CPCRI Science Team White Paper
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Water Monitoring Strategies to Inform Imperiled Species Conservation and Management in the Clinch River, Virginia and Tennessee

A White Paper Prepared by:
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Executive Summary

The Clinch River in Virginia and upstream from Norris Lake in Tennessee is among the most important freshwater bodies for biodiversity conservation in North America. Recent assessments have found mussels doing well in some parts of the Clinch River ("reference sections") but not in others ("impacted sections"). Efforts are underway to identify the stressor(s) or toxin(s) responsible for the severe declines that have occurred in certain Virginia River sections. A problem in evaluating water quality constituents for toxic effects is the lack of known ecotoxicological thresholds for such constituents that are protective of freshwater mussels. Hence, assessments of water quality data to identify potential toxins employs a reference approach, considering constituents that occur within impacted river sections at elevated levels, relative to reference sections, as potential stressors that merit further study. Targeted research employing this approach has found dissolved solids, certain major ions (including sulfates), and certain water-column metals to be at higher concentrations in an impacted river section relative to reference sections. As a means of furthering such identifications and characterizing problematic constituents' occurrence, we propose a coordinated water monitoring program for Virginia and Tennessee agencies. Such monitoring would produce benefits that include improved definition of concentrations and variability for dissolved solids and major ions at different locations, at different points in time, and under differing flow conditions in the Clinch River; such information would aid design of laboratory bioassay studies to assess physiological effects on freshwater mussels of major ions at environmentally relevant levels, should funding for such studies become available. Additional monitoring, using the protocols we describe, would also aid accurate quantification of metals and other trace elements that are elevated within the impacted sections, determining the temporal consistency of such differences; and informing the evaluation of potential transport mechanisms for metals as particle-bound forms, and importance of such forms as vectors for mussel exposure. A coordinated monitoring program across the two states will aid diagnosis of primary water-borne stressors or toxins that have negatively affected Virginia mussel assemblages, and development of management strategies for mussel conservation and protection in Tennessee and Virginia.

Introduction

The Clinch River forms in southwestern Virginia, near the town of Tazewell, and flows in a southwesterly direction for approximately 400 km before entering Norris Lake, a northeastern Tennessee impoundment in the upper Tennessee River system. The Clinch River upstream from Norris Lake is among the most important freshwater bodies for biodiversity conservation in North America. This section of the Clinch River and its tributaries support 133 species of fish (Jenkins and Burkhead, 1994) and at least 46 extant species of freshwater mussels (Neves et al., 1997; Jones et al., 2014). Of the 56 mussel species that have been reported from this section of the Clinch River (Stansbery, 1973); 29 are globally imperiled (Master et al., 1998), 20 are federally endangered, 2 are proposed endangered, and 31 are described as "at-risk" by state Natural Heritage programs (Tennessee Department of Environmental Conservation, Natural Heritage Inventory Program).

Recent assessments have found mussels doing well in some parts of the Clinch River but not in others (Jones et al. 2014; Ostby et al. 2014). Recent investigations conclude that water contaminants are a likely cause for the declines that have been observed in certain Virginia sections: contaminants are present and evidence to support other possible explanations is lacking (Ostby et al., 2014; Zipper et al., 2014). Efforts are underway to identify water contaminants that are acting as stressors or toxicants and are causing or contributing to decline. A primary means of investigation is to identify contaminants that are at elevated concentrations in river sections where mussels are doing poorly, compared to sections where mussels are thriving (CPCRI, 2009; Krstolic et al 2013; Johnson et al. 2014). Application of this approach with available agency monitoring data, however, is hampered by the dissimilar monitoring, sampling, and analysis procedures in Virginia and Tennessee (Price et al. 2014).

Here, we propose a more thorough and coordinated monitoring approach by agencies responsible for natural resource management in the Clinch River. We request involvement by environmental agencies in the effort to identify water contaminants contributing to mussel decline. The Clean Water Act has established the goal to "restore and maintain the chemical, physical, and biological integrity of the nation's waters" and to achieve “water quality which provides for the protection and propagation of fish, shellfish, and wildlife …” Maintaining viable freshwater mussel populations in the Clinch River, in both Virginia and Tennessee, is consistent with these Clean Water Act aims. Although the Clinch River’s freshwater mussels in northeastern Tennessee are doing well, the state of Tennessee has declared its section of the Clinch River as “threatened” in response to the observed decline of freshwater mussel assemblages upstream in Virginia; Tennessee’s action reflects the potential for factors causing mussel declines in Virginia to be carried downstream into Tennessee. In Virginia, mussel assemblages have experienced decline throughout the river’s extent; those declines, however, have been more significant in some river sections than in others, and signs of recovery are evident in some river sections but not in others (Jones et al. 2014). The Clinch River’s mussel population is a resource that requires an integrated management approach that transcends state boundaries. Although Virginia and Tennessee have maintained extensive water monitoring activities in the Clinch River for many years, dissimilar approaches by the two states have hampered efforts to diagnose and understand contaminant problems that appear to be affecting the Clinch River’s mussel population (Price et al., 2014).

Freshwater mussel conservation and management in the Clinch River is of direct relevance to requirements by certain federal statutes, including the Clean Water Act and the Endangered Species Act. Hence, we propose that state and federal agencies engage in collaborative activities as needed to ensure consistent, coordinated, and strategic water monitoring in the Clinch River over an extended time period. This strategic monitoring approach will provide a data record informing the diagnosis of causative agents for Clinch River mussel decline and, once those agents have been identified, management decisions.

Goal

To describe water monitoring procedures that, if carried out and coordinated in both Virginia and Tennessee segments of the Clinch River, will aid efforts to identify contaminants that are likely causes of mussel declines.

Rationale

A major scientific goal for CPCRI is to identify the specific environmental contaminant(s) that are acting as primary stressors or toxicants for freshwater mussels in the Clinch River's Virginia waters. Water monitoring data for a wide range of contaminants in the Clinch River are available. However, evaluation and assessment of those data for the purpose of identifying contaminants acting as primary stressors is problematic. One reason for this difficulty is that mussel-specific ecotoxicological thresholds are lacking for most of the environmental contaminants that occur in the Clinch River. This difficulty is compounded by the differences among mussel species in their response to individual contaminants (Naimo 1995; Keller et al. 2007; Gillis 2011). Another difficulty arises from the possibility that individual contaminants may negatively impact mussels synergistically with other contaminants present even though none exceed toxic thresholds. Laboratory toxicity tests also may inadequately estimate the response of animals *in situ* due to physiological differences from test organisms or variability in environmental conditions (Burton et al., 2006; Buchwalter et al., 2007). Such comparisons using native unionids are not available. The proposed monitoring results would provide higher confidence "best available science" for management decisions.

Given these difficulties, CPCRI scientists are proposing a monitoring plan designed to provide greater confidence and scientific certainty for management decisions. A major premise of this monitoring plan is that reaches of the Clinch River were mussels are thriving indicate contaminant levels are not harmful, whereas reaches where mussel assemblages are in decline indicate that deleterious contaminant levels are likely. River reaches where mussel assemblages are thriving and in decline are identified as reference sites and impacted sites, respectively. Considering differences among mussel assemblage status in different sections of the Clinch River, the following river sections are defined for the purpose of describing the proposed monitoring plan and its rationale:

- *Tennessee Reference:* Tennessee reference waters are defined, for the purpose of this document, as the Clinch River reach extending from Swan Island (river kilometer, RKM, 277.1; river mile, RM, 172.2), south of Sneedville, to the Virginia-Tennessee state line (RKM 325.2, RM 202.1). The reach that extends from Swan Island to Wallens Bend (RKM 309.8, RM 192.5) supports mussel assemblages that are dense, diverse, and distributed among age classes such that successful reproduction for many species is evident. The river reach extending upstream from Wallens Bend to the state line also hosts high-quality mussel assemblages, with greater density, richness, and reproduction than any river sections occurring further upstream.

- *Upstream Reference:* A secondary reference reach occurs further upstream in Virginia, extending from Cleveland (RKM 435.7; RM 270.8) to Nash Ford (RKM 449.8; RM 279.5), where mussels are reproducing and are at greater densities and richness than in other Virginia river sections. In this secondary (upstream) reference reach, however, mussels are at lower densities and richness than in Tennessee (Jones et al. 2014).

- *Impacted:* In contrast, the Virginia river reach extending from the Stock Creek confluence at Clinchport (RKM 343.3; RM 213.3) to the Lick Creek confluence near St. Paul (RKM 411.5; RM 255.7) have been described as “in severe decline with little evidence of recruitment” (Jones et al. 2014). In the text that follows, we describe this river reach as “impacted.” Within this impacted reach, the river segment extending from Clinchport to Semones Island (RKM 378.3; RM 235.1), near Dungannon, is of special concern due to the rich, diverse and reproducing mussel assemblages observed as recently as 1979 at Pendleton Island (RKM 364.2; RKM 226.3). Thus, this reach of the river is known to have physical habitat well suited to freshwater mussels; and is also known to have experienced severe and steady declines over the past 3+ decades, since 1979 (Jones et al. 2014).

Recent research and monitoring (see below) has found that certain water-column metals, associated trace elements, and major ions occur at elevated concentrations in the impacted section relative to reference river sections. Those data are from individual studies and relatively short-term monitoring efforts. We propose that state and federal agencies establish a targeted and consistent water monitoring program in the Clinch River for the purpose of further characterizing these water contaminants, their relative levels of occurrence, their correspondence with measured environmental conditions (such as streamflow), and their variability within the Clinch River’s reference and impacted sections. Such extended monitoring will aid determination of whether such patterns of occurrence are consistent, intermittent, or transitory; thus providing further definition to patterns that are becoming evident (see below).

The existence of consistently elevated levels of specific contaminants within the impacted river section, relative to reference sections, suggests that such contaminants and/or associated constituents may act as stressors or toxicants. Such results alone cannot be interpreted to define active stressors and toxicants, but identification of such contaminants is a critical step towards the goal of identifying active stressors and toxicants. The presence of multiple contaminants likely produces synergistic effects whereby physiologically negative impacts are exerted at concentration levels below single-factor toxic thresholds. Identification of such contaminants enables more focused research, including studies intended to target causal agents more directly. Greater certainty in such definitions, and greater understanding of those contaminants’ occurrences and patterns, will inform resource management strategies intended to increase both the likelihood of faunal recovery in Virginia and protection of the dense and diverse assemblages that remain in Tennessee.

Relationship of Current Proposal to Prior Studies

The monitoring program proposed by this document has been informed by results of prior studies. Those studies have revealed that certain major ions and metals are elevated in impacted river sections, relative to reference reaches. The monitoring program is intended to further characterize those constituents' occurrence. Those prior studies, and the reasoning that has led to current proposal while considering results of those prior studies, are described below.

Agency Data Analysis:

Virginia Department of Environmental Quality (VADEQ), Tennessee Department of Environment and Conservation (TDEC), and Tennessee Valley Authority (TVA) have conducted water monitoring programs in the Clinch River; the VADEQ and TDEC monitoring programs have extended for decades through the present day. Price et al. (2014) carried out an extensive analysis of agency water monitoring data obtained since the late 1960s for the Clinch River above Norris Lake. These analyses produced findings that are relatively robust for conventional pollutants that have been monitored routinely and extensively by both agencies: Surface water pH, nitrogen, ammonia, and phosphorous data provide no evidence for these constituents' current occurrence at problematic levels, and they demonstrate generally stable and declining concentrations in most river sections. The study also found, however, that total dissolved solids are rising throughout the river's extent. These monitoring records provided little recent data on the ionic composition of the total dissolved solids (TDS) and did not indicate how that ionic composition may have changed over the analysis period. Because the ionic composition of dissolved solids has been found to exert strong influence over a water's toxicity to laboratory test species (Mount et al. 1997), measurement of constituent ions and the spatial distribution relative to reference and impacted reaches is an essential objective of the monitoring program.

In contrast to conventional pollutants, observations of water column and sediment metals were sparse and inconsistent, both spatially and temporally. Sediment-quality data revealed metals contamination at levels that appear capable of impairing mussel growth throughout the river's extent (based on results from Wang et al., 2013), but Tennessee sediment-quality data are available only through the 1980s. Since the early 1990s, TDEC water-column metals data have been measured as total forms while VADEQ measured water-column metals as dissolved forms. Hence, recent data do not allow comparison of measured levels in the impacted river reach to those occurring in the Tennessee reference reach. No metals-related differences between the impacted reach and the upstream reference reach emerged from the recent CPCRI cooperative water quality monitoring project, although spatial comparisons were hampered by the nature of the data record. All Virginia observations for dissolved metals were well below EPA chronic criteria concentrations (CCC) and water-quality criteria defined by VADEQ. However, those criteria were developed without toxicity information for freshwater mussels.

USGS Study:

Johnson et al. (2014) conducted intensive analyses of water and sediment quality at two sites in the Clinch River, with most data obtained over a ~2 year period, 2009 - 2011. These researchers found that turbidity, specific conductance (SC), and water-column concentrations of several constituents, including Cl, Ca, F, K, Fe, Na, Mg, Se, and SO_4^{2-}, were significantly greater at the site in the impacted reach than at Horton Ford, in the Tennessee reference reach just south of the state line (RKM 320.4,RM 199.1). Additional water quality evaluation at 15 sites along the mainstem Clinch River showed that the spatial distributions of elevated dissolved ion (Ca, Na, Cl, and Fl) and metals (total Fe and Mn) concentrations correlated with spatial patterns of mussel decline. Johnson et al. (2014) found bed sediment contamination by metals at levels that appear capable of impairing mussel growth, but bed-sediment metal levels did not correspond with patterns of mussel decline. Caged hatchery-raised mussels exposed to ambient waters and native mussels harvested in impacted reaches accumulated higher tissue concentrations for a number of metals (e.g., Cd, Co, Cu, Fe, Pb, Ni, Th, V) than similarly treated mussels placed in reaches harboring high-quality assemblages, suggesting that water column exposure is more significant to metals uptake in mussels than are bed-sediment exposures.

The Johnson et al. (2014) findings indicate that dissolved major ion concentrations vary among river reaches but do not exceed water quality criteria. The researchers interpreted their findings to suggest that inflows to the mainstem from tributaries that drain mined areas increase dissolved constituent concentrations while water influxes from tributaries that drain land without mines cause dilution and lower concentrations. This interpretation is consistent with observations by numerous studies of elevated dissolvedsolids concentrations in waters draining lands with coal mining (e.g., Pond et al. 2008, and numerous other studies). In summary, Johnson et al. (2014) observed that concentrations of certain water-column metals (measured as total forms) and major ions correlated with patterns of mussel decline, while organic compounds, water-column nutrients, bed sediment metals, and bed sediment organic compounds did not.

Cooperative Monitoring:

Virginia DEQ and Tennessee DEC cooperated to conduct a joint monitoring program in the Clinch River over 16 months, from August 2012 through December 2013. Up to five integrated-width surface water samples were obtained at each of 8 sites on the mainstem Clinch River and from four tributaries (Indian Creek, Dumps Creek, Guest River, and Copper Creek). Mainstem sampling locations included two in the Tennessee reference river section, three in the impacted section, and two in the upstream reference section, as those river sections are defined in the above text. One sampling event was targeted to high-water conditions while others were collected at baseflow. Water quality parameters analyzed included nutrients, major ions (Ca, Mg, Na, K, SO_4^{2-}, Cl, and bicarbonate and carbonate alkalinity); 13 additional metals, with each measured as both dissolved- and total- forms; and dissolved and suspended solids. All water quality samples were analyzed by the Region 3 EPA lab in Fort Meade MD, and Virginia mainstem samples were also analyzed by the Virginia Consolidated Laboratories. There are missing data for some sample sites during specific sampling events.

Preliminary analysis of the Cooperative Monitoring data (unpublished; data analysis summary is available from C.E. Zipper) has revealed patterns concerning dissolved solids and major ions consistent with findings by Johnson et al. (2014), but the relatively small number of sampling events, combined with the inherent temporal variability of water quality, hampered the study's capability to yield strong conclusions. Total dissolved solids are nominally greater, on average, at 3 locations within the impacted reach compared to two TN reference reach sites and to two upstream reference reach sites; but this comparison does not yield a statistically significant difference at $p<0.05$. Sulfate concentrations are nominally higher in the impacted reach than in the TN reference reach; and are significantly higher in both TN reference and the impacted reaches than in the upstream reference reach. Although several trace metals were measured as both dissolved and total forms, only total Mn, dissolved Mn, and dissolved Zn were found to differ significantly among river section types; in all cases, concentrations were higher in the impacted reach than in one or both reference sections. However, trace metals comparisons were hampered by the detection limits for EPA lab analyses, as most observations for most of metals were listed as "below detect" (Table 1). Differential flow conditions among river reaches for the high-flow sampling event also hindered these comparisons. Comparisons among tributaries, conducted with only 3 observations per tributary, revealed higher SO_4^{2-} in Guest River and Dumps Creek than in Copper Creek and Indian Creek; higher dissolved solids ($p<0.05$) in Dumps Creek than in Copper Creek and Indian Creek; and nominally higher dissolved solids in Guest River than in Indian Creek and Copper Creek.

The Virginia DEQ lab, the Division of Consolidated Laboratory Services (DCLS), analyses included more trace metals and produced analytical values that were not truncated by detection limits, but did not include TN reference or tributary samples. These analyses yielded results for total dissolved solids and sulfates consistent with those from the derived from the EPA lab analyses for the VA mainstem sites. For trace metals, dissolved Al, Cu, and Ni; and total Fe, Mn, Cr, Cu, and Ni were all significantly higher in the impacted river section than in the upstream reference section. However, all of these measures were positively correlated with suspended fine sediments (STORET_SSC_FINE) which occurred at nominally higher levels within the impacted reach, suggesting that the elevated metals in the impacted reach may be adsorbed to or components of fine particles.

Table 1. Observation numbers (total observations, and those recorded less-than-detect with no analyte values) for trace-metals and metalloids, as both dissolved- and total- forms, in Cooperative Monitoring samples analyzed by EPA lab (samples from 12 sites, including 4 tributaries) and for DEQ lab samples (samples from 6 mainstem sites) which did not have reporting limits.

	- - EPA Lab Dissolved[a] - -			- - EPA Lab Total[a] - -			DEQ Dissolved[b]	DEQ Total[b]
Ele-ment	Quant. Limit (µg/L)	<QL Observ -ations	No. of Observ -ations	Quant. Limit (µg/L)	<QL Observ -ations	No. of Observ -ations	No. of Observ -ations	No. of Observ-ations
Ag								
Al	10	40	51	10	2	51	17	17
As	1	51	51	1	49	51	17	17
Ba							17	17
Be							17	17
Cd	1	51	51	1	51	51	17	17
Cr	1	36	51	1	31	51	17	17
Cu	1	49	51	1	42	51	17	17
Fe	100	49	51	100	16	51	17	17
Hg	0.2	51	51	0.2	51	51	17	17
Mn	1	0	51	1	0	51	17	17
Ni	1	0	51	1	1	51	17	17
Pb	1	51	51	1	47	51	17	17
Sb							17	17
Se	1	49	51	1	50	51	17	17
Tl	1	43	43	1	43	43	17	17
Zn	2	32	51	2	26	51	17	17

[a] EPA lab counts based on data provided for preliminary analysis, which included only 3 samples for each tributary.
[b] DEQ lab data were provided with analytical values that were not truncated at quantitative limits.

Summary:

When these studies are viewed collectively, they can be interpreted to describe an emerging pattern – but greater definition and understanding of that pattern will require additional study and data. It is becoming clear that dissolved solids, SC, and certain major ions are elevated in the impacted reach, relative to the TN reference reach (Johnson et al. 2014; and Cooperative Monitoring). This pattern is consistent with what we know of the watershed landscape, with several high-TDS tributaries entering the Clinch between the upstream and Tennessee reference sections; these include Dumps Creek and Guest River, as documented by the Cooperative Monitoring samples. Measured SC levels in the Clinch River in the impacted reach exceed the ~500 $\mu S\ cm^{-1}$ level found by Kunz et al. (2013) as toxic to one freshwater mussel species (Krstolic et al., 2013). Although the predominant ions present in the Kunz et al. (2013) solution are those that predominate water chemistry (by mass) in the Clinch River, ion ratios in the Clinch River's waters differ from those tested by Kunz et al. (2013). Nonetheless, these results can be interpreted to indicate strong concern for major ions as potential stressors or toxicants. However, major ion concentration differences between the TN reference and impacted reaches, as measured to date, are minor in magnitude relative to overall levels. Hence, more data on concentrations for dissolved solids and major ions at different locations, at different points in time, and under differing flow conditions will aid in characterizing water quality differences for these constituents among river reaches. Such information would aid design of laboratory bioassay and mesocosm studies to assess physiological effects on freshwater mussels of major ions at environmentally relevant levels, should funding for such studies become available.

Trace elements, including trace metals, are also a strong concern. Freshwater mussels are known to be highly sensitive to certain trace metals, including Cd, Cu, Ni, Pb and Zn (Havlik and Marking, 1987; Jacobsen *et al.*, 1993, 1997; Naimo, 1995; Keller *et al.*, 2007; Cope *et al.*, 2008; Wang *et al.*, 2010). Both Johnson et al. (2014) and the Cooperative Monitoring have revealed elevated trace metals, both dissolved and total, in the impacted reach; and Johnson et al. (2014) found elevated body burdens in caged mussels in the impacted reach. There is some consistency in the metals of concern identified by the two studies (e.g., Mn was identified for concern by several analyses), but other elements have been identified as being of concern by only one of these multiple analyses. Additional monitoring data, obtained using the protocols we describe below, would aid identification of trace metals and other elements that may occur at elevated levels, consistently, within the impacted section relative to reference sections and provide better information for cumulative stressor analyses.

Additional cooperative monitoring by agencies will also aid evaluating potential transport and exposure mechanisms for metals. For example, certain metals can occur within or bound to sediment particles. Such metals can enter the water column and move episodically in association with high flow; when measured as total- forms, such metals' concentrations may be correlated with measured concentrations of suspended sediments. The Cooperative Monitoring results revealed total- forms of several metals exhibiting this pattern, as expected. Those results also revealed certain "dissolved" concentrations as also occurring in association with suspended sediments, a finding that was not expected. This result would be consistent with the occurrence of such elements as within, or bound to, very fine particles that are able to pass through the 0.45 micron filters in processing waters to be analyzed for dissolved elemental forms. Such fine particles can also be expected to move in association with elevated streamflows, but with velocity thresholds for movement lower than those required for larger particles. Johnson et al. (2014) found elevated turbidity, measured at 15-minute intervals for > 1 year, at Dungannon (impacted) relative to Horton Ford in the TN reference section; this finding suggests the possibility that mussels in the impacted section are being exposed to higher concentrations of fine suspended particles; if those particles are acting as a metals' exposure vector, this finding could be of mussel-conservation significance.

Table 2. Characteristics for selected Clinch River and Guest River locations, with average channel gradients for intervening river sections (calculated).

USGS Site Number	Location	River Section	Eleva-tion (m)	River km	Drainage Area (sq km)	Gradient to Next Listed Downstream Point (m/km)
03523105	above Nash Ford near Artrip	Up Ref †	479.9	450	1,251	1.1
03524000	Cleveland	Up Ref †	466.3	437	1,380	1.4
	Dumps Creek confluence		458.0	431		1.4
0352403497	Rte 665, Carterton		449.3	425	1,513	0.2
03524055	Hwy 58 Alt, St Paul	Imp	445.9	410	1,629	0.8
03524085	below Bull Run near St Paul	Imp	435.5	397	1,735	3.8
03524500	Guest River, Coeburn		589.5	10	226	16.7
	Guest River Confluence	Imp	421.0	393		2.1
03524740	Rte 65, Dungannon	Imp †	395.9	381	2,124	0.9
03525024	Rte 619 Bridge, Fort Blackmore	Imp †	382.2	366	2,323	0.7
03525128	below Mill Creek, Craft Mill	Imp †	372.6	352	2,437	0.3
03525146	above Stock Creek, Clinchport	Imp	369.6	343	2,556	0.1
03527220	Speers Ferry		369.3	340	2,909	0.6
03527220	near Looneys Gap (Horton Ford)	TN ref †	356.9	320	2,989	0.8
03527620	Kyles Ford	TN ref †	344.9	305	3,282	0.3
03527710	Swan Island	TN ref †	335.8	277	3,636	

Notes: Site numbering, site naming, and watershed areas from Krstolic et al. (2013). All sites on Clinch River except Guest River, Coeburn. Elevations prepared by Patricia Donovan, Virginia Tech, from USGS (2009). River sections are Upstream Reference, Impacted, and Tennessee Reference, as described elsewhere in this document. † are locations at or close to sampling points proposed by this document (See Figure 1, Table 3).

Channel gradient likely influences the spatial distribution of contaminants and bioavailability in the Clinch River. The average gradient in and above the impacted reach is greater than that within the TN reference reach (Table 2). This channel gradient configuration is consistent with an hypothesis of long-duration particle-bound metals exposure that could be occurring preferentially in the impacted reach. The upstream half of the impacted reach also receives inputs from high-gradient tributaries draining areas northwest of the mainstem that host urbanized areas, active coal mines, abandoned mines and remnant coal-refuse, and other industrial activities that are potential sources for metals that may become mobilized and enter water courses. Among these tributaries is the Guest River, a 4th order stream with a high gradient that enters the Clinch River just above Dungannon (Table 2). Those potential pollutant source areas combined with known channel configurations potentially create a biological-effect mechanism by particle-bound metals: (1) mobilization in upper watershed areas; (2) transport of those particles by faster-flowing waters in tributaries' steeper channel segments into the mainstem due to turbulence-generated particle suspension; (3) transport of those particles into the impacted reach facilitated by steep mainstem channel gradients above the impacted reach; and (4) preferential exposure of mussels in the impacted reach to such solid-phase or particle-bound metals. This scenario is consistent with an hypothesis that particle-bound metals may be a significant source of mussel toxins. The declining channel gradient directly below the impacted reach, and the very low gradient within the reach extending from Kyles Ford to Swan Island, where mussel densities are greatest, would also be consistent with this mechanism's influence, as there would be less likelihood for hydrologic processes to chronically transport such particle-bound contaminants from the impacted reach to the TN reference reach, relative to the likelihood of transport from upper-tributary locations into the impacted reach. The fact that bed-sediment analyses also revealed metals' contamination at numerous locations in the mainstem (Johnson et al. 2014; Price et al. 2014) adds further evidence for potential influence by particle-bound metals.

Other explanations for mussel-status differences among river reaches, however, would also be possible. For example, watershed drainage area above the TN reference reach is 30-40% greater than that above the impacted reach which could dilute stressors or toxicants. It is also possible that stressors and toxicants may be assimilated by biological or physical processes as they move downstream reducing the negative impacts on mussels. Additional monitoring data, obtained using the protocols we describe below, would aid in resolving such questions.

Proposed Water Monitoring Program Design

In order for the proposed monitoring design to yield useful data, comparable sampling and analysis procedures would be required in both Tennessee and Virginia. Here, we describe such procedures focusing on the primary features of the monitoring program.

Monitoring Locations:

We propose that compatible and complementary procedures be employed in both states, which would enable Tennessee data to be interpreted as a reference for use in assessment of Virginia data from the impacted river section. Should a parameter within the impacted reach differ significantly from the same parameter in Tennessee, that parameter would be identified as a contaminant of concern.

The monitoring program would be designed to enable comparison of measured water parameters among Clinch River locations supporting mussel assemblages of differing status (Table 3). Three sets of monitoring points (TN Reference, Impacted, and Upstream Reference) have been defined for the purpose of such analyses. Three sampling points have been defined within each of the TN Reference and Impacted reaches so as to aid the analyses of interest, which will compare water quality between these two sections. Having three points within both sections will aid statistical comparisons of data from these two river sections. Only two points have been defined within the upstream reference reach, considering both its limited spatial extent and the cost required for each additional sampling point.

Each of the three site types includes or is close to a USGS stream gage. Hence, water flows at sampling times can be known and interpreted to better understand particle-bound metals transport.

Table 3. Proposed monitoring locations.

River Mile	Site Type	Description	Rationale
172.3	TN Ref	Clinch River mile 177.4, Rt 33 bridge south of Sneedville.	Reference for best-case mussel populations. Site is both easily accessible and upstream from the Swan Island sampling site (mile 172.4), which has been sampled for multiple years, upstream of Briery Creek (mile 174.5, sampled in 2006), and immediately downstream from the mile 178.4 sampling site at Sneedville (2006).
~189	TN Ref	Route 70 bridge near Kyles Ford, Tennessee (downstream from mile 189.6 sampling site, Kyles Ford)	Reference for best-case mussel populations (Kyles Ford, sampled 2004); downstream from North Fork Clinch River tributary influx. Provides a bridge for sampling, unlike the Wallens Bend (mile 192.5) location, sampled by Cooperative Monitoring, which requires wading.
199.1†	TN Ref	Clinch River mile 199.1 at bridge, Horton Ford, Tennessee.	Reference for high quality mussel populations; also at USGS gage.
~ 218	Impacted	Rt. 645 bridge at Craft Mill	Closer to Pendleton Island, and thus more clearly within the impacted reach, than Clinchport sampling point used for Cooperative Monitoring. Upstream from USGS gage at Speers Ferry. [note: a possible alternative sampling site would be the Slant swinging bridge at river mile 223.5, which is closer to Pendleton Island and a VDGIF mussel sampling site].
227.25 †	Impacted	Ft Blackmore, either Rt. 72 or Old 72 Bridge.,	Within impacted reach, close to and upstream from Pendleton Island
236.89 †	Impacted	Dungannon, Rt. 65 Bridge	Within impacted reach.
271.50 †	Upstream Ref	Cleveland, Rt. 82 Bridge	Downstream end of recovering reach, and close to USGS streamflow gage.
~279.4	Upstream Ref	Nash Ford, Rt. 645 Bridge	Upstream end of recovering reach.

† Sampling locations for TDEC-VADEQ Cooperative Monitoring study.

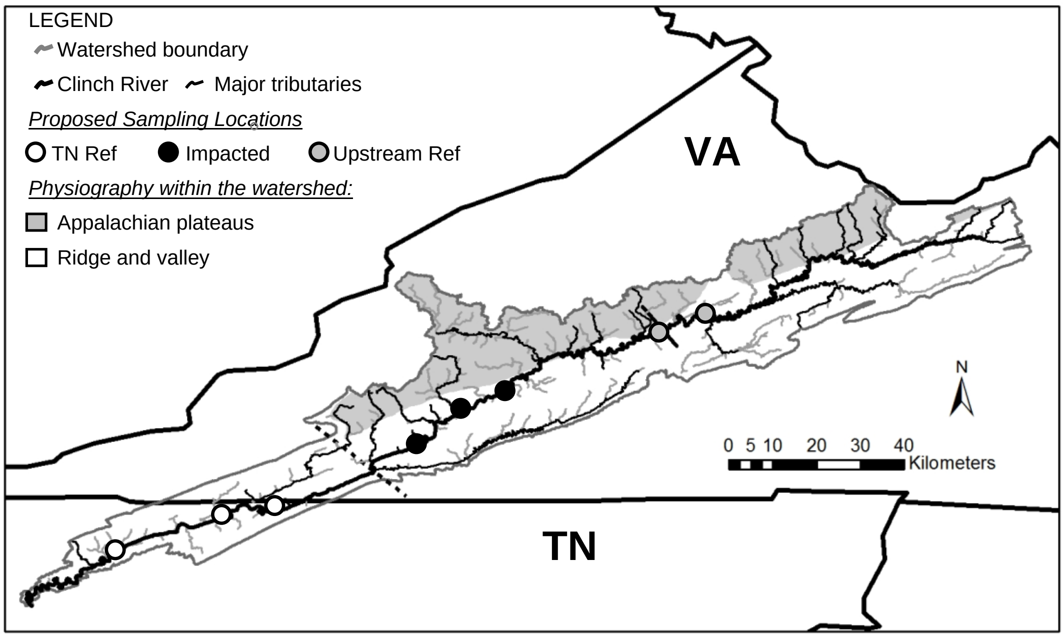

Figure 1. Approximate sampling locations, as proposed, within a section of the Clinch River watershed.

Water Contaminant Priorities:

Given the scientific evidence reviewed above, primary focus would be placed on trace metals, certain non-metallic trace elements of special interest, and major ions. All metals and trace elements would be obtained in both total (unfiltered) and dissolved (filtered at 0.45 microns) forms, so as to enable greater certainty concerning whether contaminants are creating primary exposures as “dissolved” (including very fine particulate) or particle-bound forms. To evaluate how trace metals are being transported, we also request any and all standard analyses to characterize transported particles by size category and/or composition (i.e., mineral vs. organic). We suggest that other basic water-quality characterization data should be obtained.

Table 4. Proposed water constituents for sampling and analysis.

Parameter Group (listed by priority)	STORET CODE	VADEQ GROUP CODE	Comment
Major Ions, Other Salinity Indicators			
CALCIUM, DISSOLVED (mg/L AS CA)	00915	DCMET1	Negatively correlated to multiple mussel health metrics (Johnson et al. 2014)
MAGNESIUM, DISSOLVED (mg/L AS mg)	00925	DCMET1	Higher body burdens for caged mussels in impacted section vs. TN (Johnson et al. 2014).
SODIUM, DISSOLVED (mg/L AS NA)	00930	PROB4	Negatively correlated to multiple mussel health metrics (Johnson et al. 2014)
POTASSIUM, DISSOLVED (mg/L AS K)	00935	PROB4	Higher body burdens for caged mussels in impacted section vs. TN (Johnson et al. 2014).
CHLORIDE, DISSOLVED IN WATER mg/L	00941	PROB4	
SULFATE, DISSOLVED (mg/L AS SO4)	00946	PROB4	Higher water-column levels in impacted section vs. TN (Johnson et al. 2014).
Bicarbonate, dissolved			Bicarbonate is often elevated in mining-influenced headwater streams (Pond et al. 2008). .
SPECIFIC CONDUCTANCE (UMHOS/CM @ 25C)	00095	PROB4	Installation of continuous conductivity loggers would be desirable, if such were to be within the routine capability of personnel operating the monitoring program. However, that is not requested here.
RESIDUE,TOTAL FILTRABLE (DRIED AT 180C),mg/L	70300	PROB4	Proxy for total dissolved solids
Trace metals, other trace elements			These elements are included in the VADEQ TCMET and DCMET lab series.
ALUMINUM, DISSOLVED (µg/L AS AL)	01106	DCMET1	
ALUMINUM, TOTAL (µg/L AS AL)	01105	TCMET1	
ANTIMONY, DISSOLVED (µg/L AS SB)	01095	DCMET1	
ANTIMONY, TOTAL (µg/L AS SB)	01097	TCMET1	
ARSENIC, DISSOLVED (µg/L AS AS)	01000	DCMET1	
ARSENIC, TOTAL (µg/L AS AS)	01002	TCMET1	

Table 4 (continued). Proposed water constituents for sampling and analysis.

Parameter Group (listed by priority)	STORET CODE	VADEQ GROUP CODE	Comment
BARIUM, DISSOLVED (µg/L AS BA)	01005	DCMET1	
BARIUM, TOTAL (µg/L AS BA)	01007	TCMET1	
BERYLLIUM, DISSOLVED (µg/L AS BE)	01010	DCMET1	
BERYLLIUM, TOTAL (µg/L AS BE)	01012	TCMET1	
CADMIUM, DISSOLVED (µg/L AS CD)	01025	DCMET1	Higher body burdens for caged mussels in impacted section vs. TN (Johnson et al. 2014).
CADMIUM, TOTAL (µg/L AS CD)	01027	TCMET1	
CHROMIUM, DISSOLVED (µg/L AS CR)	01030	DCMET1	
CHROMIUM, TOTAL (µg/L AS CR)	01034	TCMET1	
COPPER, DISSOLVED (µg/L AS CU)	01040	DCMET1	Historic data shows high levels of Cu throughout the system (Price et al. 2014). Higher body burdens for caged mussels in impacted section vs. TN (Johnson et al. 2014).
COPPER, TOTAL (µg/L AS CU)	01042	TCMET1	
IRON, DISSOLVED (µg/L AS FE)	01046	DCMET1	Higher body burdens for caged mussels in impacted section vs. TN (Johnson et al. 2014).
IRON, TOTAL (µg/L AS FE)	01045	TCMET1	
LEAD, DISSOLVED (µg/L AS PB)	01049	DCMET1	Higher body burdens for caged mussels in impacted section vs. TN (Johnson et al. 2014).
LEAD, TOTAL (µg/L AS PB)	01051	TCMET1	
MANGANESE, DISSOLVED (µg/L AS MN)	01056	DCMET1	
MANGANESE, TOTAL (µg/L AS MN)	01055	TCMET1	
MERCURY-TL,FILTERED WATER,ULTRATRACE METHOD ng/L	50091	DCMET1	
NICKEL, DISSOLVED (µg/L AS NI)	01065	DCMET1	
NICKEL, TOTAL (µg/L AS NI)	01067	TCMET1	
SELENIUM, DISSOLVED (µg/L AS SE)	01145	DCMET1	
SELENIUM, TOTAL (µg/L AS SE)	01147	TCMET1	
SILVER, DISSOLVED (µg/L AS AG)	01075	DCMET1	
SILVER, TOTAL (µg/L AS AG)	01077	TCMET1	
THALLIUM, DISSOLVED (µg/L AS TL)	01057	DCMET1	

Table 4 (continued). Proposed water constituents for sampling and analysis.

Parameter Group (listed by priority)	STORET CODE	VADEQ GROUP CODE	Comment
THALLIUM, TOTAL (µg/L AS TL)	01059	TCMET1	
ZINC, DISSOLVED (µg/L AS ZN)	01090	DCMET1	Historic data shows high levels of Zn throughout the system (Price et al. 2014)
ZINC, TOTAL (µg/L AS ZN)	01092	TCMET1	
Strontium, Dissolved			Johnson et al. (2014) found Sr accumulating in caged mussels preferentially within the impacted section. Sr is sometimes used as a coal-influence tracer.
Strontium, Total			
RESIDUE, TOTAL NONFILTRABLE (mg/L)	00530	PROB4	Proxy for total suspended solids
SUSPENDED SED SIEVE DIAMETER,% FINER THAN .062MM	70331	SSC-C2	
SUSP. SED. CONC. - >62 µm, mg/L, (Method C)	SSC-COARSE	SSC-C2	
SUSP. SED. CONC. - <62 µm, mg/L, (Method C)	SSC-FINE	SSC-C2	Cooperative Monitoring program results, VDEQ lab samples, revealed positive correlations of SSC fines with dissolved Al, Cu, Ni.
SUSP. SED. CONC. TOTAL, mg/L,(Method B)	SSC-TOTAL	SSC-C2	
<u>Basic Characterization</u>			
Field parameters (DO, SC, Temp, pH)	FDT		
<u>Nutrients</u>			With the exception of ammonia, we do not see nutrients as essential. They are suggested expecting cost to be relatively low and understanding the role of nutrients as human activity indicators. Nutrients would be a lower priority than metals/trace elements and major ions.
NITROGEN, AMMONIA, TOTAL (mg/L AS N)	00610	NUT4	Freshwater mussels are highly sensitive to ammonia (reference).
NITROGEN, TOTAL (mg/L AS N)	00600	PROB4	
PHOSPHORUS, TOTAL (mg/L AS P)	00665	TNUTL	Negatively correlated to multiple mussel health metrics (Johnson et al. 2014)
CARBON, TOTAL ORGANIC (mg/L AS C)	00680	TOC	

Sampling and Laboratory Analysis Procedures:

It is essential that identical and comparable procedures be employed across all sampling locations. Because primary monitoring targets are trace metals that occur at part-per-billion levels, "Clean Hands" procedures and pre-cleaned equipment that are intended to minimize risks of sample contamination should be employed rigorously. Laboratory procedures should report sub-ppb analyte levels for most trace metals for the data to be useful in achieving primary stated goals. The recommended methodology that is currently being used by the Virginia Division of Consolidated Laboratory Services for trace elemental determinations is that of Methods of Standard Additions which does not suffer from large percent variances at low concentrations. For all VADEQ ambient monitoring data, uncensored values and Practical Quantitation Limits (generally the lowest concentration above zero from the calibration curve), and the Method Detection Limit (MDL, 40 CFR 136 Appendix B) are reported. These laboratory procedures have been in place for more than a decade, have been thoroughly vetted and reviewed, and enable use of <MDL uncensored values for statistical analysis. We would request that any lab conducting analyses for this study be capable of using the Method of Standard Additions (a method in which samples are consistently spiked to generate a regression curve to calculate true analyte concentrations within sample matrix interferences (U.S. Environmental Protection Agency, 1980)) or another similarly sensitive method for detecting very low levels of analyte.

Integrated depth- and width- sampling would be the most desirable sampling method if it can be implemented with available resources. All proposed sampling sites are accessible from a bridge, which would enable integrated depth- and width- sampling at less effort and cost than if bridges were not available. If the cost of integrated depth- and width- sampling were to prove prohibitive, an alternative procedure at each sampling event would be to perform an initial survey of conductivity moving laterally across the river, and to take a depth-integrated sample at the thalweg if conductivity does not vary by >10% laterally across the river. If conductivity does demonstrate >10% lateral difference, and depth- and width- integrated sample would be taken.

Sample splits to multiple containers should be achieved using a method that ensures similar particle distributions among the multiple containers, such as a churn splitter or the VADEQ clean-metals bridge bottle

Laboratory analysis procedures should also be identical or provide identical results between split samples. Ideally, all samples would be sent to the same lab and, for each sampling event, analyzed in the same batch so as to ensure comparability. If agencies were to agree on the Virginia DCLS as the best option for analysis, Tennessee samples could be transported to the consolidated lab at minimal cost if delivered to the Virginia DEQ office at Abingdon.

Sample Timing and Scheduling

The primary goal is to compare contaminant levels among various river sections. Coordination of scheduling will enable this goal to be achieved with greater statistical certainty than if sampling schedules are not coordinated.

Table 5. Proposed scheduling and timing guidance, with supporting rationale.

Attribute	Scheduling Request	Rationale
Time Period	Multiple years – at least 3 years if possible. Data will be analyzed and evaluated by the CPCRI Science Team after the first year. Based on that analysis, the Science Team will recommend if the site configuration described here should be maintained or if some sampling sites should be relocated to enable stressor source identification or for other purposes. Any recommendations for relocation would be developed in consultation with VDEQ and TDEC, so as to ensure that a newly recommended suite of sites can be sampled with the resources being made available for this study by TDEC, VDEQ, and supporting entities.	Weather and stream conditions vary from day to day and from year to year. Water quality is influenced by weather and stream conditions.
Baseflow Sampling Frequency	More samples per year are better than fewer samples per year. Considering agency procedures, we request bi-monthly sampling (6x per year) as a goal, with a minimum of 4x samples per year. Regardless of frequency, baseflow samples should be distributed throughout the year.	Larger numbers of samples allow characterization of contaminant concentrations under varying stream conditions and seasons, and allow increased certainty in data interpretation.
Stormflow Sampling Frequency	Ideally, baseflow sampling schedule would be supplemented by obtaining stormflow samples. We propose 2x stormflow samples per year as a sampling goal – one during the warm weather months and the other during vegetation dormancy – with a minimum of 1x stormflow samples per year.	Particle-bound contaminants can be expected to move preferentially during high-flow events.
Sampling Dates	We request that sampling dates and times be consistent as possible. Ideally, all samples for each sampling event would be collected on the same day.	Our goal is to compare VA contaminant levels to those occurring in TN. The statistical power of comparisons will be enhanced if they can be analyzed as paired samples. That can only occur if sampling schedules are consistent between VA and TN.
Seasonality - baseflow	We request that baseflow samples be evenly distributed over the year; and that the sampling time frames be repeated from year to year.	Such a sampling regime would enable improved characterization of seasonal patterns (if present). The existence of seasonal patterns may aid diagnosis of source.

Table 5 (continued). Proposed scheduling and timing guidance, with supporting rationale.

Attribute	Scheduling Request	Rationale
Seasonality - stormflow	We request that stormflow samples be evenly distributed among leaf-on and leaf-off seasons, to the extent that scheduling allows.	Such a sampling regime would enable improved characterization of seasonal patterns (if present).
Hydrograph timing for stormflow samples	We request that stormflow samples be obtained from hydrograph rising, when possible.	Rising-limb samples will enable better characterization of particle mobilization by the stormflows. We make this request, understanding the logistical difficulty of the rapid mobilization that will be required to obtain rising-limb samples. Falling-limb samples would also be of value when mobilization required for a rising-limb sample is not possible.
Upstream vs. downstream sampling direction	We request sampling in an upstream direction.	The direction of movement by the sampling team during sampling events will influence sampling outcomes. The Science Team will consider this question as the document develops.

Prioritization

We have proposed a thorough and comprehensive monitoring program, which we see as justified given the nature of the biological resource, and threats to that resource, that are present in the Clinch River. Nonetheless, we recognize that funding limitations are a reality. Given that recognition, we propose the following principles for prioritization if resources become available but those resources prove inadequate to implement to full monitoring program as described:

We suggest that a high priority be placed on inclusion of a wide suite of metal and trace element contaminants, to be monitored as both dissolved- and total-forms to be analyzed with high precision, and to be sampled using procedure that ensure against sample contamination. We see inclusion of major ions and suspended solids in the sampling program as essential.

Monitoring location priorities would be:

- TN ref and Impacted: Top priority. Horton Ford should be a high priority within the TN Ref group due to USGS stream gage proximity.
- VA ref: second priority. Cleveland should be a high priority within the VA Ref group due to USGS stream gage proximity.

We recognize that coordination of sample timing may prove problematic, especially if the program were to be implemented by having VADEQ and TDEC personnel sample the Virginia and Tennessee locations, respectively. However in such situation, we would strongly encourage the two agencies to coordinate sample timing such that Impacted and TN Reference samples are closely aligned temporally, so as to enable statistical analysis approaches that incorporate paired comparisons. Although it would be desirable for all sampling to be aligned temporally, we see the Impacted vs. TN Ref comparisons – and, hence, their temporal alignment – as most critical.

Execution

Given the state agencies' water monitoring programs and capabilities, we believe that VADEQ and TDEC personnel could do an excellent job with the proposed monitoring program, if the agencies were able to provide or were provided with adequate resources to support the sampling and analysis. In order for the proposed monitoring program to achieve intended goals successfully, interstate coordination would be required for sampling procedures, analytical procedures, analytical precision, and sample timing. If the coordination and personnel allocation required for the proposed sampling would not be feasible for the state agencies within context of other demands, USGS personnel would also have the capability to conduct the proposed monitoring if adequate resources were made available.

Intended Outcomes

Better information will enable better management.

A collaborative monitoring program, coordinated to ensure statistical comparability among monitoring locations supporting mussel populations of differing ecological status, would identify specific water constituents for further study. Contaminants occurring at elevated levels at locations with low-quality mussel assemblages, relative to locations supporting higher-quality assemblages, would become suspect as potential stressors or toxicants or as indicators that are associated with potential stressors or toxicants. Improved knowledge concerning which contaminants occur at elevated levels in the impacted reach, relative to TN Ref (and to Upstream Ref if possible) would enable resource managers to implement contaminant reduction strategies based on best available science and inform future research concerning potential effects by such contaminants on freshwater mussels. Should further research conclude that any of the constituents targeted by the proposed monitoring program, or directly associated constituents, are acting as stressors or toxicants, data derived by the proposed monitoring program would aid development of strategies for management of such stressors or toxicants.

Contributing Authors

This document was assembled by the Clinch-Powell Clean Rivers Initiative (CPCRI) Science Team. The CPCRI is a voluntary coalition of agencies, research scientists, conservation organizations, and industry leaders that works to protect and restore water quality in North America's most important river for rare and imperiled freshwater animals.

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References

Buchwalter, D. B., D. J. Cain, W. H. Clements, and S. N. Luoma. 2007. Using biodynamic models to reconcile differences between laboratory toxicity tests and field biomonitoring with aquatic insects. Environmental Science & Technology 41(13):4821-4828.

Burton, G. A., M. S.Greenberg, C. D. Rowland, C. A. Irvine, D. R. Lavoie, J. A. Brooker, L. Moore, D. F. N. Raymer, and R. A. McWilliam. 2006. *In situ* exposures using caged organisms: a multi-compartment approach to detect aquatic toxicity and bioaccumulation. Environmental Pollution 134:133-144.

CPCRI. 2009. Science Plan for the Clinch-Powell River System: Identifying Environmental Stressors Responsible for Biodiversity Decline. Prepared by the Science Team, Clinch-Powell Clean Rivers Initiative. Abingdon, VA. 26 pp.

Cope, W.G., R.B. Bringolf, D.B. Buchwalter, T.J. Newton, C.G. Ingersoll, N. Wang, T. Augspurger, F.J. Dwyer, M.C. Barnhart, R.J. Neves, and E. Hammer. 2008. Differential exposure, duration, and sensitivity of Unionoidean bivalve life stages to environmental contaminants. Journal of the North American Benthological Society 27:451-462.

Gillis, P. L. 2011. Assessing the toxicity of sodium chloride to the glochidia of freshwater mussels: Implications for salinization of surface waters. Environmental Pollution 159: 1702-1708.

Havlik, M.E., and L. Marking. 1987. Effects of contaminants on Naiad mollusks (Unionidae): A review. Resource Publication 164. U.S. Fish and Wildlife Service, Washington, DC.

Jacobson, P.J., J. Farris, D. Cherry, and R. Neves 1993. Juvenile freshwater mussel (Bivalvia: Unionidae) responses to acute toxicity testing with copper. Environmental Toxicology and Chemistry 12:879–833.

Jacobson, P.J., R. Neves, D. Cherry, and J. Farris. 1997. Sensitivity of glochidial stages of freshwater mussels (Bivalvia: Unionidae) to copper. Environmental Toxicology and Chemistry 16:2384–2392.

Jenkins, R., and N. Burkhead. 1994. Freshwater Fishes of Virginia. American Fisheries Society, Bethesda, Maryland. ISBN-13: 978-0913235874.

Johnson, G.C., J.L. Krstolic, B.J.K. Ostby, and W.F. Henley. 2014. Influences of water and sediment quality and hydrologic processes on mussels in the Clinch River. Journal of the American Water Resources Association (In review).

Jones J., S. Ahlstedt, B. Ostby, B. Beaty, M. Pinder, N. Eckert, R. Butler, D. Hubbs, C, Walker, S. Hanlon, J. Schmerfeld, and R. Neves. 2014. Quantitative monitoring of freshwater mussel populations in the Clinch River, Tennessee and Virginia, from 2004-2009, and collapse of the fauna at Pendleton Island since 1979. Journal of the American Water Resources Association (In review).

Keller, A., M. Lydy, and S. Ruessler. 2007. Unionid mussel sensitivity to environmental contaminants. P. 151-167, in: J.L. Farris, J.H. Van Hassel. Freshwater Bivalve Ecotoxicology. CRC Press, Boca Raton. ISBN-13: 978-1420042849.

Krstolic, J.L., G.C. Johnson, and B.J.K. Ostby. 2013. Water Quality, Sediment Characteristics, Aquatic Habitat, Geomorphology, and Mussel Population Status of the Clinch River, Virginia and Tennessee, 2009–2011. U.S. Geological Survey Data Series 802 (including data tables, http://pubs.usgs.gov/ds/0802/)

Kunz, J.L., J.M. Conley, D.B. Buchwalter, T.J. Norberg-King, N.E. Kemble, N. Wang, and C. G. Ingersoll. 2013. Use of reconstituted waters to evaluate effects of elevated major ions associated with mountaintop coal mining on freshwater invertebrates. Environmental Toxicology & Chemistry 32:2826-2835.

Master, L., S. Flack, and B. Stein (eds). 1998. Rivers of Life: Critical Watersheds for Protecting Freshwater Biodiversity. The Nature Conservancy, Arlington, Virginia: 71 pp. ISBN: 1-886765-09-X. http://www.natureserve.org/library/riversoflife.pdf

Mount, D. R., J. M. Gulley, J. R. Hockett, T. D. Garrison, J. M. Evans. 1997. Statistical models to predict the toxicity of major ions to Ceriodaphnia dubia, Daphnia magna, and fathead minnows (Pimephales promelas). Environmental Toxicology and Chemistry, 16:2009–2019.

Naimo, T.J. 1995. A review of the effects of heavy metals on freshwater mussels. Ecotoxicology 4: 341–362.

Neves, R., A. Bogan, and J. Williams, S. Ahlstedt, and P. Hartfield. 1997. Status of aquatic mollusks in the southeastern United States: A downward spiral of diversity. p. 43-85, *in*: G. Benz and D. Collins (eds.). Aquatic Fauna in Peril: The Southeastern Perspective. Lenz Design and Communications, Decatur, GA. ISBN-13: 978-0965484107

Ostby, B.J.K., J.L. Krstolic, and G.C. Johnson. 2014. A reach-scale comparison of geomorphology, stream bed composition, fish habitat, riparian condition and current mollusk assemblages for select sites in the Clinch River. Journal of the American Water Resources Association (In review).

Pond G.J., M.E. Passmore, F.A. Borsuk, L. Reynolds, C.J. Rose, 2008. Downstream effects of mountaintop coal mining: comparing biological conditions using family- and genus level macroinvertebrate bioassessment tools. Journal of the North American Benthological Society 27:717-737.

Price, J., C. Zipper, J. Jones, and C. Franck. 2014. Water and sediment quality in the Clinch River, Virginia - Tennessee, over nearly five decades. Journal of the American Water Resources Association (In review).

Stansbery, D. 1973. A preliminary report on the naiad fauna of the Clinch River in the southern Appalachian mountains of Virginia and Tennessee (Mollusca: Bivalvia: Unionoida). Bulletin of the American Malacological Union, pp. 20-22.

U.S. Environmental Protection Agency. 1980. Method of Standard Additions and Effects of Dilution. Report EPA 903/9-80-004. Annapolis Field Office. 17 pp.

U.S. Geological Survey (USGS). 2009. Nationial Elevation Dataset (NED) 1/3 arc second. http://ned.usgs.gov

Wang, N., C.G. Ingersoll, C.D. Ivey, D.K. Hardesty, T.W. May, T. Augspurger, A.D. Roberts, E. van Genderen, and M.C. Barnhart. 2010. Sensitivity of early life stages of freshwater mussels (Unionidae) to acute and chronic toxicity of lead, cadmium, and zinc in water. Environmental Toxicology and Chemistry 29:2053-2063.

Wang, N., C.G. Ingersoll, J.L. Kunz, W.G. Brumbaugh, C.M. Kane, R.B. Evans, S. Alexander, C. Walker, and S. Bakaletz. 2013. Toxicity of sediments potentially contaminated by coal mining and natural gas extraction to unionid mussels and commonly tested benthic invertebrates. Environmental Toxicology and Chemistry 32:207-221.

Zipper, C.E., B. Beaty, G.C. Johnson, J.W. Jones, J.L. Krstolic, B.J.K. Ostby, W.J. Wolfe, P. Donovan. 2014. Freshwater mussel population status and habitat quality in the Clinch River, Virginia and Tennessee, USA: A featured collection. Journal of the American Water Resources Association (In review).

Appendix: Document Development Process and Authors

The document development process is described below:

Genesis: Science Team discussion at CPCRI planning meeting, 13 November 2013, Abingdon.

First round: 24 February draft was circulated among Braven Beaty, Christine Bergeron, Greg Cope, Jess Jones, Jen Krstolic, Roger Stewart, Carl Zipper prior to 28 February meeting among those parties which included further discussion.

Second round: 4 March draft circulated among Braven, Jess, Roger, Carl as preliminary revision in follow-up to the meeting.

Third round: 17 March draft circulated among Braven Beaty (TNC), Greg Cope NCSU), Greg Johnson (USGS), Jess Jones (FWS), Brad Kreps (TNC), Jen Krstolic (USGS), Allen Newman (VDEQ), Roger Stewart (VDEQ), Carl Zipper (VT), Sherry Wang (TDEC) and Beverly Brown (TDEC).

Fourth round: 14 April draft circulated to CPCRI Science Team.

Fifth round (18 June): Final review by CPCRI Science Team.

The document was prepared under leadership by Braven Beaty and Carl Zipper. CPCRI Science Team members are listed below:

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