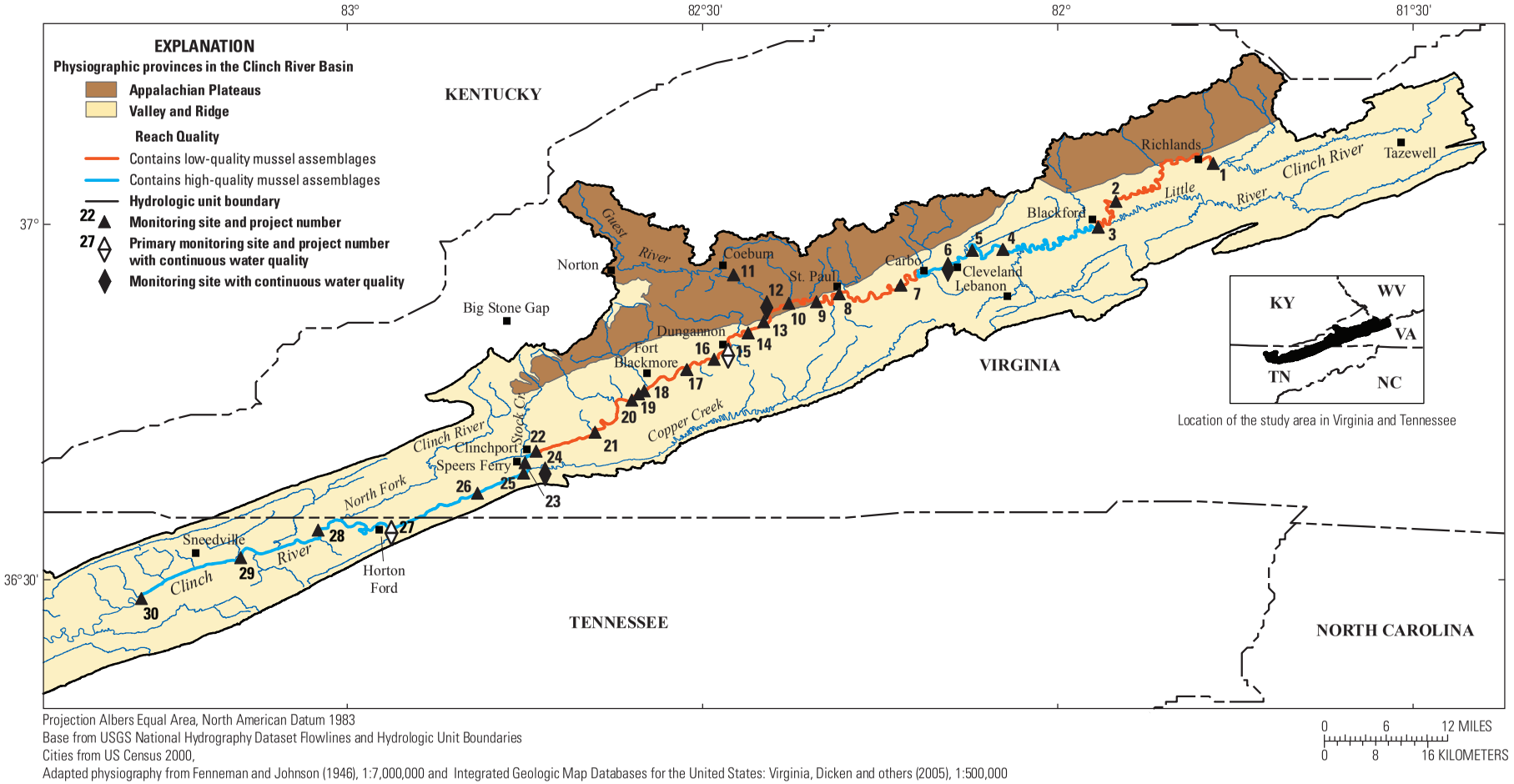

Figure 1. Clinch River study sites and freshwater mussel health in Virginia and Tennessee. Monitoring site numbers refer to project numbers in table 1.

Discrete Water-Quality Sampling

Discrete surface-water sample sets were collected at Dungannon and Horton Ford from April 2009 through December 2011. The largest tributary within the low-quality reach, the Guest River, was added to the discrete sample sets in February 2011. All of the discrete water-quality samples were collected using depth-integrated, equal-width-interval collection methods (U.S. Geological Survey, 2006). Samples were processed onsite or at the USGS field offices using methods from Wilde and others (2004). Laboratory analyses were conducted by the USGS National Water Quality Laboratory (NWQL) in Denver, Colorado, the USGS Kentucky Sediment Laboratory (KSL) in Louisville, Kentucky, and the USGS Eastern Energy Environmental Laboratory (EEEL) in Reston, Va. Field-measured water-quality data for properties such as temperature, dissolved oxygen, pH, specific conductance, turbidity, acid-neutralizing capacity, and alkalinity were collected using protocols described in Wilde (variously dated). Sixteen sample sets were collected at base-flow conditions (table A4), 6 of which included the Guest River, and 10 sample sets were collected during stormflow conditions (table A5), 3 of which included the Guest River. Whole water samples were analyzed for major ions, nutrients, selected trace metals, and suspended sediment fraction. Seven samples were analyzed for mercury, but analysis was discontinued after January 2010 because of low concentrations. Four storm samples were analyzed for organic compounds in the suspended sediment and six filtered storm samples were analyzed for dissolved-phase organic compounds in the water column (table A6). Beginning in May 2010, filtered storm samples were analyzed for dissolved phase metals as well.

Base-flow major-ion sampling was conducted twice during 2011 at 15 sites (table 1) along the mainstem of the Clinch River from an upstream site at Richlands, Va., to Kyles Ford, Tenn. Sampling locations included sites with a range of low-quality to high-quality mussel assemblages. Each mainstem sampling location represented one major tributary input and an increase in drainage area of approximately 100 mi^2. Discrete water-quality samples were collected at each site and analyzed for dissolved-phase major ions (table A7). Discharge measurements were made where no USGS gaging stations were present, and field-measured water-quality properties (pH, specific conductance, turbidity, temperature, and alkalinity) completed the water-quality datasets (table A7).

Bed-Sediment Chemistry

Bed-sediment samples were collected at 10 sites in the Clinch River in 2010 and at two sites in 2011 (table 1) using protocols described in Shelton and Capel (1994). In 2010, bed-sediment samples were collected from upstream at Artrip, Va., to downstream at Swan Island, Tenn. (table 1, project numbers 5, 6, 7, 9, 15, 19, 25, 27, 29, and 30). Bed-sediment samples were composited from multiple depositional zones at each site; the samples then were sieved to 2 millimeters (mm) for the organics analyses and determination of the light weight fraction organic matter (coal) or sieved to 63 microns for the metals analysis. Samples collected in 2010 were sent to the USGS Mineral Resources Program Laboratory (MRP) within the Central Mineral and Environmental Resources Science Center in Denver, Colo., for metals analysis (table A8), the USGS KSL for size-class determination and percentage coal content (table A9), and the USGS EEEL for organic compound analysis (table A6). In 2011, bed-sediment samples were collected at Semones Island, Va., which is less than 1 mile from Dungannon Va., and at Horton Ford, Tenn. (table 1, project numbers 16 and 27). Four bed-sediment samples were collected from different depositional zones at each site, with one sample split as a duplicate sample. The 2011 samples were sent to the same three laboratories (KSL, EEEL, MRP) used for analysis of the 2010 samples (tables A8 and A9). Split replicates of each 2011 bed-sediment metals sample and a few duplicate samples were also sent to the USGS Energy Geochemistry Laboratory (EGL) of the Central Energy Resources Science Center in Denver, Colo. (table A10), to compare results with those from the USGS MRP Laboratory.

Time of Travel Study

A time of travel and dye dispersion study was conducted during base-flow conditions during September 9–17, 2009, using methods described in Wilson and others (1986) and Kilpatrick and Wilson (1989). Rhodamine WT, a nontoxic fluorescent dye, was released into the river at separate times at three locations, and each resulting plume was monitored as it moved downstream (table 1). Water samples were collected at seven locations along the river (table A11). As a dye plume passed a measurement site, 30 samples were collected at regular intervals depending on the expected duration of the plume. Samples collected at the first measurement site, closest to an injection site, were collected by hand as grab samples from the centroid of flow. Samples collected at sites with longer expected duration times were collected with automated barge samplers using 20 milliliter (mL) syringes or as grab samples. Dye concentrations were determined in the field by using either a Turner TD-700 or a Turner 10 field fluorometer. Dye concentrations for a subset of samples were verified at the University of Tennessee Department of Earth and Planetary Sciences by using a Perkin Elmer LS55 luminescence spectrophotometer. The travel time, duration, and velocity of the dye plumes are summarized for each reach in three sections of the Clinch River—from Dungannon to Fort Blackmore, from Fort Blackmore to Speers Ferry, and from Speers Ferry to Horton Ford (table A11). Final calculations were made to estimate the time of travel between Dungannon, Va., and Horton Ford, Tenn., and the rate at which a plume of soluble pollutant might disperse (table A12).

Aquatic Habitat and Geomorphology

Fluvial geomorphologic and habitat characteristics were measured from April 2009 through October 2010 at the same 10 sites where bed-sediment samples were collected (table 1). Geomorphologic and local habitat assessments were focused on measures of hydraulic stability, local land use, and features important to fish hosts, all of which are known to be associated with variation in mollusk assemblages. Freshwater mussels reach their highest density and species richness in riffles, runs, and shoals in the Clinch River (Ostby, 2005), thus sampling was focused on these habitats. A shoal was defined as a cobble and gravel formation where water depth was relatively shallow (depth less than or equal to 1 meter (m); 3.28 feet (ft)) and flow was of moderate velocity (0.3 to 0.5 meter per second (m/s); 0.98 to 1.6 feet per second (ft/s)). Shoals are comparable to fast, shallow runs but are not quite as steep or turbulent as riffles. At each of the 10 sites, geomorphologic, habitat, and mollusk sampling was centered at the head of the most extensive riffle or shoal.

The U.S. Environmental Protection Agency (USEPA) Environmental Monitoring & Assessment Program (EMAP) techniques and metrics for visually and quantitatively assessing habitat were adapted in this study to develop quantifiable characteristics for as many properties as possible (Lazorchak and others, 1998). Some EMAP metrics were modified while attempting to stay true to intended outcomes of metrics. For example, EMAP protocols for quantifying bankfull shear stress and relative bed stability called for the use of a handheld clinometer to measure slope directly. To obtain more precise measurements for these metrics, an auto level and stadia rod were employed with repeated measurements to confirm findings. Ideally, the spatial extent of a site would include a linear reach of river extending a length from 5 times the bankfull width upstream and 5 times the bankfull width downstream from the head of the riffle (10 times bankfull width in total); however, because mean bankfull width ranged from 52 to 103 m, study reach length was standardized to 500 m (1,640 ft) to make measurements manageable in a day. For other metrics, such as classification of human disturbance and riparian vegetation, standard EMAP techniques were followed.

At each site, 11 transects (labeled A to K from downstream to upstream) were placed at longitudinal intervals of 50 m (table A13). The middle transect (F) was always placed at the head of a riffle or shoal so that five transects were placed upstream and five were downstream. When multiple channels conveyed flow through a reach, transects were placed in each channel following the same configuration. Measurements of water depth, substrate roughness, and substrate particle size were made at distances of 0.1, 0.3, 0.5, 0.7, and 0.9 times the wetted width along each transect (table A13). Visual estimates of silt depth and embeddedness were made at the same locations (tables A13 and A14).

Instream riparian cover was approximated using a convex densiometer at 0.1, 0.5, and 0.9 times the wetted width along each transect (tables A13 and A15). Bank slope, bankfull height, terrace height, and riparian characteristics were determined at each transect on both banks of a channel. Riparian cover and fish habitat were classified according to EMAP protocol (Lazorchak and others, 1998) by assigning canopy, understory, and ground cover to categories according to type and density for an area one times the stream width (tables A15 and A16). Riparian cover on banks was approximated using a convex densiometer.

Unlike EMAP protocol, water-surface slope of the reach was measured from the head of the nearest riffle upstream of the central transect F to the nearest riffle downstream from the center. Relative water-surface elevation was measured with an automatic level and stadia rod (table A17). If riffle formations were too far away (greater than 500 m) to accurately measure, slope was measured from similar habitat units located above and below the central transect (such as glide to glide or pool to pool). In all cases, the entire sampled reach was bracketed between upstream and downstream measurements of relative water-surface elevation for similar habitat types. Slope and depth measurements were used to derive shear stress at base flow and bankfull flow for the reach. Substrate particle size was used to derive critical shear stress in Newtons per square meter (N/m^2) and measures of relative bed stability for base flow or bankfull flow for the reach (table A17).

Additional slope, depth, and substrate measurements were made for the most extensive riffle or shoal where quantitative mussel sampling was conducted in each reach (tables A14 and A17). The slope of each riffle or shoal was measured from the upstream extent of the habitat to the downstream extent (table A17). A standard Wolman pebble count (Wolman, 1954) was conducted in association with the quantitative mussel sampling. Habitat-specific shear stress estimations and relative bed stability ratios were made for each riffle or shoal in the same manner as was done for the entire reach using depth, slope, and substrate values collected only in those habitats (table A17).

Standard substrate metrics, such as median particle size (D50), 16th percentile particle size (D16), and 84th percentile particle size (D84), were derived for both the reach (all transect measurements) and the dominant riffle or shoal habitat in a reach (table A14). Because many point measurements fell on bedrock, two sets of substrate metrics were calculated—one including bedrock and one without. Bedrock was assigned a value of 5,000 mm, clay a value of 0.001 mm, silt a value of 0.01 mm, and sand a value of 0.5 mm. The percentage of point measurements from bedrock (PBED) was also calculated.

Channel roughness was approximated so that shear stress values could be calculated in a hydraulically accurate context where large particles can influence local flow conditions (table A13). Most roughness estimates were calculated using the 84th percentile substrate value for each reach (REACH_D84(XBED) in tables A13 and A14); however, for some channels with larger substrate particles such as bedrock ridges and boulders, values greater than the D84 were selected for roughness calculations.

Freshwater Mussel and Mollusk Assemblage Sampling

Mollusk assemblages were surveyed between April 2009 and October 2010 at the same 10 sites as habitat assessments (table 1) with emphasis on detecting recruitment and accurately quantifying population density and richness. The goals for sampling the mollusk assemblage were to (1) sample a reach thoroughly enough to detect species present at low densities (for example, 0.01 individuals per square meter); (2) sample reaches in a uniform manner so that results can be compared among reaches; (3) limit sampling efforts to those that can be completed thoroughly in a day by a crew of three; and (4) use methods that will not be biased by varying levels of sampler experience. To achieve these goals, qualitative and quantitative sampling protocols were used. As with habitat measurements, the sampling reach was centered at the head of the most extensive riffle or shoal. At each transect (A though K), visual searches were conducted of the substrate surface within a 4-m wide search area (qualitative sampling). Depending on water depth, view scopes, snorkel, or SCUBA were used to conduct surveys. Live mussels observed in each transect were identified to species, counted, and measured to the nearest millimeter (table A18). All live mussels were immediately returned to the precise locations where they were detected. Dead mussels (shells) were noted, as were the presence of other mollusks (live or shell). Using wetted width and transect width (4 m), the total area searched in a reach was calculated. The sampling equation from Smith (2006) was used to calculate post hoc detection rates for the reach (table A18).

Mussels often are buried in the substrate and undetectable during a surface survey. A growing body of evidence suggests that only 10 to 30 percent of mussels are present at the surface and therefore detectible with surface searches. Additionally, at least one species, *Hemistema lata*, and also juveniles and young adults of all species are rarely detectable without excavating at least 10 centimeters (cm) into the substrate. To better detect the presence of younger mussels and to better quantify the density of mussels and snails, quantitative excavation sampling was conducted in the riffle or shoal habitats at the center of each reach. Sixty 0.25 m^2 quadrats were systematically assigned from three random starting points in the area between transects E and G. Live mussels in each quadrat were identified to species, counted, measured to the nearest millimeter, and replaced (table A18). Live *Io fluvialis* were counted in each quadrat. Because densities were much higher for other snails and *Corbicula fluminea*, less than half of the quadrats were sampled for selected snail species and *Corbicula fluminea* density estimates. Differentiating among the snail species of the genus *Elimia* was time consuming and difficult, so these species were combined at the genus level when counted.

Quantitative sampling allowed the calculation of density estimates for live mussel species and for all mussels combined in each reach as well as quantitative richness (table A18 and table 2). Density estimates were also derived for common snail species and *C. fluminea*. Semiquantitative visual searches and quantitative excavations were used in combination to derive live species richness and (dead) shell mollusk species richness as well as shell length statistics for mussels. In other words, any individual observed at a site, whether observed at the streambed surface along the transect or observed in the subsurface from quadrat excavations, was included in the total live mussel species richness, total shell species richness, and shell length metrics for the site. Summary mussel metrics for each site are presented in table 2.

Mussel Growth and Mortality Study

In situ growth and survival experiments were conducted with 372 hatchery-propogated mussels deployed at sites in both the low-quality and high-quality reaches as an assessment of water-column chemistry effects on juvenile mussels. Mussel silos containing juvenile *Villosa iris* (*V. iris*) were deployed at four Clinch River sites: Cleveland Island, Semones Island, Pendleton Island, and Horton Ford (table 1). Eight silos per site were deployed near the upstream extent of the dominant riffle or shoal at equal intervals from the right descending wetted margin of the channel to the left descending wetted margin of the channel. Concrete silos were constructed on the basis of the Barnhart silo design (Barnhart and others, 2007). Each silo contained 11–12 *V. iris* juveniles with a mean length of 6.02 mm (standard deviation = 0.91 mm) ranging from 4.06 to 8.40 mm. These juvenile *V. iris* were propogated at the Virginia Department of Game and Inland Fisheries (VDGIF) Aquatic Wildlife Conservation Center (AWCC) at Buller Hatchery near Marion, Va.

A set of silos was initially deployed in late May 2009; however, half of the replicates failed because of a silo structural design error. Because failures were detected early in the deployment and each site suffered loss of replicates, replacement silos were distributed equally among sites by June 2009. Deployment was delayed until June because of high flows and inclement weather conditions. Due to the interrupted deployment schedule, the mid-July measurement was considered the baseline measurement of length and survival. Beginning July 14, 2009, length and survival were measured every 2 months until November 2010 if conditions were suitable and safe to access silos (tables A19 and A20). Due to high flow conditions or below-freezing air temperatures when flows were low, no sampling was completed between November 2009 and April 2010. Silos were cleared of sand and other sediments following major storm events because it was determined that the cold air temperatures might cause mortality while silos were out of the water.

All measurements were made by taking multiple photographs of *V. iris* juveniles against a scaled background using a macro setting on a digital waterproof camera. In the laboratory, lengths were measured to the nearest 0.01 mm using U.S. National Institutes of Health image measurement software,

ImageJ (Rasband, 1997–2012). Survival was also verified from photographs (tables A19 and A20). Over the course of the study, nine silo replicates were lost during storm events. Surviving juveniles in the 21 remaining silos were collected and measured in November 2010 after 18 months of exposure.

Temperature data from continuous water-quality monitors (tables 1 and A3) close to three of the four silo sites (project numbers 6, 16, and 27) were used to compare temperature regimes at three of the four silo sites (tables 1 and A21). Total growth degree days (GDD) were calculated from the continuously monitored temperature data for the period of deployment (525 days). GDD is equal to the average of the daily maximum temperature and the daily minimum temperature minus a baseline temperature of 10 degrees Celsius (°C), (GDD = ((max+min)/2)–10). Additionally, a representative sample of particulate nitrogen and organic carbon (Shelton and Capel, 1994) and chlorophyll *a* and phytoplankton (Moulton and others, 2002) was collected during the growing season in August 2010 to quantify nutrient availability among sites (table A22).

Mussel Tissue Chemical Composition

To measure the uptake of chemicals into freshwater *V. iris* mussel tissue at the conclusion of the growth and survival study in 2010, wet tissue was shucked from shells, frozen, and delivered to FWS contract laboratories for analysis of major ions, metals (table A23), and polycyclic aromatic hydrocarbons (PAH) in the tissue (table A24). Individuals from the same brood stock of *V. iris* that had remained in the hatchery during the growth and survival study were also processed and sent to the FWS contract laboratories for analysis of major ions, metals (table A23), and PAH in the tissue (table A24). Living, native pheasantshell mussels (*Actinonaias pectorosa*) were harvested from two sites in 2011 for comparison with tissue concentration analysis of the hatchery propogated juvenile *V. iris* mussels and in conjunction with the 2011 replicate bed-sediment samples (tables A8, A9, and A10). At each site, eight *A. pectorosa* of similar size were harvested. Wet tissue was shucked from shells, a section of organ tissue was removed and preserved for independent histological analysis, and the remaining tissue was frozen and delivered to the USGS NWQL for major ions and metals tissue concentration analysis (table A25).

Quality Control Data

Throughout each phase of water-quality and sediment-quality data collection, quality control samples were collected. Equipment and field blanks were collected to ensure there was no contamination of samples from residue on equipment. Replicate samples were used to determine if field methods were followed in a manner that produced consistent results. These samples included 3 blanks and 2 replicates for discrete water-quality sampling, 1 replicate and 1 blank for base-flow water-quality sampling, and 3 replicates for bed-sediment sampling. Additionally, all the 2011 bed-sediment samples were sent to two laboratories (MRP and EGL) for metals analyses as laboratory split replicates, or in a few cases as duplicates. In general, variability among water-quality replicates was within an acceptable range, less than 10 percent for most samples, and less than 0.02 milligram per liter (mg/L) for constituents with low concentrations. Water-quality blanks were within expected and acceptable ranges of detections; all values were less than the specified detection limit for each constituent. Bed-sediment sample replicates for 2010 and 2011 were generally within 10 percent of each other for the same laboratory analyses and within 20 percent for samples split among the MRP and EGL laboratories.

Laboratory Methods

Laboratory methods used for sample analysis are presented below for the seven laboratories involved in the analysis of samples for this study. Brief descriptions of published methods are referenced.

Streambed-sediment size distribution and light weight fraction organic matter (coal) content were analyzed at the USGS Kentucky Sediment Laboratory. Surficial streambed-sediment size distribution is based on standard methods using dry sieve and 5-point pipette analysis (Guy, 1969). Coal was separated from the sediment with lithium heteropolytungstate using procedures modified from Carver (1971) and Horowitz and others (1993). Coal content was calculated as the ratio of the mass of separated coal to original dried sample mass expressed as a percentage.

Water quality and adult *Actinonaias pectorosa* freshwater mussel tissue samples were analyzed at the USGS NWQL. Water samples submitted for nutrient analysis were analyzed using methods described in Fishman (1993), Patton and Truitt (2000), Patton and Kryskalla (2003), and Patton and Kryskalla (2011). Water samples submitted for analysis of major ions, trace metals, carbon (total, inorganic, and organic), and total nitrogen were analyzed using a variety of methods (Fishman and Friedman, 1989; Hoffman and others, 1996; U.S. Environmental Protection Agency, 1997; Garbarino and Struzeski, 1998; Garbarino and others, 2006). *Actinonaias pectorosa* freshwater mussel tissue major ion and metals concentrations were analyzed using methods described in Hoffman (1996), USEPA (1996), and Garbarino and others (2006).

Bed-sediment metals were analyzed at the USGS Mineral Resources Program Laboratory within the Central Mineral and Environmental Resources Science Center in Denver, Colo. Mercury was analyzed using methods described in Hageman (2007). Samples were prepared and minerals were analyzed using protocols described in Taggart (2002). Split replicate bed-sediment metals samples were analyzed at the USGS Energy Geochemistry Laboratory of the Central Energy Resources Science Center in Denver, Colo., using methods described online at *http://energy.usgs.gov/GeochemistryGeophysics/GeochemistryLaboratories/GeochemistryLaboratoriesMethods.aspx* (accessed August 24, 2012).

Villosa iris juvenile mussel tissue was analyzed at the FWS Trace Element Research Laboratory at Texas A&M University and Geochemical & Environmental Research Group at Texas A&M University. The tissue samples were extracted by the National Oceanic and Atmospheric Administration Status and Trends Method (MacLeod and others, 1985) with minor revisions (Wade and others, 1988; Brooks and others, 1989). Trace element and PAH analyses are documented online at *http://www.fws.gov/chemistry/methods_terl_lab.htm* and *http://www.fws.gov/chemistry/methods_gerg_lab.htm* (accessed August 24, 2012).

Bed sediment, suspended sediment, and discrete stormwater samples were analyzed for PAHs and other organic constituents at the USGS EEEL. Sediment samples were analyzed following methods in Olson and others (2003), and stormwater samples were analyzed following methods in Orem and others (2007).

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Table 1. Sampling locations, drainage area, and summary of data collected in the Clinch River Basin in Virginia and Tennessee, 2009–2011.

[USGS, U.S. Geological Survey; VA, Virginia; TN, Tennessee; *V. iris, Villosa Iris; A. pectorosa, Actinonaias pectorosa*; VT, Virginia Tech. Green shaded rows represent sites with high-quality mussel assemblages sampled during this investigation. Orange shaded rows represent sites with low-quality mussel assemblages sampled during this investigation]

Project number upstream to downstream	USGS station number	USGS station name	Drainage area[1] (square miles)	Latitude (decimal degrees[2])	Longitude (decimal degrees[2])	Continuous monitoring of water quality or discharge	Discrete water quality base and storms 2009– 2011	Synoptic water quality July 2011	Synoptic water quality August 2011
1	03521500	Clinch River at Richlands, VA	137	37.0862	–81.7809	X[3]		X	X
2	0352190828	Clinch River at Route 636 at Swords Creek, VA	220	37.0324	–81.9179			X	X
3	03522519	Clinch River at Route 80 at Blackford, VA	355	36.9959	–81.9433			X	X
4	03523105	Clinch River above Nash Ford near Artrip, VA	483	36.9652	–82.0769			X	X
5	0352365258	Clinch River at Route 661 at Artrip, VA	511	36.9631	–82.1206				
6	03524000	Clinch River at Cleveland, VA	533	36.9448	–82.1549	X[4]		X	X
7	0352403497	Clinch River at Route 665 at Carterton, VA	584	36.9154	–82.2210			X	X
8	03524055	Clinch River at Highway 58 Alternate at St Paul, VA	629	36.9023	–82.3079			X	X
9	0352405765	Clinch River at Burtons Ford near St Paul, VA	648	36.8912	–82.3399				
10	03524085	Clinch River below Bull Run near St Paul, VA	670	36.8893	–82.3785			X	X
11	03524500	Guest River at Coeburn, VA	87	36.9293	–82.4563		X		
12	03524550	Guest River near Miller Yard, VA	100	36.8787	–82.4060	X[5]			
13	0352455225	Clinch River above Townes Tunnel near Dungannon, VA	776	36.8541	–82.4265			X	X
14	0352455262	Clinch River above Sinking Spring near Dungannon, VA	776	36.8427	–82.4332				
15	03524740	Clinch River at Route 65 at Dungannon, VA	820	36.8254	–82.4640	X[5]	X	X	X
16	03524748	Clinch River at Semones Island near Dungannon, VA	823	36.8100	–82.4836				
17	03524790	Clinch River below Dingus Branch near Grays Island, VA	843	36.7961	–82.5224				
18	03525024	Clinch River at Route 619 Bridge at Fort Blackmore, VA	897	36.7659	–82.5827				
19	03525025	Clinch River above Pendleton Island at Fort Blackmore, VA	898	36.7618	–82.5904			X	X
20	03525027	Clinch River above Suck Branch near Fort Blackmore, VA	898	36.7529	–82.5996				
21	03525128	Clinch River below Mill Creek at Craft Mill, VA	941	36.7080	–82.6520				
22	03525146	Clinch River above Stock Creek at Clinchport, VA	958	36.6756	–82.7420				
23	03525530	Clinch River above Copper Creek near Speers Ferry, VA	989	36.6559	–82.7456			X	X
24	03526990	Copper Creek above Mouth at Speers Ferry, VA	133	36.6554	–82.7432	X[5]			
25	03527000	Clinch River at Speers Ferry, VA	1,123	36.6487	–82.7504	X[6]		X	X
26	03527060	Clinch River near Flat Rock, VA	1,136	36.6219	–82.8172				
27	03527220	Clinch River near Looneys Gap (Horton Ford), TN	1,154	36.5728	–82.9388	X[4]	X	X	X
28	03527620	Clinch River at Kyles Ford, TN	1,267	36.5695	–83.0413			X	X
29	03527690	Clinch River at Frost Ford, TN	1,351	36.5305	–83.1509				
30	03527710	Clinch River at Swan Island, TN	1,404	36.4731	–83.2899				

Table 1. Sampling locations, drainage area, and summary of data collected in the Clinch River Basin in Virginia and Tennessee, 2009–2011.—Continued

[USGS, U.S. Geological Survey; VA, Virginia; TN, Tennessee; *V. iris, Villosa Iris; A. pectorosa, Actinonaias pectorosa*; VT, Virginia Tech. Green shaded rows represent sites with high-quality mussel assemblages sampled during this investigation. Orange shaded rows represent sites with low-quality mussel assemblages sampled during this investigation]

Project number upstream to downstream	USGS station number	Mollusk assemblage 2009–2010	Habitat assessment 2009–2010	Bed sediment collection 2010; trace metals, organics, and percent coal	Growth and survival *V. iris* juveniles silos 2009–2010	*V. iris* tissue analysis for trace metals and organics	Chlorophyll and dissolved organic carbon at silo stations	Native *A. pectorosa* tissue analysis for trace metals; VT histological study	Bed sediment collection 2011; trace metals, organics, and percent coal	Time of travel study for dissolved constituents 2009
1	03521500									
2	0352190828									
3	03522519									
4	03523105									
5	0352365258	X	X	X						
6	03524000			X	X	X	X			
7	0352403497	X	X	X						
8	03524055									
9	0352405765	X	X	X						
10	03524085									
11	03524500									
12	03524550									
13	0352455225									
14	0352455262									X
15	03524740	X	X	X						X
16	03524748	X	X		X	X	X	X	X	
17	03524790									X
18	03525024									X
19	03525025	X	X	X	X	X	X			X
20	03525027									X
21	03525128									X
22	03525146									X
23	03525530									
24	03526990									
25	03527000	X	X	X						X
26	03527060									X
27	03527220	X	X	X	X	X	X	X	X	X
28	03527620									
29	03527690	X	X	X						
30	03527710	X	X	X						

[1] Tennessee Valley Authority (1970).

[2] North American Datum of 1983 (NAD 83) coordinate system.

[3] Discharge at Richlands is a crest stage gage only.

[4] Continuous water quality and discharge.

[5] Continuous water quality only.

[6] Continuous discharge only.

Table 2. Freshwater mussel survey summary statistics for the Clinch River, 2009–2010.

[Quantitative richness, Richness determined from quantitative methods only; Total density, Mean individuals per square meter based on quantitative quadrat sampling; Total standard error, Standard error for density in individuals per square meter; Total CV, coefficient of variation in individuals per square meter; Total recruit, Total number of species with evidence of recent recruitment both quantitative and semiquantitative methods; Total live mussel richness, Total number of live species observed at site from both quantitate and semiquantitative methods; Total shell, Total number of species observed in shell collection or richness determined from shell; Total recruiting proportion, Proportion of total recruit to total live mussel richness; Live species proportion, Proportion of live to shell or total live mussel richness/total shell]

Project number[1]	Summary mussel survey statistics												
	Quantitative richness	Total density	Total standard error	Total CV	Total recruit	Total live mussel richness	Total shell	Total recruiting proportion	Live species proportion	Number Federal T&E species[2]	Number globally listed species	Number State listed species	Total reach area searched (square meters)
5	9	6.89	1.01	0.15	3	14	18	0.21	0.78	2	4	2	1,864
7	2	0.20	0.11	0.57	0	9	19	0.00	0.47	1	1	1	1,994
9	5	0.40	0.16	0.39	1	5	17	0.20	0.29	1	1	1	2,428
15	2	0.20	0.15	0.74	0	4	13	0.00	0.31	1	1	1	3,400
16	4	1.04	0.45	0.43	0	8	18	0.00	0.44	2	1	2	2,868
19	8	1.39	2.67	0.24	1	17	29	0.06	0.59	6	3	6	2,992
25	9	3.26	0.58	0.18	6	19	30	0.32	0.63	2	4	5	2,412
28	17	6.77	0.85	0.13	7	16	18	0.44	0.89	4	3	5	2,968
29	20	38.9	3.71	0.10	15	27	30	0.56	0.90	11	7	14	2,806
30	13	23.5	4.90	0.20	9	22	27	0.41	0.81	6	5	7	2,635
30	19	29.4	4.05	0.14	14	22	28	0.64	0.79	8	6	10	2,635

[1]Project numbers from table 1.

[2]Includes candidates.

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