United States
Environmental Protection
Agency

Office of Water
4305T

EPA 800-R-13-003
August 2013

Technical Support Document for Conducting and Reviewing Freshwater Mussel Occurrence Surveys for the Development of Site-specific Water Quality Criteria for Ammonia

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U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology
Standards and Health Protection Division
National Water Quality Standards Branch
Washington, DC

Acknowledgements

This technical support document (TSD) was prepared by Trish Rider, Lars Wilcut, Shari Barash, and Grace Robiou with written and technical support provided by Environmental Protection Agency (EPA) contractor Great Lakes Environmental Center, Inc. External expert peer review was conducted by Eastern Research Group, Inc., and peer reviewers included Alex M. Barron (Virginia Department of Environmental Quality), Jerome Diamond (Tetra Tech, Inc.), Celeste Mazzacano (The Xerces Society for Invertebrate Conservation), David Strayer (Cary Institute of Ecosystem Studies), and John Van Hassel (American Electric Power). Additional input on and assistance with this TSD were also provided by Robert Angelo (EPA's Region 7 office in Kansas City), Tom Augspurger (United States Fish and Wildlife Service), Candice Bauer (EPA's Region 5 office in Chicago), Heidi Dunn (Ecological Specialist, Inc.), Thomas Gardner (EPA's Office of Science and Technology), Edward Hammer (EPA's Region 5 office in Chicago), Gretchen Giannelli (ORISE Research Participant, EPA's Office of Science and Technology), Stephen McMurray (Missouri Department of Conservation), and David Smith (United States Geological Survey). Please submit comments or questions to Trish Rider, U.S. EPA, Mail Code 4305T, 1200 Pennsylvania Ave., NW, Washington, DC 20460 (e-mail: rider.trish@epa.gov).

Disclaimer

This TSD does not impose legally binding requirements on EPA, states, tribes, or the regulated community, nor does it confer legal rights or impose legal obligations upon any member of the public. The Clean Water Act (CWA) provisions and the EPA regulations described in this document contain legally binding requirements. This TSD does not constitute a regulation, nor does it change or substitute for any CWA provision or EPA regulation.

Executive Summary

Since the publication of the United States Environmental Protection Agency's (USEPA or EPA) 1999 Clean Water Act (CWA) § 304(a) national ambient water quality criteria recommendations for ammonia (USEPA 1999), additional toxicity testing has confirmed data on the effects of ammonia on sensitive freshwater invertebrate species in general and freshwater mollusk species (e.g., freshwater mussels in the Order Unionoida) in particular. EPA's 2013 national ammonia criteria recommendations (USEPA 2013a) expand the freshwater toxicity database for ammonia and result in national criteria recommendations that are protective of the aquatic community as a whole, which includes sensitive freshwater mollusk species.

EPA 304(a) national criteria recommendations that are developed using EPA's 1985 *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (USEPA 1985) are based on the premise that toxicological data for the species used to derive the national criteria recommendations are representative of the sensitivities of appropriate untested species (see Section III, p. 11 of the 1985 Guidelines referenced above). The acute and chronic datasets included in EPA's national criteria recommendations are generally from tests with aquatic species that are sensitive to many pollutants, but these and comparably sensitive species might not occur at a site; conversely more sensitive species could occur at a site. For example, freshwater mussels in the Order Unionoida are included in the 2013 national criteria dataset for ammonia but may not occur at all sites.

To facilitate the state and tribal adoption and implementation of the 2013 national criteria recommendations for ammonia, EPA has developed *Revised Deletion Process for the Site-Specific Recalculation Procedure for Aquatic Life Criteria* (USEPA 2013b), which describes a procedure and includes a spreadsheet that may be used to derive site-specific water quality criteria for the protection of aquatic life in order to better reflect the organisms that occur at a specific site. The Recalculation Procedure is intended to allow site-specific criteria that appropriately differ from national criteria recommendations (i.e., concentrations that are higher or lower than national recommendations) where there are demonstrated differences in sensitivity between the aquatic species that occur at the site and those that were used to derive the national criteria recommendations.

This technical support document (TSD) has been prepared explicitly to provide information to help states and tribes determine whether freshwater mussels in the Order Unionoida are present or absent at a particular site. If unionid mussels are determined to be absent at a particular site, states and tribes may decide to adopt site-specific criteria based either on the alternative criteria values provided in Appendix N of the 2013 national ammonia criteria recommendations, or on their own criteria values resulting from application of the Recalculation Procedure.

This TSD summarizes commonly used mussel survey techniques, sampling methods, and data sources and provides an overview of various study approaches, considerations, and limitations for individuals without mussel survey experience who may be involved in conducting or reviewing a freshwater mussel study in connection with state or tribal site-specific criteria for ammonia.

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Introduction

In 2013, the United States Environmental Protection Agency (USEPA or EPA) published updated Clean Water Act (CWA) § 304(a) national ambient water quality criteria recommendations for ammonia to incorporate the latest science, which includes new toxicity studies on freshwater mussels (USEPA 2013a). The updated ammonia criteria recommendations apply to all fresh waters for the protection of the overall aquatic community, including fish, mussels, and other mollusks.

Because mussels in the Order Unionoida (hereafter referred to as "freshwater mussels" or "mussels") include some of the most sensitive species in the national dataset for the ammonia criteria recommendations but may not be present in all waters, EPA anticipates that some states and tribes may consider site-specific criteria where there are demonstrated differences in sensitivity between the aquatic species that occur at a given site and those that were used to derive the national criteria recommendations. In the case of ammonia, where a state or tribe can demonstrate that mussels are absent on a site-specific basis, a state or tribe can use the site-specific criteria values provided in Appendix N of the *Aquatic Life Ambient Water Quality Criteria for Ammonia – Freshwater 2013* (USEPA 20103a), or use the Recalculation Procedure to derive their own site-specific criteria values [see *Revised Deletion Process for the Site-Specific Recalculation Procedure for Aquatic Life Criteria* (USEPA 2013b) and EPA's *Water Quality Standards Handbook* (USEPA 1994a)].[1,2] Removal of the mussel species from the national criteria dataset may better represent the species present in the waterbody. In this case, the Recalculation Procedure may result in criteria (and associated water quality-based effluent limits based on such criteria) with higher concentrations than EPA's recommendations but that are still protective of the designated use of the waterbody.

Purpose

This document provides a basic overview of freshwater mussel survey techniques, sampling methods, and data sources as well as additional sources of information for individuals without mussel survey experience who may be involved in conducting or reviewing a freshwater mussel survey.

Specifically, the purpose of this document is two-fold:

1. To assist state and tribal staff in determining whether freshwater mussels in the Order Unionoida are present or absent (i.e., do not occur) at a particular site.

[1] Throughout this document, use of the terms "freshwater mussels" or "mussels" refer specifically to mussels in the Order Unionoida.

[2] Throughout this document, the term "states and tribes" also refers to U.S. territories. The terms "tribes" and "tribal" refer to tribes authorized for treatment in a manner similar to a state under CWA § 518 for purposes of § 303(c) water quality standards.

2. To assist EPA staff in reviewing state and tribal water quality standards (WQS) submissions that contain site-specific criteria for ammonia (either from Appendix N of the 2013 national ammonia criteria recommendations, or derived using EPA's Recalculation Procedure) and a demonstration that mussels are absent (i.e., do not occur) at the site.[3]

Development of this Document

In developing this document, EPA undertook several efforts to collect information on existing mussel survey techniques, sampling methods, and data sources. EPA received information from mussel experts in academia, industry, and government; state natural heritage programs; state fish and game agencies, environmental protection agencies; and natural resource and conservation agencies. Combined with a literature review, the information EPA received formed the basis for the background and general content and scope of a draft *Technical Support Document for Conducting and Reviewing Freshwater Mussel Occurrence Surveys for the Development of Site-specific Water Quality Criteria for Ammonia*. The draft technical support document (TSD) was then sent to five independent external expert peer reviewers who were charged with answering specific questions concerning the content, scope, completeness, and adequacy of this TSD. EPA then revised the TSD to reflect the recommendations of the peer reviewers.

Information presented in this document neither represents an exhaustive list of available techniques, sampling methods, and approaches for conducting mussel surveys nor recommends an approach that states and tribes must undertake to make mussel presence/absence determinations in support of site-specific criteria development for ammonia. Instead, this document represents the information EPA compiled during its information collection efforts, and the brief overview provided in this document is for informational purposes only. EPA is not endorsing or directing states or tribes to use any particular method or approach, as states and tribes choosing to utilize the Recalculation Procedure for ammonia have the flexibility to elect any method they deem appropriate to demonstrate that mussels are absent on a site-specific basis as long as the chosen method is scientifically defensible. However, based on information acquired during the development of this document and the subsequent peer review, EPA believes that much of the information provided below under "General Approach to Mussel Presence/Absence Determinations" represent the key elements upon which states and tribes may base an approach to support a scientifically-defensible rationale for their decision-making processes. Consequently, EPA anticipates that approval of site-specific criteria may rely on the inclusion of some or all of these key elements (or similar fact-finding information) in order to provide the most transparent, high quality, and scientifically-defensible rationale for a decision that aligns with the goals of the CWA.

[3] Although this document is primarily intended to assist state and tribal water quality regulators with developing and EPA staff with reviewing site-specific criteria for ammonia, EPA is fully aware that other parties may be interested in pursuing site-specific water quality criteria development for ammonia and may also use the information in this document to justify those decisions and situations where site-specific criteria might be appropriate. However, EPA only considers the information submitted by the state or tribe when reviewing adopted state water quality criteria.

Background on Water Quality Criteria and the Recalculation Procedure

Water Quality Criteria

The term "water quality criteria" has two different meanings under the CWA. Under § 304(a), EPA publishes water quality criteria recommendations that consist of scientific information regarding concentrations of specific chemicals or levels of parameters in water that protect aquatic life and human health. States and tribes may use these recommendations as the basis for developing enforceable WQS. Water quality criteria are also elements of state and tribal WQS adopted under § 303(c) of the CWA. According to the federal WQS regulations at 40 CFR § 131.11(a)(1), states and tribes must adopt water quality criteria that meet the following requirements:

- Protect the designated use(s) of a waterbody.
- Be based on a sound scientific rationale.
- Contain sufficient parameters or constituents to protect the designated use.
- Support the most sensitive use of the waterbody.

Additionally, the regulation at 40 CFR § 131.11(b)(l)(ii) provides that states and tribes may adopt water quality criteria that "… reflect site-specific conditions." Site-specific criteria are intended to come closer than the national criteria recommendations to providing the intended level of protection to the aquatic life at the site, usually by taking into account the biological and/or chemical conditions (i.e., the species composition and/or water quality characteristics) at the site. Site-specific criteria, as with all water quality criteria, must be based on a sound scientific rationale and protect the designated use.

When states and tribes adopt new and/or revised WQS (which include water quality criteria), they are required under CWA § 303(c) to submit such standards to EPA for review and approval/disapproval. EPA reviews the standards following the requirements of § 303(c) of the CWA to ensure that the use designations, water quality criteria, and antidegradation policy meet minimum requirements. EPA also ensures that standards are scientifically defensible and that they adhere to regulatory and statutory requirements.

According to EPA's *Water Quality Standards Handbook: Second Edition* (USEPA 1994a), some of the general elements of an EPA review include, but are not limited to, the following:

- EPA determines whether the state's or tribe's water quality criteria are sufficient to protect the designated uses by ensuring that all numeric criteria are based on CWA § 304(a) guidance, § 304(a) guidance modified to reflect site-specific conditions, or other scientifically-defensible methods. EPA's decision to approve or disapprove criteria based on site-specific calculations or alternative scientific procedures is based on whether the site-specific criteria are also sufficient to protect the designated use and on a determination of the validity and adequacy of the supporting scientific procedures and assumptions. EPA's decision to approve or disapprove site-specific criteria is not based on whether the resulting criteria are more or less stringent than EPA guidance.

- EPA ensures that designated uses and/or criteria provide for the attainment and maintenance of downstream standards.
- Where the analyses supporting any changes in the WQS are inadequate, EPA identifies how the analyses should be improved and suggests the type of information or analyses needed.
- EPA reviews whether the revised or new state or tribal WQS are consistent with the CWA and EPA's implementing WQS regulations.

The Recalculation Procedure

In *Guidelines for Deriving Numerical Aquatic Site-specific Water Quality Criteria by Modifying National Criteria* (USEPA 1984), EPA first described three procedures that can be used to derive site-specific aquatic life water quality criteria:

1. The Recalculation Procedure, a taxonomic composition adjustment (revised in 2013).
2. The Indicator Species Procedure, a bioavailability adjustment now called the Water-Effect Ratio Procedure.
3. The Resident Species Procedure, a little-used approach effectively superseded by combined application of the Recalculation and Water-Effect Ratio Procedures.

The Recalculation Procedure may be used to derive site-specific criteria concentrations that are higher or lower than the national criteria recommendations where demonstrated differences in sensitivity exist between the aquatic species that occur at the site and those that were used to derive the national criteria recommendations. The Recalculation Procedure allows for the creation of a site-specific toxicity dataset that is appropriate for deriving site-specific aquatic life criteria through correction, addition, and/or deletion of test results in the national toxicity dataset for the pollutant of concern (e.g., ammonia).[4] Due to the complexity of the relationship between ammonia toxicity and pH and temperature across different aquatic organisms, EPA has re-calculated site-specific criteria removing mussels from the national dataset and provided these values in Appendix N of the 2013 ammonia criteria document.

Deletion is based on taxonomic composition of the site under consideration. The deletion procedure does not provide for simplistic deletion of all species that do not occur at the site because some tested species might be necessary to represent untested species that occur at the site. Rather the concept is to consider which tested species are most closely related to those occurring at the site and to delete those for which another tested species would better represent the species occurring at the site. Because the 2013 national criteria recommendations for ammonia are driven in part by the sensitivity of freshwater mussels[5] and these animals may not be present at all locations throughout a particular state or tribal land, EPA anticipates that some states and tribes may consider using either the alternate criteria values provided in Appendix N of the 2013 national ammonia criteria recommendations or the updated deletion process of the

[4] Only corrections and additions approved by EPA may be made. All corrections and additions should be made before the deletion process is performed (USEPA 1994b).

[5] Freshwater aquatic snails, although sensitive to ammonia, are somewhat less sensitive than mussels; it is also assumed that their distribution is ubiquitous. Therefore they are not the focus of this document: the use of the Recalculation Procedure for deriving site-specific ammonia criteria.

Recalculation Procedure to derive site-specific criteria where there is a demonstrated difference in sensitivity between the aquatic species that occur at a particular site and those that were used to derive the national criteria recommendations.

The Recalculation Procedure is dependent on the species that occur at the site. As stated in *Revised Deletion Process for the Site-Specific Recalculation Procedure for Aquatic Life Criteria* (USEPA 2013b), the equivalent terms "resident" and "occur at the site" include life stages and species that meet one of the following elements:

- Are usually present at the site.
- Are present at the site only seasonally due to migration.
- Are present at the site intermittently because they periodically return to or extend their ranges into the site.
- Were present at the site in the past, are not currently present at the site due to degraded conditions, but are expected to return to the site when conditions improve, or
- Are present in nearby bodies of water, are not currently present at the site due to degraded conditions, but are expected to be present at the site when conditions improve.

The terms "resident" or "occur at the site" do not include life stages and species that meet one of the following elements:

- Were once present at the site but cannot exist at the site now due to permanent (physical) alterations of the habitat or other conditions that are not likely to change within reasonable planning horizons.
- Are still-water life stages or species that are found at a flowing-water site solely and exclusively because they are washed through the site by stream flow from a still-water site.

Special provisions apply if a "critical species" occurs at a site. A critical species is a resident species that is commercially or recreationally important at the site, listed as threatened or endangered under Section 4 of the Endangered Species Act, or a species for which there is firm evidence that its loss would yield an unacceptable impact on the site's commercially or recreationally important species, endangered species, abundances of a variety of other species, or structure or function. The deletion process should not be undertaken unless toxicity data are available for at least one species in each class of aquatic plants or animals that contains a critical species. Similarly, states and tribes should be mindful of areas where there is designated critical habitat for any endangered or threatened species listed under Section 4 of the Endangered Species Act when determining if they should pursue site-specific criteria, particularly if those critical habitat areas fall within the site.

When site-specific criteria are derived using the Recalculation Procedure, all species that occur at the site should be taken into account when deciding what species, if any, are to be deleted from the dataset. Perhaps the most important condition in defining species residency is that the taxa that occur at the site cannot be determined merely by a one-time sampling downstream and/or upstream of the site. The approach below describes one way to ensure a comprehensive,

scientifically-defensible determination of the absence of mussels at a given site, or in a watershed.

General Approach to Mussel Presence/Absence Determinations

In practice, the effective use of the deletion process in the Recalculation Procedure is predicated on the determination of species that occur/do not occur at the site (i.e., presence/absence). To this end, several peer reviewers advocated for a tiered or phased approach to mussel presence/absence determinations that is similar to the approach described below. The concept of this approach is to reduce the required effort needed to make a decision that mussels are present and add increased scrutiny and effort for "mussels-absent" decisions. For example, a state or tribe may choose to follow the phased approach below to determine if mussels are absent when pursuing site-specific criteria for ammonia using the Recalculation Procedure:

1. Delineate the site (study area) and define presence and absence.
2. Check databases, literature, and reports for mussel survey records (historical and recent).
3. If no records of mussel presence are available, conduct a mussel survey(s) at the site.
4. If after steps 1-3 mussels are still not detected, develop site-specific criteria using the Recalculation Procedure.
5. Re-evaluate the site-specific criteria as needed but at least once every three years in conjunction with the state or tribe's triennial WQS review process.

At any point during the phased approach, if freshwater mussels in the Order Unionoida are found at the site, the state or tribe may choose to discontinue the development of site-specific criteria. Note, however, that the Recalculation Procedure may still afford some flexibility in a limited number of instances even where freshwater mussels are found to be present in the waterbody. At sites where only one or a few untested mussel species are present, the state or tribe may want to consider conducting toxicity tests on such species rather than relying on the species in the national dataset to serve as surrogates for the untested species.[6]

The remainder of this document is organized following the steps indicated above because the general approach outlined highlights many important nuances and elements that a "mussels-absent" decision might contain. This approach may be useful to states and tribes because it identifies several elements that should be included in a record of decision to support any decision a state or tribe might reach regarding mussel presence or absence. In providing this information, EPA is not advocating for the use of any one specific approach over another but, instead, encourages states and tribes to develop their own process as necessary (i.e., states or tribes may add to, modify, or remove any of the steps above or base their decisions on an entirely different approach at their discretion).

[6] While this document is intended to address the approach a state or tribe may follow to provide adequate justification for a "mussels-absent" determination, correct use of the Recalculation Procedure requires consideration of all species that occur at the site.

Throughout the process of using the Recalculation Procedure, a state or tribe must be mindful of downstream waters. For example, as with all designated uses and criteria in a state's or tribe's WQS, 40 CFR § 131.10(b) states the following:

> In designating uses of a water body and the appropriate criteria for the uses, the State shall take into consideration the water quality standards of downstream waters and shall ensure that its water quality standards provide for the attainment and maintenance of the water quality standards of downstream waters.

States and tribes should take into account downstream waters when they consider any changes to site-specific criteria at a given site.

Phase 1. Delineate the Site and Define Presence and Absence

Step 1. Delineate the Site

Fundamental to derivation of any site-specific criteria is delineating the site to which the criteria will apply. There are several key issues in delineating the site, particularly as it pertains to WQS, the Recalculation Procedure, ammonia, and mussels. Because the rationales for site-specific criteria are usually based on differences in species sensitivity, physical and chemical characteristics of the water, or a combination of the two, the concept of a site should be consistent with this rationale. In the general context of site-specific criteria, a "site" may be a region, watershed, waterbody, or segment of a waterbody. The site-specific criteria are to provide adequate protection for the entire site no matter how the site is defined.

For example, if the species occurring at a site are toxicologically comparable to those in the national criteria dataset for a pollutant of interest and physical and/or chemical water characteristics are the only factors supporting modification of the national criteria recommendations, the site can be defined on the basis of expected changes in that pollutant's biological availability and/or toxicity due to physical and chemical variability of the site water. If, however, physical and chemical characteristics of the water are not an important consideration, the site can be as large as a generally consistent biogeographic zone permits. For example, large portions of the Chesapeake Bay, Lake Michigan, or the Ohio River may be considered as one site if their respective aquatic communities do not vary substantially [see section 3.7.3 of EPA's *Water Quality Standards Handbook: Second Edition* (USEPA 1994a)]. Communities with a unique taxonomic composition may also justify a designation as a distinct site. In general, the number of taxa that occur at the site will decrease as the size of the site decreases.

Exactly how the site is defined is a matter of state or tribal discretion as long as the definition is scientifically defensible and transparent. Examples of site definitions include the following:

- A stream, river, lake, reservoir, or wetland.
- A segment of a stream, river, lake, reservoir, or wetland.
- A watershed or part of a watershed.
- Some specified distance upstream and downstream of a point-source discharge.
- Some other geographical feature or extent, as defined in the state's or tribe's WQS.

With ammonia in particular, delineating the extent of the site (e.g., site boundaries) may require characterization of expected worst-case instream conditions or a study of the transport and fate of ammonia from a point source discharge at a site. Such characterization should include how far downstream the effects of the ammonia discharge are observed or expected and the potential impact of that discharge on the downstream waters. A permit limit might be controlled by a criterion that applies outside (e.g., downstream of) the site. Furthermore, a scientifically-defensible approach to delineating a site that is subject to a specific source of pollution (i.e., discharge from a wastewater treatment plant) should take into consideration upstream waters or other nearby reference waters that are free of pollution to help determine the likelihood for mussel habitation at the site. Surveying exclusively within the spatial extent of the discharge could confound the survey results, as mussels may already have been extirpated from that site. Although sedentary as adults, freshwater mussels are capable of colonizing new territory when they are attached to their fish hosts in the larval stage. Knowledge of the presence/absence of mussels both below and above the source of an ammonia input (specifically in areas of suitable habitat) into the waterbody can help determine if the source of pollution may be cause for their absence. Characterizing the discharge from a point source can aid in the determination of proper boundaries for the site, which should include areas beyond the zone of anticipated effects (area of direct impact) to determine the potential for mussel colonization within the delineated site.

Step 2. Define Mussel Presence and Absence

As stated above, the Recalculation Procedure is dependent on the species that occur at the site, and the definition of "occur at the site" relies on the term "present," which can be interpreted in different ways for different species. For example, for freshwater mussels, presence can be defined in terms of the existence of live mussels, mussel tracks, recently dead mussels' shells, unweathered shells, suitable habitat, and/or historical presence data.[7] Similarly, information that could indicate that mussels are absent at a site could include the lack of live mussels, shells, fish hosts, historical presence data, and records in any database and published and unpublished literature as well as the existence of only weathered or sub-fossil shells without evidence of live mussels.[8] The results of any mussel survey will depend, in part, on how the state, tribe, surveyor, or other entity chooses to define mussel presence and absence.

While presence of mussels can be a rather straight forward definition, most experts agree that it is difficult to determine true absence of a species in a waterbody, which may be contingent on many considerations other than simply not finding live mussels during a particular survey. Moreover, sampling efforts generally are limited due to self-imposed temporal and spatial

[7] According to the external peer reviewers, using the existence of suitable habitat as the sole criteria for mussel presence and the lack of suitable habitat for mussel absence should be used sparingly and with extreme caution because different species of mussels have different habitat and microhabitat preferences. Furthermore, even experienced malacologists can be surprised where they find mussels (e.g., even in deep silt or on solid bedrock). Habitat descriptors used by most malacologists to define mussel habitat do a poor job of predicting where mussels occur (see Strayer and Ralley 1993 for more information).

[8] Mussels have a unique life history where their eggs develop into parasitic larvae (glochidia). During this stage, the young mussels are expelled from the mother and must quickly attach to the gills or fins of a fish host (Harrold and Guralnick 2010). Because glochidia cannot swim, many are lost at this stage while the others stay attached to their host fish until they are strong enough to drop off and find a place in the substrate to grow into adults. Some mussels have a specific host, but others will attach to any fish that comes along (Harrold and Guralnick 2010).

sampling constraints and may not be sufficient to support a declaration of true absence. Therefore, the terms “no mussels observed” or “mussels not detected” are generally used rather than “absent.”

Because of the difficulty in determining true absence of a species, there are no existing standardized protocols to determine absence of mussels with 100% accuracy. However, statistical models can be applied with specific survey types and designs to estimate the likelihood that mussels are absent or that a specific rare species is absent (Strayer and Smith 2003; Smith 2006). Such models have generally been used to estimate the likelihood of mussel absence only at targeted sites rather than entire streams or watersheds. When the ability to detect a species is low (such as with a rare species), mussel surveys may be better able to gauge the probability that mussels are present in an area rather than document their absence. For example, one possible definition of absence might be if actual mussel survey data supported the conclusion that the probability of mussel presence given the survey effort was less than some predetermined threshold (e.g., 5%). In this example, one could allow the conclusion that mussels are absent (i.e., occurred at a density less than X) if the survey data supported the conclusion that the probability of a density greater than X were below some threshold (e.g., 5%). Both the density X and the threshold should be established by state or tribal regulators before any surveys begin. The literature on the conservation of rare/extinct species provides additional guidance on the problem of interpreting absence (or extinction) from actual survey data (Smith 2006).

The absence of mussels at one point in time does not guarantee that they will not be present even a few months later. As stated above, mussels are capable of colonizing new territory when they are carried on their fish hosts in the larval stage. In Virginia, for example, an absence survey is considered valid for only two years.

However a state or tribe chooses to define presence and absence, the definition should be clear, transparent, reasonable, scientifically defensible, and available to the public.

Phase 2. Check Databases, Literature, and Reports for Mussel Survey Records

This step of the phased approach to mussel presence/absence determinations utilizes the information that is already available to the state or tribe. Specifically, it provides an initial screen of available mussel occurrence data to help the state or tribe determine and/or prioritize areas that may warrant pursuit of site-specific criteria for ammonia. One readily-available database that can be accessed easily and with little time and effort is NatureServe© Explorer. This online searchable database has information on more than 70,000 plants, animals, and ecosystems of the U.S. and Canada. Appendix A provides a step-by-step guide describing how to access the mussel distribution data in NatureServe© Explorer at the state, county, and watershed levels. A state or tribe may want to prescreen their waters using this database and/or other databases and sources of information to determine which waterbodies may benefit from site-specific ammonia criteria and which may not, based on survey records of mussels presence. However, it should be noted that NatureServe© Explorer does not contain all available data, so a state or tribe may find it necessary to look for data from other sources as well. Other sources of mussel distribution data provided from states/entities and external peer reviewers are included below and in Appendix B.

Importantly, because of the difficulty in determining true absence, data sources vary with regard to the amount and type of absence data that are reported. Some states report both presence and absence data (e.g., West Virginia, Delaware, Maryland, Georgia, Illinois, Ohio, New Mexico, Missouri, Kansas, North Dakota, Montana, Arizona, and Idaho), while other states do not report absence data (e.g., Virginia, North Carolina, Tennessee, Indiana, Colorado, Utah, and Wyoming). In some states, absence data are reported only in certain circumstances. For example, New York and South Dakota record absence data only if there is a previous record of species presence, and Kentucky reports absence data only if they pertain to a federally-listed species.

Available Data

Sources of Available Data

The three main sources of data that states and other entities rely on to make mussel presence/absence decisions are published and unpublished literature, mussel and macroinvertebrate surveys and databases, and data from other experts including environmental consulting firms and agencies. Other sources include museum specimens, university survey data, citizen reports, personal communications, and any other reliable source.

While several states and other entities maintain databases to aid in mussel presence/absence determinations, no single database contains all of the available mussel data. Even within a state there may not be a single source of mussel presence/absence information. For example, the Virginia Department of Game and Inland Fisheries (VDGIF) is responsible for maintaining a database of mussel distribution data, while the Virginia Natural Heritage Program maintains a database only for species of concern. The two databases do not completely overlap, so neither contains all available information for Virginia waters. Additionally, there may be other sources of data within a state that have not been included in a database such as peer-reviewed publications, student theses, contractor reports, and other gray literature. Furthermore, the majority of available data have been generated through efforts to determine the presence of threatened and endangered species rather than common species. Therefore, mussel presence/absence data may be incomplete in any given database. Appendix B lists some of the databases and literature and other important data sources that are available for locating mussel presence/absence data.

Frequency of Data Collection

States survey mussel populations with varying frequency. Some states survey each year (e.g., Connecticut) or once every five years (e.g., West Virginia). Other states conduct occasional surveys that may only target rare, threatened, endangered, or other high priority species (e.g., Kentucky and Maryland), and the frequency may be dependent on the availability of grant funds and timing (e.g., Delaware). Still other states note mussel presence only when conducting regular benthic surveys (e.g., North Carolina) or basin-wide surveys (e.g., Ohio). Some states (e.g., Wisconsin) conduct surveys in association with construction projects such as bridge crossings, pipeline crossings, gravel dredging, channel maintenance, or any other project that would disturb the bottom of the river. A number of states do not survey mussel populations, but there are usually at least some records available in various sources for such states (see above and Appendix B).

Historical and Current Data

At a minimum, to protect existing uses of the waterbody, the use of historical data should be considered for presence determinations if the survey found mussels on or after November 28, 1975.[9] This position is similar to that previously expressed by EPA in 1999 for determination of the presence of early life stages (ELS) of fish, which is quoted below:

> According to the Clean Water Act, States and Tribes are to protect existing uses, and therefore should protect for the most sensitive uses that have occurred in a given waterbody since November, 1975. 40 CFR 131.12(a)(1) and 40 CFR 131.3(e). Hence, States and Tribes should consider both current and historical species that have used a waterbody for spawning and rearing since November, 1975. Even where water quality is protective of designated uses, the current species composition in a waterbody may not reflect all species that have used the waterbody for spawning or rearing since 1975. It is EPA's position that any ELS-absent provision should not prevent the return of any species associated with an existing or designated use. Therefore, States and Tribes should evaluate both current and historical data back to November, 1975, in determining a presence or absence of sensitive life stages (Environmental Protection Agency, FRL-6513-6, Notice of availability, 64 Federal Register 245 (December 22, 1999), pp. 71973-71980).

Accordingly, a state or tribe that has mussel presence survey data dating on or after November 28, 1975, should assume mussels are present to protect existing uses.

Among states, the definition of historical and current data varies. In general, “current data" are less than 10-20 years old, while “historical data” are older than this range. However, some states consider records older than 1970 to be historical. Similarly, West Virginia categorizes its data into one of three different groupings: historical (information collected prior to 1975), so-called “Taylor data” (collected from 1977-1989), and new data (collected 1990-present).

[9] Existing uses are “those uses actually attained in the water body on or after November 28, 1975, whether or not they are included in the water quality standards.” Existing uses are known to be “attained” when both the use *and* the water quality necessary to support the use have been achieved (see http://water.epa.gov/scitech/swguidance/standards/upload/Smithee-existing-uses-2008-09-23.pdf).

The use of historical and current data also varies among states and other entities. In some states, data older than 10-20 years either do not carry as much weight or are not considered when assessing current conditions in relation to regulatory decision-making (e.g., Georgia, New Jersey, and Illinois). Likewise, Maryland assumes that data less than 20 years old are representative of current conditions unless enough evidence is available to contradict that assumption. In Montana, data older than 10 years are considered to be unreliable without backup verification. In contrast, some entities use historical data dating back to 1919 (e.g., The Partnership for the Delaware Estuary) and do not think that a specific time frame for acceptable data should be used due to the recolonization potential of mussels.

Biologists began tracking and recording precise locations of imperiled and rare mussel species in the 1970s and, in some locations, even before then. Some of these historical data have been retained and used because they serve as a reference to show how mussel populations and distributions have changed over time. Historical data may also prove to be the only available data for some sites. Often, historical data are retained because of the difficulty and/or lack of ability to conduct mussel surveys every year over the entire geographic range of the state. However, a state or tribe may determine that a long time frame for data acceptability is warranted due to the longevity of mussels and the length of time it may take mussels to recolonize a site or waterbody. Regardless of age and intended use, most state agencies and other entities retain all the data they collect.

By evaluating the presence/absence of mussel occurrence data, the state or tribe may determine that the pursuit of site-specific ammonia criteria using the Recalculation Procedure is not warranted. A state or tribe may find that areas or sites at which mussels have been found historically or threatened or endangered mussels have been found may not be worth pursuing. There may be situations where the abundance of occurrence data provides sufficient justification to make a mussel presence determination. Conversely the lack of available information may not provide enough justification to make a mussels-absent decision. As with all survey data, the state or tribe should be aware of the type of survey conducted and its objectives and goals to determine the appropriate conclusions that can be drawn from it.[10] The applicability and usefulness of information from previous studies should be determined on a case-by-case basis, but in many situations, such studies can provide useful information. EPA acknowledges, however, that there may be situations in which some waterbodies have been thoroughly studied specifically for mussel absence (and absence data specifically have been recorded), and the use of previous survey results may provide sufficient justification to create a scientifically-defensible record for a mussels-absent determination. Again, these situations should be determined on a case-by-case basis, but the majority of states and tribes will likely need to conduct additional mussel surveys to provide sufficient justification for a mussels-absent decision. To aid states and tribes in this process, the information below is intended to provide an overview of the different

[10] Not all occurrence data are the same. Depending on the type of survey conducted, not finding any mussels may be a function of the study design and objective and not a true reflection of the species diversity at the site. For example, most aquatic macroinvertebrate surveys are not designed for mussel detection, and a surveyor may not always record mussel presence. Therefore caution should be used when considering benthic macroinvertebrate surveys for mussel-absence determinations or as part of the rationale for mussel-absence records. In many cases, lack of mussel presence in benthic macroinvertebrate surveys alone will not be sufficient rationale for demonstrating that mussels are truly absent from a site.

kinds of mussel surveys with some recommendations of key elements that a good survey should contain.

Phase 3. Conducting Mussel Surveys

This section presents the general steps for conducting mussel surveys and provides summaries of the various approaches and methods employed by states and other entities to determine whether mussels are present or absent in waterbodies of various types. The steps include defining the study objectives and choosing a sampling approach, design, and method. A proper characterization of the site (e.g., size, depth of water, turbidity, and hard or soft-bottomed substrate) is necessary to determine the best survey approach to meet the objective(s) of the study before sampling is conducted.

In terms of making mussel presence and absence determinations, it is important to note that, while less comprehensive studies can be used for presence determinations, they may not be warranted or scientifically defensible for absence determinations. Therefore, states and tribes may find it necessary or worthwhile to initially survey mussels with a less comprehensive, lower cost method and then survey again with a more comprehensive method only where the initial survey did not detect mussels. For example, a state or tribe may use brail bars or dredges to confirm presence of freshwater mussels suspected in a large area of a river segment with relative ease and over a short period of time. Thus, the state or tribe may find this two-step sampling process less burdensome and resource intensive where the initial survey detects mussels.

In the steps outlined below, the state or tribe has discretion to determine the appropriate amount of rigor and effort needed to adequately justify a mussels-absent determination. However, to successfully, accurately, and precisely survey for freshwater mussels, especially where populations are likely to occur at low densities, requires a substantial amount of time and effort regardless of the methods used. The results of these surveys should be included in the decision record so that the state or tribe can provide a clear justification for its decision to develop site-specific criteria for a particular site. EPA anticipates that different surveys will require varying levels of effort depending on the physical properties of the sites in question and on the density and patchiness of any resident mussel populations.[11] EPA will review site-specific ammonia criteria and supporting documentation and either approve or disapprove such criteria depending on the specific facts of the situation, which include the defensibility of the state's or tribe's mussels-absent determination.

Step 1: Define the Study Objective

The most important aspect of designing a mussel survey for a site is a careful consideration of the objectives (Dunn 2000; Strayer and Smith 2003; Wisconsin Department of Natural Resources (WDNR) 2005). The specific objectives of the survey help determine the appropriate sampling approach, design and method for the size of the site to be covered, any limitations of the survey,

[11] In some cases, a less comprehensive survey may be appropriate for specific waterbodies (e.g., the waterbody is outside the known range of certain mussel species, the waterbody is not connected hydrologically to any waterbody known to contain populations of mussels, the waterbody is a first order headwater stream with unsuitable habitat, and/or the waterbody is an arid waterbody subject to prolonged periods with no flow). All of these decisions should be made on a case-by-case basis.

and the strength (i.e., scientific rigor) of the conclusions that can be drawn. The information presented below is meant to highlight the important elements and nuances of conducting mussel surveys so that states and tribes can better address the following study objective: determine whether freshwater mussels in the Order Unionoida are present or absent at a particular site. Other approaches are also included below as a means of evaluating their appropriateness and utility in addressing other related study objectives.[12]

Step 2: Choose a Sampling Approach

Four primary sampling approaches were identified through EPA's information collection efforts for this TSD: reconnaissance, qualitative, semi-quantitative, and quantitative. These terms appear to be used loosely, and many studies often utilize a combination of these different sampling approaches to address various study objectives. The terms are used to convey the general scope and type of effort involved.

Reconnaissance (Exploratory/Preliminary)

This approach involves a cursory visual and/or tactile search of the most promising habitats in a waterbody to obtain a preliminary understanding of mussel presence or absence (Dunn 2000). It is often used to determine if further study is warranted because it can reveal valuable information (e.g., site characteristics, conditions, and hazards) before a more comprehensive survey is undertaken (Strayer and Smith 2003). In the context of site-specific criteria, this approach may be adequate to determine mussel presence, thus eliminating the need for a more comprehensive survey. Also, if fresh shells or live mussels are found, a site might be considered for further investigation to determine family, genus, and species type(s), distribution, density, or community characteristics depending on the study objectives.

This approach requires the surveyor to have some *a priori* knowledge of expected mussel distribution and habitat requirements. For example, in low gradient systems, mussels may be present in areas with high flow, while in high gradient systems, mussels are more likely to be found in flow refugia.

Qualitative

This primarily visual sampling approach tends to be more comprehensive than reconnaissance surveys. This approach can be used to determine mussel presence, richness, and, to a limited extent, density (Angelo et al. 2007; Dunn 2000). Often this approach is selected for use in a well-defined area for a specific length of time, which is called a timed-search (Dunn 2000; WDNR 2005). Overall, this approach may be the best for detecting mussel presence or demonstrating a

[12] In using the Recalculation Procedure, it may only be necessary to identify resident mussels to the order level. However, in some situations, the state or tribe may decide to identify resident mussels to the species level because the Recalculation Procedure may afford some limited flexibility even where mussels are present. The survey approaches for these two objectives may be entirely different. EPA is including the additional information related to study objectives other than presence/absence of mussels in Order Unionoida to assist those states that may choose to identify mussels to a species level (i.e., study objective: determine what kind of mussels in Order Unionoida are present) and/or define mussel presence/absence in terms of a density (i.e., study objective: determine how many mussels in Order Unionoida are present per unit area).

reasonable probability of mussel absence. Smith (2006) provides specific guidance on the level of effort necessary to detect rare species with high probability, which, in some cases, could be directly applicable to support the adoption of site-specific criteria for ammonia. The approach can also be used to gage the approximate level of effort necessary to perform quantitative mussel surveys, particularly in low-density situations.

Qualitative sampling sometimes involves excavation of sediment in selected habitats in addition to a visual and tactile search of the substrate surface and adjoining shoreline area for live mussels and spent shells materials (Angelo et al. 2007). Qualitative surveys can more easily canvass long stream reaches, and when combined with some level of substrate excavation, they can provide a reasonable degree of assurance of mussel presence or absence, especially in small streams (Angelo et al. 2009).[13]

Semi-quantitative

Semi-quantitative sampling entails sampling a given area both visually and tactually and often is used to determine mussel distribution, species composition, and relative abundance (Dunn 2000; McRae et al. 2004). This approach generally involves sampling the substrate surface along transects or within grid cells such that an area may be searched systematically. Smith's (2006) survey design for detecting rare mussels with high probability of detection provides another example of a semi-quantitative sampling approach. Such approaches are not considered truly quantitative because substrate is not excavated. A significant proportion of most mussel communities tends to be buried and will not be detected through sampling of the substrate surface alone.

Quantitative

Quantitative sampling techniques are generally used to estimate freshwater mussel density, relative species abundance, and/or age or size class distributions within individual mussel populations (Dunn 2000). Quantitative sampling represents the most time consuming and labor intensive form of sampling in part because it generally entails systematic excavation of the substrate (Dunn 2000; Miller and Payne 1993; Smith 2006; Smith et al. 2001; Strayer and Smith 2003). This type of sampling approach is generally not needed unless the study objective includes defining mussel community metrics or comparing these metrics over time.

Step 3: Choose a Sampling Design

The sampling design in a survey plan defines "what" is to be sampled in the survey. To effectively address the objective(s) of the survey, a good survey plan will be explicit in terms of what will be sampled and where sampling will occur in the waterbody.

The information summarized in this section is largely based on the section titled "Sampling Design" in *A Guide to Sampling Freshwater Mussel Population*s (Strayer and Smith 2003). Note that virtually all of the literature and other information reviewed and summarized herein refer to

[13] Because a substantial portion of the mussel community may be below the substrate and not found from visual only searches, excavation may be necessary to determine all the mussel species present in the waterbody.

the use of one of the sampling designs described in the book, which provides an overview of each specific sampling design and its limitations. Only the subset of those sampling designs applicable to mussel presence/absence determinations is highlighted below.

Informal Sampling (Non-probabilistic)

Informal sampling includes reconnaissance, qualitative, and semi-quantitative sampling approaches. This type of sampling is considered informal because it is not probability based and cannot be used to compare mussel communities over time or space like a formal (i.e., probabilistic) quantitative sampling design can. This approach to sampling may be useful in preliminary survey approaches to determine mussel presence, absence, or distribution as well as to obtain a relative understanding of species composition. It can also be used to define a polygon for random sampling or define strata for a stratified sampling design. This sampling design is not, however, useful for estimating population size, relative abundance, or some other community metrics.

Simple Random Sampling

Simple random sampling design divides the spatial area of interest into non-overlapping distinct units of the same size. Then a random sample of those distinct units is surveyed for mussels. This approach is different from informal sampling because it allows for estimations of sampling probabilities, which can then be used to calculate the variance of the estimate. However, this design may not be the most appropriate approach for mussel presence/absence determinations because mussels can be clumped at several spatial scales (Strayer and Smith 2003), and it is inefficient at detecting mussels. Furthermore, partially because of clumping, it is possible with this approach to miss the mussel population present in the area and incorrectly label a waterbody as having no mussels. In other words, because a simple random sample does not sample the entire area, the subset of the area surveyed may not contain mussels even if the entire area does contain mussels.

Figure 1 displays an example of a simple random sample design; the site is divided into equally sized units, and coordinates are selected at random indicating which units will be sampled (highlighted grey).

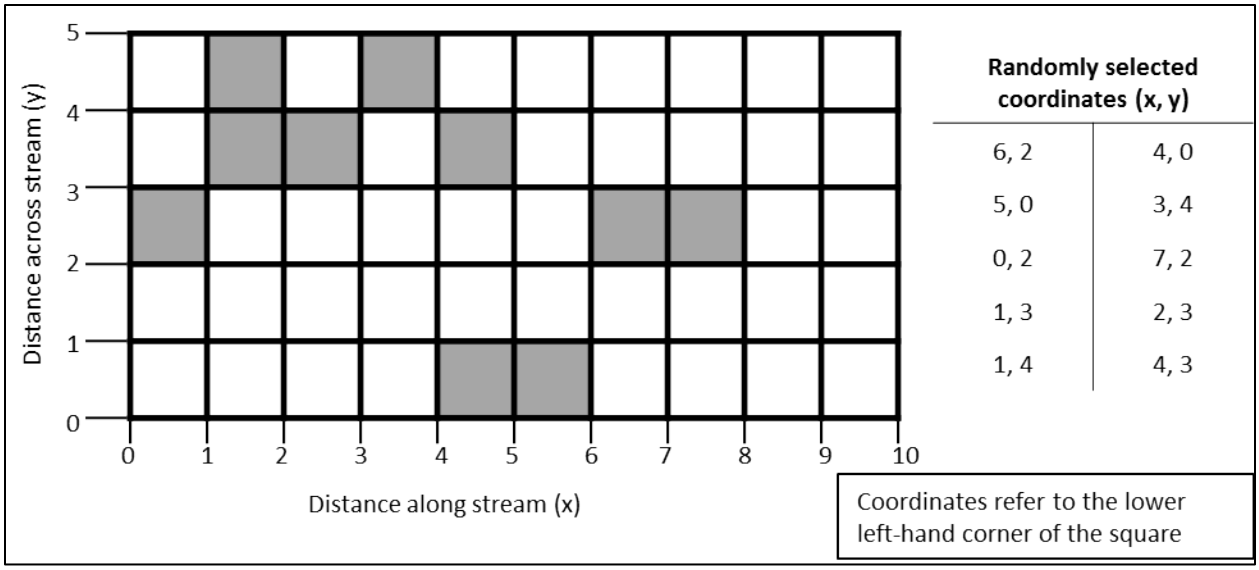

Figure 1: Example of a simple random sampling design (adapted from Strayer and Smith 2003)

Systematic Sampling

This design is similar to simple random sampling except that the samples are spatially distributed throughout the area such that relatively complete coverage of the site is achieved. In essence, a small number (i.e., two to three) of random locations/coordinates are selected and then the pattern is repeated throughout the survey area. This design can be used at many spatial scales, from the placement of individual quantitative samples to the selection of qualitative sites along a river. Systematic sampling with two or three random starts has been shown to be more effective than simple random sampling (Pooler and Smith 2005) and is preferred for sampling rare, spatially clustered populations in the absence of prior information on distribution.

Figure 2 displays an example of a systematic sampling design with three random starts. The start locations are chosen randomly, and additional samples are then selected at a specified distance (3 units in this example) from the start location and its subsequent selected locations. In Figure 2, the start location and the units that are selected based on the specified distance are all highlighted the same color gray and numbered according to the start location. For example, the random start location for starting coordinates "1, 0" is represented by "2-S," and the additional sample locations are indicated by the number "2."

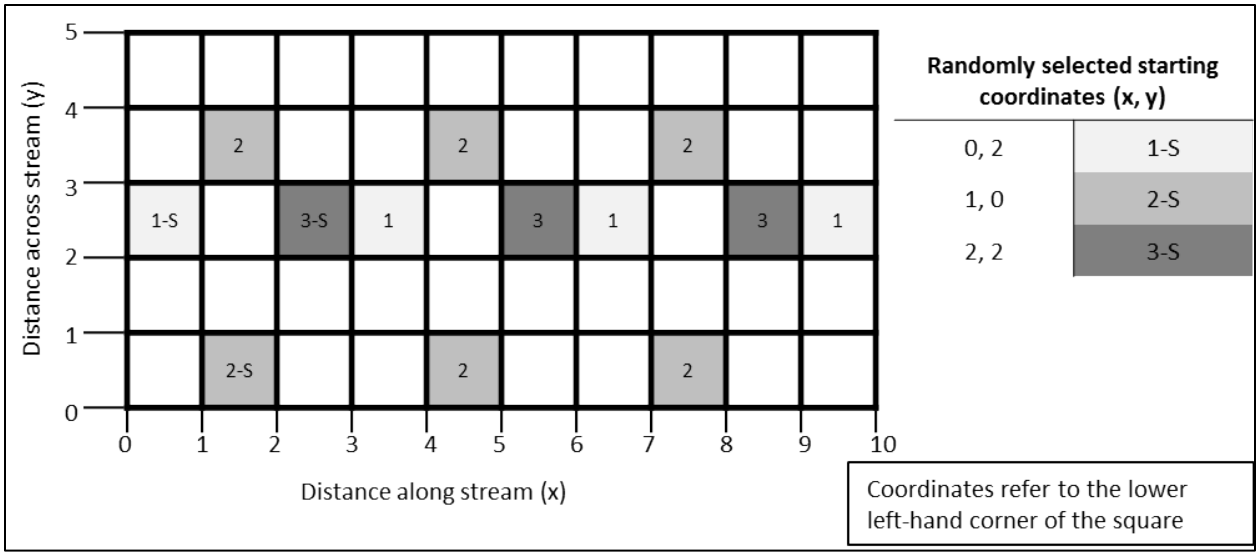

Figure 2: Example of a systematic sampling design (adapted from Strayer and Smith 2003)

Double Sampling

Double sampling can be applied to both qualitative and quantitative samples. In double sampling for quantitative surveys, an observer samples a number of distinct units/quadrants using a visual survey method in the upper part of the substrate and then samples a subset of those quadrants again using excavation to detect mussels not visible at the surface. This type of design can be used to determine the percentage of the population that is buried compared to those at the substrate surface. However, double sampling may still miss the target mussel population. As stated above, because the entire area is not surveyed, the subset of the area surveyed may not contain mussels even if the entire area does contain mussels. This problem has been solved by some investigators by combining a double sampling design with a systematic sampling design using multiple random starts.

This design is perceived by at least one expert peer reviewer to be the best for surveying a large area and is considered to combine high levels of accuracy and precision with a feasible input of time and funding. In fact, some literature suggests that this approach with specific sample quadrant sizes may be the best approach to find new mussel beds (Pooler and Smith 2005; Smith et al. 2001; Vaughn et al. 1997). An example of this type of design for a mid-sized stream is provided in Appendix C, and it is considered an appropriate approach to surveying a large area.

Stratified Sampling

This sampling design involves dividing the sampling area into different strata, which can be defined in any number of ways. For example, the study area could be divided by depth and the cost of sampling at each depth. In this case, the more expensive diving sampling method (see below) could be used in deep water, while a less expensive snorkeling/wading sampling method could be used in the shallow water. The study area could also be divided into habitat regions or areas (e.g., riffles and pools) where mussels are likely to be present versus those areas where

they are typically not present.[14] In each stratum, the mussel surveyor might choose a different sampling design and sampling method. The stratified sampling design keeps the cost of the survey low but ensures that high priority areas receive special attention.

Figure 3 displays an example of a stratified sample that divides the area into two zones: deep water (highlighted gray) and shallow water (not highlighted). Sample locations are chosen randomly with twice as many sample locations in the shallow water compared to the deep water. Each sample location is indicated with an "x." This example illustrates where a less expensive method sampling method (e.g., wading) is used in the shallow water (and is, therefore, able to cover more sampling area) and a more expensive sampling method (e.g., diving) is used in the deeper water.

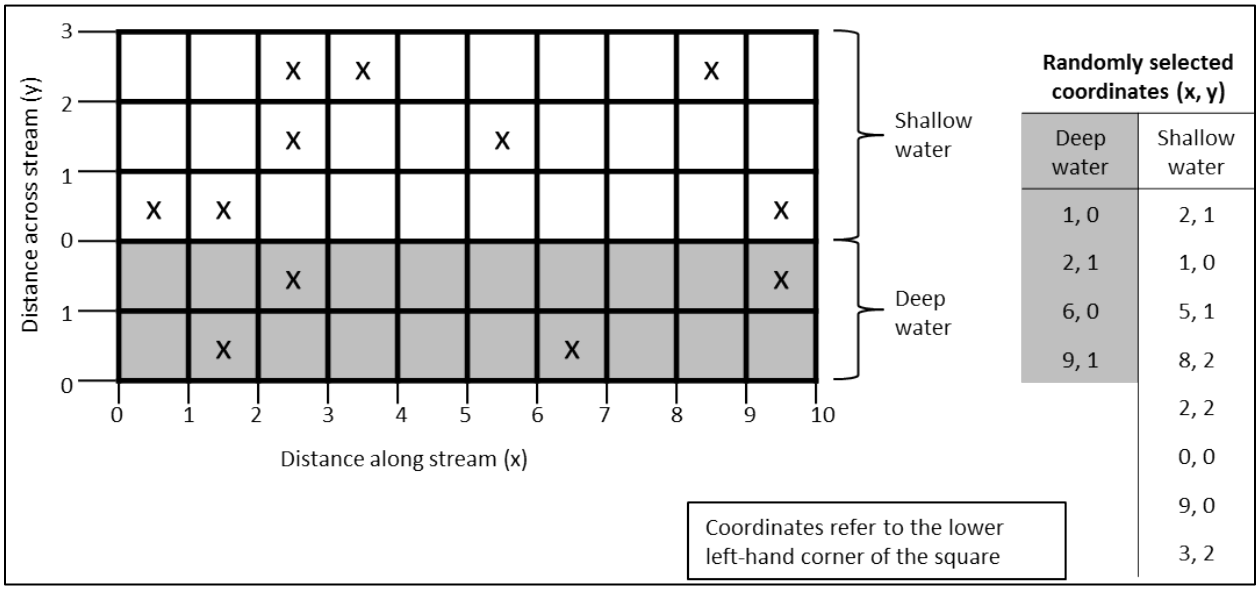

Figure 3: Example of stratified sampling design with random site selection (adapted from Strayer and Smith 2003)

Complete Coverage

Complete coverage entails sampling with sufficient effort to collect all or most mussels in a study area and is typically limited to salvage efforts or intensive research projects. Surveyors should consider the habitat damage that may ensue from the use of this method. Complete coverage assumes the same sampling method will be used throughout the entire study area. A challenge with this sampling design is that, depending on the type of sampling method, the cost of the mussel survey can be prohibitively expensive on all but the smallest streams. In large waterbodies, complete coverage may prove too costly to undertake and even impractical, but in smaller waterbodies, this approach may be a viable option due to the lower cost of surveying smaller areas.

[14] This approach assumes that the survey designer has a thorough working knowledge of the preferred types of habitat where mussels are likely to be present. Designs based on habitat preferences should be used with caution because even experienced malacologists can be surprised where mussels are found. One reviewer noted finding fatmuckets in small pool under a bridge despite the rest of the stream being dry.

Step 4: Choose a Sampling Method

There are several different types of sampling methods for mussel surveys. In this section, the sampling method is defined as how the mussel population will be surveyed. Often the sampling method is tied to the sampling design (e.g., timed or distance transects are often combined with either snorkeling or diving). Another key factor is the cost of each method, which varies greatly by waterbody, locality, and the size of the site.

Table 1 presents general cost guidelines in terms of the level of effort required for various types of survey sites/sampling methods and sampling approaches, with '1' being the least costly. The units are simply a level of effort, and actual costs would vary based on site-specific factors. As mentioned above, the physical characteristics of the site will also help determine both the design and methods used for sampling (e.g., it is often unrealistic to sample a large river site using a complete coverage design, and therefore, some form of timed search along transects via diving or a double sampling design is often used).

Table 1: Generalized costs (level of effort) for mussel survey types

Method or Habitat	Informal	Qualitative	Semi-quantitative	Quantitative
Wadable Streams	1	2	4	5
Snorkeling or Scuba	2	3	5	6
Navigable Waterways	3	4	6	8

This section is divided by rank from the least effective to the most effective method that might be employed to sample the entire mussel population at a given site. [15] However, while some of the less comprehensive methods may not be useful to determine mussel absence, the low cost of these methods may warrant their use in preliminary or exploratory surveys to determine mussel presence. It should be noted that not all methods below are approved for use (in various survey protocols) by different states or the United States Fish and Wildlife Service (USFWS) due to potential damage to habitat and species.

Shoreline Searches

Shoreline searches are useful in reconnaissance surveys. This method includes walking along the waterbody looking for live mussels in the water and shells on the shore (Nedeau et al. 2009). This approach is safe and easy and can be useful when the water levels are low (Nedeau et al. 2009). This method can include muskrat shell midden searches. Muskrats are capable of eating large numbers of mussels and often leave the shells in neat piles (middens) along the stream bank (Strayer and Smith 2003). However, because muskrats are selective eaters, the piles are of limited use for species type and relative abundance (Dunn 2000; Strayer and Smith 2003). Figure 4 shows a photo of a muskrat shell midden. Other predators such as river otters, raccoons,

[15] Effective in this context means least likely to miss finding mussels and, therefore, least likely of leading to inaccurate conclusions.

skunks, gulls, and shorebirds eat mussels and also leave spent shells along shorelines, which can be used to infer presence. Additionally, floods typically deposit as many shells as they wash away, which can persist for decades and result in large accumulations in depositional locations and along shorelines.

Figure 4: Photo of a muskrat shell midden (Photo from Nedeau et al. 2009)

Overall, shoreline searches are the least expensive method for screening mussel presence or absence. While it is not recommended to determine true mussel absence from a waterbody, it can be useful in determining mussel presence.[16] Where a shoreline search determines mussels are present, a more comprehensive (and likely more expensive) method may not be warranted depending on the objective(s) of the study.

Brail Bars and Dredges

Brail bars and dredges are also useful reconnaissance survey approaches and are commonly used by commercial fishermen to collect mussels in a large area in a relatively short period of time. A brail bar, used primarily in large rivers, is dragged slowly by boat across the bottom of the waterbody. The mussels clamp down on the brail hooks and are pulled up to the boat (Strayer and Smith 2003). Figure 5C displays an image of a brail bar. A dredge, a shovel-like apparatus used primarily in marine waters, is dragged by boat across the bottom of the waterbody to scoop up the mussel population. The use of these two methods is limited by environmental characteristics (i.e., water must be deep enough for a power boat, there must not be too many snags to impede equipment, and the substrate must not be too stony for dredges).

[16] One expert peer reviewer noted that, in one half mile survey reach where hundreds of mussels were present in the water, not one shell was seen on shore. The shells can wear out, be washed away during floods, or the appropriate predators may not be present in the area.

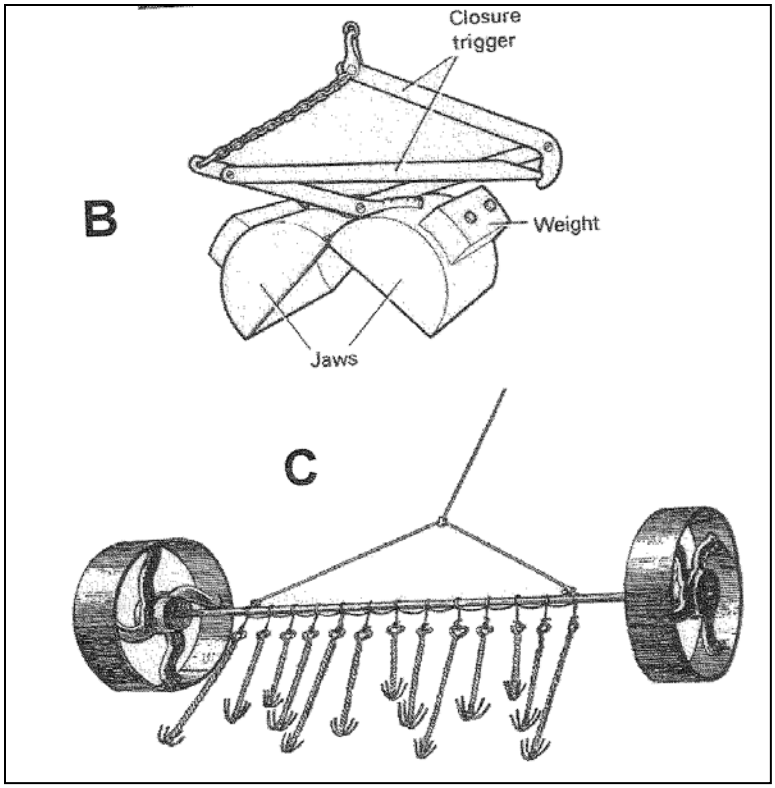

Figure 5: Example of PONAR grab (B) and brail bar (C) (Image from Strayer and Smith 2003)

Both methods are similar in usefulness for mussel presence/absence surveys. It should be noted, though, that these methods can be highly disruptive to the entire habitat and may lead to mortality among mussels and other aquatic species present in the substrate. Results of a brail bar are affected by brail hook type, substrate conditions, water temperature, time of day, turbidity, species behavior, mussel size, gender, time of year, and collector experience (Dunn 2000). The same area can be sampled several times under different conditions and produce different results (Dunn 2000). Dredges are not widely used to sample mussels in freshwater but have similar limitations as brail bars.[17] Both methods are relatively inexpensive when compared to some other sampling methods. The usefulness of these methods is similar to that of shoreline searches because they are relatively easy methods to determine mussel presence. With both techniques, though, a more comprehensive method should be used for making mussels-absent determinations due to the wide variability in their results and ability to detect mussel presence.

Searches while Wading

This type of sampling can be used in reconnaissance or qualitative sampling approaches. It describes a visual search for mussels that is conducted by wading in the waterbody and looking with eyes alone for mussels. However, in some cases, it can be accompanied by tactile searches.[18] This method can be supplemented with the use of glass-bottomed buckets called

[17] However, dredges may be necessary in deep water where diving is not safe.

[18] Tactile searches performed while wading include gently fanning away the sediment (where appropriate) and trailing the fingertips along the substrate to feel for mussels.

aquascopes (Dunn 2000). The surveyor places his/her head in the bucket and views the bottom of the waterbody through the glass bottom of the bucket, thereby eliminating the glare from the sun on the water surface (Young et al. 2001). This method can cover a lot of ground quickly (Strayer and Smith 2003) and can be effective in fast riffles too shallow for snorkeling. Figure 6 shows a photo of surveyors using aquascopes while wading during a stream survey.

Figure 6: Example of an aquascope (Photo from Nedeau and Victoria 2003)

This method is most useful in clear, shallow (i.e., less than three feet) water (Nedeau et al. 2005) because mussels are more difficult to detect in turbid water than in clear water. This method has a high catch rate when the mussels are on the top of the substrate. However, this method is not as useful when the mussels are small or for those species that bury deep in the sediment (Strayer and Smith 2003). According to Strayer and Smith (2003), juvenile mussels are far more likely to bury deeper than adults, and mussels in general are most likely to be buried during fall and winter than in spring and summer. Additionally, a large part of the mussel population present may often be buried. Stagliano (2010) confirmed this problem with visual only searches, noting that mussels less than 30 millimeters will not be visible on the substrate surface and that visual surveys tend to be biased toward larger individuals. The Ohio River Valley Ecosystem Team (ORVET) Mollusk Subgroup assumed in their draft protocol for mussel surveys that only 50% of the mussel community is visibly present at the substrate surface (ORVET 2004). This assumption depends a lot on the species and age of the mussel, time of year, and the habitat characteristics, but it is still assumed that a substantial fraction of the mussel community may be out of sight. This visual limitation can be compounded by species whose shell sculpture (i.e., shape) may make them hard to distinguish from gravel and cobble (Miller and Payne 1993).

Snorkeling

Snorkeling can be used in reconnaissance, qualitative, semi-quantitative, and quantitative sampling approaches. The objective is for the observer's eyes and hands to be as close to the substrate as possible. Snorkeling should be limited to water depths less than an arm's length (approximately one meter). Snorkeling is similar to wading and the use of aquascopes except that, depending on the waterbody depth, some tactile methods may also be used. A tactile search can include gently running a finger over the sediment, fanning away fine sediment, and removing loose non-embedded material (Smith et al. 2001; Strayer and Smith 2003). Snorkeling is slower than wading and aquascopes but can be used in deeper water.

Snorkeling may be a more efficient and suitable method for detecting small or cryptic mussels than wading and aquascopes because it should be a slower and, therefore, more comprehensive search method. However, there is no good documentation to confirm this performance (Strayer and Smith 2003). Snorkeling and tactile methods may be more effective when surveying in fine-grained sediment (e.g., sand and mud) than in coarse-grained sediment (e.g., cobble) because it is easier to detect mussels tactilely than visually in the fine-grained sediment. On the other hand, snorkeling can be useful in coarse-grained sediment to visually distinguish mussels from cobble or other small rocks. Despite possible advantages, snorkeling is still unable to detect all of the mussel community located deep in the substrate.

Diving

Diving is almost identical to snorkeling in its utility but can be used in deeper waters (e.g., greater than one meter) (Smith et al. 2001).[19] Diving is more labor intensive than snorkeling but allows the surveyor to spend more time closer to the substrate, thereby improving the opportunity and ability to detect mussels. In fact, Dunn (2000) states that diving may be the method least biased by sampling conditions when compared to wading, brail bar, and dredge searches because of the greater amount of time the surveyor can spend closer to the substrate. While diving is similar to snorkeling in terms of its effectiveness for detecting mussels, the main differences in these two methods are the depth of the waterbody that is being sampled and the cost of each method, with diving being significantly more expensive.[20] Diving using self-contained underwater breathing apparatus (SCUBA) also has limitations, and in navigable waterways and deep mussel beds in the southeast, surface-supplied diving may be the safest and most efficient method due to boat traffic and other hazards. Because surface-supplied diving often utilizes diver-surface communications, many sampling tasks can be accomplished *in situ* by trained surveyors, and the data can be relayed to the surface and recorded.

Excavation

Excavation is the most effective sampling method that is able to detect the entire mussel community; however, this method is also the most invasive and time consuming. Excavation involves digging up a small amount of substrate (usually 10-15 centimeters), depending on habitat and target species) and sieving (often 2-8 millimeters) the material to find all buried and non-buried mussels. This method is the slowest of all the methods and, therefore, usually the most costly. Substrate is usually excavated by hand or trowel via wading, snorkeling, or diving depending on water depth and placed into a mesh bag or bucket. The material is then sieved on the shore, boat, or *in situ*. Excavation can also include the use of grabs (see Figure 5B above) in areas where site characteristics (e.g., flow and depth) may make collecting samples by hand difficult. Due to the increased invasiveness and the amount of time needed to sample, excavation is primarily conducted on a subset of transects or quadrants in quantitative surveys (i.e., double sampling) and limited to selected habitats (e.g., gravelly riffles) during qualitative sampling.

[19] In this context, diving may include surface-supplied air, SCUBA, or other supplied air devices.
[20] This assertion may be dependent on site conditions because diving may be used in less than ideal conditions, particularly in poor light and high turbidity. Furthermore, diving is slower than snorkeling, and therefore, the time available to perform sampling can limit this method's effectiveness due to less area being sampled.

Excavation of an entire reach would rarely be feasible but may be the only true quantitative means of collecting mussels depending on the objective(s) of the study.

Other Considerations for Mussel Surveys

In addition to the four steps above, there are a number of other considerations that should be taken into account for a well-designed and well-executed mussel survey.

Time of Year

Surveyors should sample the site when they are most likely to find mussels. Some sources stress the importance of sampling only from spring through early fall (i.e., April to October) (Angelo et al. 2009; ORVET 2004; Shearer et al. 2005; Smith et al. 2001; Sovell and Guralnick 2004; USFWS and VDGIF 2008; WDNR 2005). During the April to October time period, conditions are best for viewing live mussels in the substrate because river flow tends to be low with high water clarity (Smith et al. 2001). In addition, during the summer, high proportions of some mussels are at the substrate surface rather than buried deep in the sediment (Amyot and Downing 1991; Balfour and Smock 1995), and during the cooler months, mussels tend to be located deeper in the substrate, which makes them more difficult to find by visual methods alone (USFWS and VDGIF 2008). The Wisconsin Department of Natural Resources (WDNR) guidelines for sampling mussels suggest that surveys be conducted in Wisconsin waters from mid-June to late September when mussels are more active (WDNR 2005). Furthermore replicate surveys performed in different seasons or in different years may provide a greater assurance that mussels are truly absent at a given site.

Special Considerations for Small or Rare Species and Vertical Migration

Relatively recent research by Chris Eads and Jay Levine (2007) at North Carolina State University shows that smaller (and sometimes rare) mussel species tend to spend less time on the sediment surface than larger mussel species, possibly as a means to avoid predation or being swept away by the current. However, vertical migration through the substrate can be affected for any species by water temperature, time of year (e.g., males releasing sperm and females preparing to broadcast glochidia), and changing water levels. At least some excavation should be used when sampling in cooler water, when looking for endangered/rare species, and/or other site-specific circumstances in which the state or tribe decides that excavation is necessary to defensibly demonstrate mussel absence.

Visibility Requirements

The ORVET (2004) draft protocol identifies a minimum visibility requirement of 0.5 meters with or without lights at the depth of the survey. The protocol specifies that the surveyor must quantify the actual visibility. If the visibility prerequisite is not met, either the survey must be rescheduled or a different protocol should be used (e.g., a more intensive quantitative survey). In some rivers, this visibility level will never be realized, and the surveyor will need to depend on tactual collection. Tactual searches can be less efficient than visual searches, and some excavation and sieving of substrate might be considered.

Experienced Investigators/Surveyors

The majority of mussel sampling methods include some level of visual search and the ability to identify, by sight alone, a mussel when encountered in the substrate. Therefore, it is important to have surveyors or surveying crews who are experienced with mussel sampling and have expert knowledge of the species habitat and life history. Such experience is crucial because it is often difficult to find small, juvenile, or cryptic mussels and distinguish them in the substrate. An experienced sampler will also be able to identify sections of the sample area that will most likely support mussel populations. Inexperienced collectors can also be utilized, but their work may need to be calibrated by quantitative sampling, sampling paired with an experienced collector for an initial period, or sampling the area again by an experienced investigator. In Virginia, only qualified, pre-approved mussel surveyors can conduct surveys (USFWS and VDGIF 2008). Those not pre-approved must submit their qualifications before conducting any survey.

The need for experienced and qualified mussel investigators is also highlighted in the Freshwater Mussel Survey Protocol for the Southeastern Atlantic Slope and Northeastern Gulf Drainages in Florida and Georgia (Carlson et al. 2008). In this protocol, surveyors must have sufficient knowledge of the mussel species likely present in the area as well as the basin they propose to survey. This knowledge includes species-specific biology and ecological requirements and the ability to identify freshwater mussels (Carlson et al. 2008).

In addition to the general academic knowledge surveyors should possess, surveyors should have adequate field experience, which includes documented field time; the ability to execute mussel survey methods independently; the ability to locate and identify federally-listed species; and experience in the safe care and handling of threatened, endangered, or candidate mussels. This knowledge and experience should be documented, and a letter of recommendation may be requested prior to any surveys being conducted (Carlson et al. 2008). Some external peer reviewers suggested that having experienced mussel surveyors may be the single most important aspect of mussel sampling. Experienced surveyors can bring more rigor to a qualitative survey and, in some cases, reduce the necessity for conducting formal quantitative surveys. States and tribes may want to consider requiring that all mussel surveys performed for purposes of utilizing the Recalculation Procedure be conducted by an experienced individual or team, especially when the results are used to justify a "mussels-absent" determination.

Appropriate Permits

Surveyors must have appropriate permits from state and federal officials before the survey is conducted.[21] State permits may be required because some states (e.g., Virginia) are responsible for the conservation and management of all freshwater mussel species within the state. However, federal permits may also be required because the USFWS is responsible for the conservation and management of all federally-listed mussel species. Permits for waterbodies potentially containing federally-listed species are necessary because the USFWS (and some states like Virginia) control unlawful take of threatened and endangered species. Under Section 7 of the Endangered Species

[21] In some areas of the country, it may be necessary to consult with the state fish and wildlife agencies to ensure that mussel surveys do not disturb native fish spawning areas (i.e., salmonid egg redds) or other aquatic species because mussel sampling often involves some disturbance to the substrate.

Act, take is defined as harass, harm, pursue, hunt, shoot, wound, kill, trap, capture or collect, or to attempt to engage in any such conduct. The USFWS views mussel surveys as harassing mussel species and, thus, could be considered "take." Some states like South Carolina only require a fishing permit to conduct mussel studies.

A good survey will include coordination with state game and fisheries or natural resources departments and the USFWS, especially if there is potential for federally-listed species to be present at the site. Additionally, many states now have mussel sampling protocols that should be followed (e.g., see Appendix C for a summary of West Virginia's mussel survey protocols).

Safety

From time to time, field personnel participating in mussel surveys may encounter potentially dangerous situations. In addition to the routine possibility of automobile, boating, or equipment accidents, surveyors may encounter aggressive animals, belligerent people, surface waters contaminated with toxic substances or infectious microorganisms, swift and/or deep water, uneven and/or slippery surfaces, submerged obstacles, dangerous weather (e.g., lightening, heavy rain, strong wind, high heat index), or other threatening situations. Such situations could potentially lead to injuries or illnesses and, from a quality assurance perspective, deprive field crews of the services of valuable members. To minimize these risks, field personnel should be cognizant of the potential safety issues involved in sampling at a particular site and observe any safety requirements set forth in the sampling protocol or established standard operating procedures. Additionally, field personnel should plan ahead and take proper precautions, which may include the following actions, to minimize potential risks and be prepared in the event of an emergency or accident:

- Sample in crews of at least two individuals that remain within hearing distance.
- Carry fully charged cellular phones.
- Demonstrate familiarity with applicable safety procedures and the use of available safety equipment.
- Obtain certification in adult cardiopulmonary resuscitation and basic first aid.
- Obtain any necessary certification with the use of particular sampling equipment or methods (e.g., SCUBA).
- Equip vehicles and vessels with appropriate safety gear including first aid kits, fire extinguishers, spare tires and tire changing equipment, rain gear, road reflectors and/or flares, flashlights and batteries, life vests, and flotation devices.
- Maintain vehicles and vessels in proper operating condition.

Checklist of Key Elements in a Mussel Survey Protocol

This checklist provides a list of key elements that should be considered when selecting or reviewing a suitable protocol for determining whether mussels are present or absent at a particular site. This information is provided as general guidance and does not necessarily mean that state and tribal water quality criteria based on a protocol that contains all of the elements below will be approved by EPA. EPA reviews and either approves or disapproves new or revised state and tribal water quality criteria on a case-by-case basis.

Preliminary Information

- □ The surveyor/contractor is qualified to survey the geographic area, waterbody type, and potential mussel fauna of the region (i.e., The surveyor/contractor has been pre-approved to conduct mussel surveys in the region/state and has provided adequate credentials/certifications including number of hours worked or trained, etc.).
- □ The objective of the study is clearly stated.
- □ The state or tribal definitions of presence and absence are clearly defined.
- □ The waterbody or watershed/region of interest was investigated to determine if any occurrence data (via historical records, other survey data, etc.) indicate mussels are/were present. [Note: Records, databases, studies etc. that were searched should be explicitly stated along with the results, if any]. In systems influenced by anthropogenic impacts, it may be beneficial to include information from similar nearby waterbodies, and to include waters upstream and downstream from dischargers.
- □ The surveyor/contractor has all appropriate state and federal permits (e.g., in the case of a rare species being found).
- □ A thorough study plan has been developed with proper quality assurance/quality control elements and a safety plan.
- □ The study plan has been prepared in cooperation with, reviewed by, or approved by an individual with demonstrated expertise in conducting mussel studies as well as a state natural resources or federal U.S. Fish and Wildlife Service official.

Study Design

- □ The study area is thoroughly delineated (i.e., a map has been created showing all aspects of relevance within the area of interest such as study boundaries, vertical and horizontal in-stream demarcations, quadrats/cells to be sampled, etc.).
- □ The study area is thoroughly described (e.g., coordinates of location, qualitative and quantitative instream features, water quality, channel stability, impoundments, riparian features, road crossings, and other unique natural and anthropogenic features) in relation to the stream/segment that would be subject to any resulting site-specific criterion.
- □ If the study area does not encompass the entire site for which site-specific criteria are to be developed, the study plan explains how the results of the survey can be extrapolated to the entire site.
- □ The survey method is thoroughly described and appropriate for the waterbody and potential mussel fauna present, and relevant research studies are cited to support the sampling approach, design, and method.

□	The appropriate state and federal authorities/experts have reviewed and approved the design.
□	The method includes more than one surveyor, and surveyor names are provided with an indication of the level of training or experience of each surveyor.
□	The proposed sampling date(s) fall within the recommended time frame for the region and mussel fauna potentially present (e.g., April to October or other time frame based on current research information).
Reporting	
□	A final report has been prepared containing author contact information, study objective(s), and a thorough description of protocol, survey results/findings, and conclusions.
□	All forms/field data sheets have been made readily available, upon request, for quality review.
□	A provision for continued monitoring of the site/stream segment is included in the study plan if results indicate that mussels are absent. The provision stipulates the return frequency and protocol and provides a scientific justification.
□	A provision for documentation with appropriate authorities and archives (e.g., U.S. Fish and Wildlife Service, state natural heritage programs, academic institutions) is included in the study plan if results indicate that mussels are present.

Phase 4. Develop Site-specific Criteria Using the Recalculation Procedure

In the case of ammonia, where a state or tribe can demonstrate that mussels are absent on a site-specific basis, the Recalculation Procedure may be used to remove the mussel species from the national criteria dataset to better represent the species present at the site. The scope of this effort involves gathering the appropriate data, creating a list of species that occur at the site (i.e., a resident species list), comparing that resident species list to the species list provided in *Aquatic Life Ambient Water Quality Criteria for Ammonia – Freshwater 2013* (USEPA 2013a), and then carrying out the step-wise process of deleting (or retaining) taxa from the national toxicity dataset [see *Revised Deletion Process for the Site-Specific Recalculation Procedure for Aquatic Life Criteria* (USEPA 2013b)]. Standard procedures are used to recalculate site-specific acute and chronic criteria values using the site-specific (resident) species dataset. Due to the complexity of the relationship between ammonia toxicity and pH and temperature across different aquatic organisms, EPA has recalculated site-specific criteria removing mussels from the national dataset and provided these values in Appendix N of *Aquatic Life Ambient Water Quality Criteria for Ammonia – Freshwater 2013* (USEPA 20103a) . For convenience and consistency, states and tribes may propose these values directly for site-specific criteria, as appropriate, for sites at which the state or tribe determines that mussels are absent.

For example, many of the commonly occurring freshwater bivalves (e.g., pea clam) are more closely related to the veneroid (i.e., Order Veneroida) fingernail clam *Musculium* (which is the fourth most sensitive genus in the national dataset for the chronic criterion) than to the unionid (i.e., Order Unionoida) mussels *Lampsilis* and *Villosa* (which are the two most sensitive genera in the national dataset for the chronic criterion). EPA presumes that for the majority of sites where all bivalves present are more closely related to *Musculium* than to *Lampsilis* and *Villosa* (i.e., where mussels in Order Unionoida are absent at the site), the Recalculation Procedure may be used to remove *Lampsilis* and *Villosa* from the dataset because they would not be representative of the species present at the site. The retention of *Musculium* in the dataset would represent the veneroid bivalves present at the site, so the veneroid bivalves would still be protected if *Lampsilis* and *Villosa* were removed from the dataset. However, at sites where both unionid and veneroid bivalves are present, all three bivalves in the national dataset (i.e., *Lampsilis*, *Villosa*, and *Musculium*) would be retained because they would represent the species present at the site. The Recalculation Procedure describes how to compare the taxa at the site with the taxa in the national criteria dataset.

The number of tested genera (N) in the criteria calculations must be updated where genera such as *Lampsilis* and *Villosa* are removed from the dataset. For example, if only the two unionid mussels are removed from the dataset for the national chronic ammonia criterion, N would be reduced from 16 genera in the national dataset to 14 genera in the site-specific dataset.

As discussed earlier, when choosing an appropriate site-specific criterion, a state or tribe must be mindful of downstream waters. For example, as with all designated uses and criteria in a state's or tribe's WQS, 40 CFR § 131.10(b) states the following:

> In designating uses of a water body and the appropriate criteria for the uses, the State shall take into consideration the water quality standards of downstream waters and shall ensure that its water quality standards provide for the attainment and maintenance of the water quality standards of downstream waters.

States and tribes should take into account downstream waters when they consider any changes to site-specific criteria at a given site.

Phase 5. Re-evaluate as needed

Pursuant to 40 CFR § 131.20, each state or tribe is required to review its water quality standards, which include site-specific criteria, at least once every three years. However, one peer reviewer of this draft TSD considers two years to be a prudent time frame upon which an "absent" finding may still be valid. The reasons for re-evaluating a "mussels-absent" finding are many, the first being that juvenile mussels spend at least the first year of life buried deeply in the substrate. Thus, juvenile mussels may be missed by certain sampling methods (e.g., qualitative sampling via brailing) as might other species that are able to tolerate more silted conditions such as many of the western *Anodonta*. Additionally, the proportion of mussels at the surface of the substrate varies greatly depending on water temperature, mussel gender, mussel species, and time of year. Again, these may be missed by certain sampling methods. Finally, not only do smaller species spend less time at the sediment surface, vertical migration through the substrate can be affected

in general for any species by water temperature, time of year (e.g., males may be releasing sperm and gravid females may be preparing to broadcast glochidia), and changing water levels. All these factors contribute to a high degree of year-to-year variability with regard to sampling efficiency. Because the Recalculation Procedure states that species that occur at the site cannot be determined by a one-time sampling event, a state or tribe may find it necessary or beneficial to sample over a two- or three-year time period (or more) and use the results of multiple surveys to bolster a mussels-absent decision.

Table 2 below illustrates why "mussels-absent" findings from mussel surveys should have a limited lifespan. It shows the difference in mussel presence for several mussel species from 2006 to 2012 in a segment of Craig Creek in Virginia as part of a relocation study for on-going ford maintenance activities. While it is clear from this table that sampling efficiency differs greatly from one year to the next, the results vary from year to year and within seasons. At the site, up to six species were found in every year of sampling except 2007, when no mussel species were found even though they had been documented as present in other years using the same sampling methods (i.e., timed searches). Had a single survey been used to determine mussel absence in 2007, the present mussel population would not have been detected, which is not indicative of the true characteristic of the waterbody. This example also suggests that a single mussel survey may not accurately characterize the waterbody. It may be that mussels were buried or field conditions were difficult for sampling in 2007.

Table 2: Mussel survey data for the Carter's Ford segment of Craig Creek in Virginia (Johnson and Neves 2012)

Number of Individuals found at Carter's Ford in Craig Creek, VA.						
Year	***Elliptio complanata***	***Elliptio fisheriana***	***Fusconaia masoni* (threatened)**	***Pluerobema collina* (endangered)**	***Strophitus undulatus***	***Villosa constricta***
2006	3	44	0	2	1	30
2007	**0**	**0**	**0**	**0**	**0**	**0**
2008	1	33	3	0	2	10
2009	0	14	7	2	1	8
June 2010	1	45	6	0	1	19
August 2010	0	6	6	0	0	25
2011	1	43	2	1	4	25
July 2012	0	65	5	2	1	18
2012	0	2	0	0	0	1

Given the above and the fact that a substantial fraction of the mussel community may be out of sight during sampling, states and tribes should consider stipulating return frequency and the appropriate survey method if no mussels are found during a particular survey, especially if the first survey does not find any mussel species. Requiring new or multiple surveys over a specified time, especially when a survey returns a finding of "absent" and before a "mussels-absent" decision (for the purposes of site-specific criteria) is made, not only helps ensure that mussel colonization or recolonization is documented, it also increases the probability that juvenile mussels that were initially missed in an earlier survey (because they were buried and/or too small

to be detected) will have grown substantially such that they are more likely to be found in subsequent surveys.

West Virginia's mussel survey protocols (Clayton et al. 2013) states that survey data collected at a specific site will be considered valid for five years from the date the survey was conducted. A shorter time frame was selected for the Draft Freshwater Mussel Guidelines for Virginia (USFWS and VDGIF 2008), where a negative survey (i.e., no mussels found) is only valid for 2 years.

When considering any stipulations on return frequency/re-evaluation, states and tribes should recall that adoption of site-specific criteria is subject to full public participation requirements. After adoption, additional public review of a site-specific criterion could be accomplished in conjunction with the public review required for permit issuance.

Summary

This technical support document provides a basic overview of the considerations and nuances that states and tribes should be aware of in demonstrating mussel absence for the purposes of developing site-specific criteria for ammonia using either the alternate criteria values provided in Appendix N of the 2013 national ammonia criteria recommendations or the updated Recalculation Procedure. In particular, this document attempts to address most of the issues that are important for a state or tribe to contemplate when evaluating whether mussels are absent at a site. Some of these items include delineating the site where the site-specific criteria apply, creating a scientifically-defensible definition of mussel presence and absence, reviewing and analyzing previous survey records, and the steps involved in designing and conducting mussel studies. EPA does not advocate one approach over another to make these decisions but, in the process of reviewing a state's or tribe's WQS, will evaluate whether the site-specific criteria and the methods used to derive them are scientifically defensible, sufficient to protect the designated uses, provide for the attainment and maintenance of downstream standards, and are consistent with the CWA and WQS regulations.

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Appendix A: How to Use NatureServe© Explorer to Query Mussel Distribution Data

I. What is NatureServe© Explorer?

NatureServe© is a non-profit conservation organization whose mission is to provide a scientific basis for informed decisions on managing natural resources. It represents an international network of biological inventories operating in North and South America including all 50 U.S. states. The objective scientific information about species and ecosystems developed by NatureServe© is used by conservation groups, government agencies, corporations, academia, and the public. NatureServe© Explorer is a product of NatureServe© and is the searchable database for information on more than 70,000 plants, animals, and ecosystems of the U.S. and Canada.

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Note:

All species and ecological community data presented in NatureServe Explorer at http://www.natureserve.org/explorer were updated to be current with NatureServe's central databases as of October 2012.

Note:

This report was printed on June 21, 2013

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> Citation for web site data including State Distribution, Watershed, and Reptile Range maps: NatureServe. 2012. NatureServe Explorer: An online encyclopedia of life [web application]. Version 7.1. NatureServe, Arlington, Virginia. Available http://www.natureserve.org/explorer. (Accessed: June 21, 2013).

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II. What data are available in NatureServe© Explorer?

NatureServe© Explorer contains data on plants, vertebrates, invertebrates, and ecological units (associations and systems). Users can search by scientific or common names of species; plant or animal group; location by state, county or watershed; or conservation status.

For each species, the data available to users include the following:

- Distribution data and maps
- Images
- U.S. invasive species impact rank
- Economic attributes
- Life histories and conservation needs

For each ecological unit, the data available to users include the following:

- Classification
- Global conservation status
- Distribution
- Vegetation structure
- Dynamic processes

III. How Can Searches be Performed Using NatureServe© Explorer?

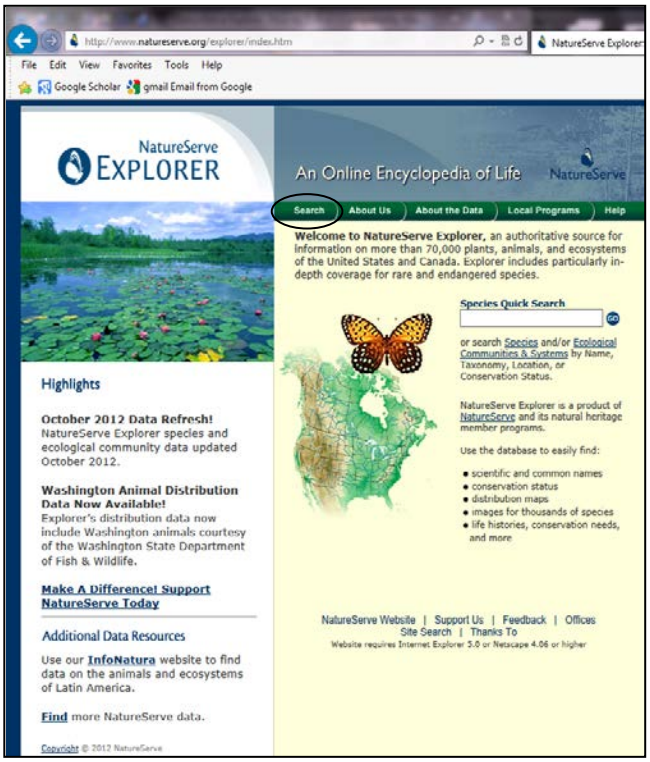
- Name (Common name, Species, or Species Group)
- Location (U.S. States & Canadian Provinces, U.S. Counties, or U.S. Watersheds)
- Status (conservation status)
- Any combination of the three items above

[NOTE]: Functionality of this website is designed for use with Internet Explorer (version 5.0 or newer) or Netscape (version 4.06 or newer) browsers, and JavaScript 1.2 is required to run data searches. However, the steps outlined below were also tested with Firefox, Safari, and Chrome browsers, and all browsers preformed adequately except where noted below. Most of the instructions are also found in the help menus on the NatureServe© website, and the steps outlined below are presented in a similar fashion.

IV. Beginning a plant or animal search

1. From the NatureServe© Explorer home page (www.natureserve.org/explorer),
2. Click **Search.**
3. Click the tab for Plants and Animals.
4. Click the tab for **Name**, **Location**, or **Status**. (You can search by one or any combination of these in any order.)

[NOTE]: Only the two most applicable searches for mussel distribution queries are presented below (i.e., searches by **Name** and **Location**).



Tips for name searches:

- Use a singular name, never plural. NatureServe Explorer searches by a scientific or common name, which is always singular.
- Use wildcards (*) with partial names.
- Use AND/OR for multiple word searches.
- Use ignore punctuation to broaden your search.

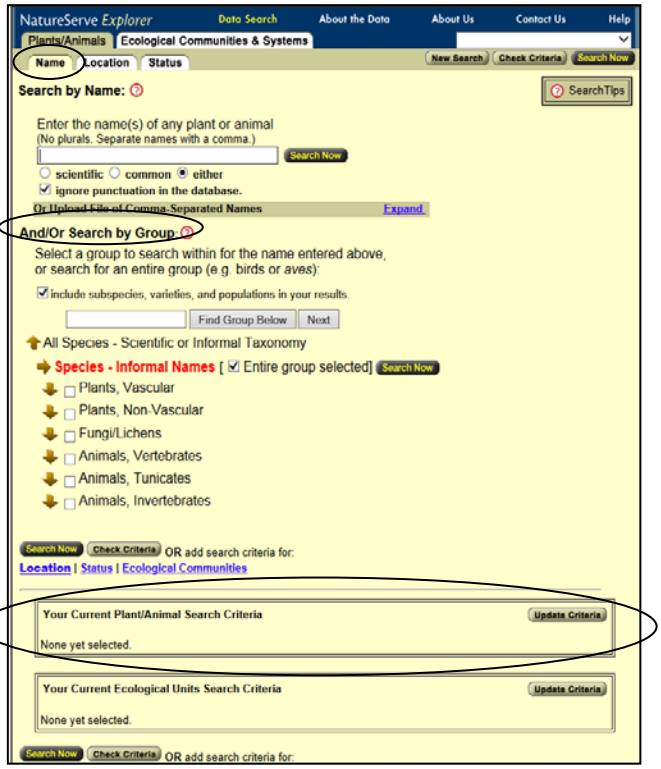
V. Searching by Name

1. In the **Plants/Animals** tab, click **Name**.
2. Type the scientific or common name of a plant or animal under **Search by Name**.
3. Click
   - **Scientific** for only scientific names.
   - **Common** for only common names.
   - **Either** for either scientific OR common name.

   Or, you can search by uploading a Comma-Separated (.csv) file.

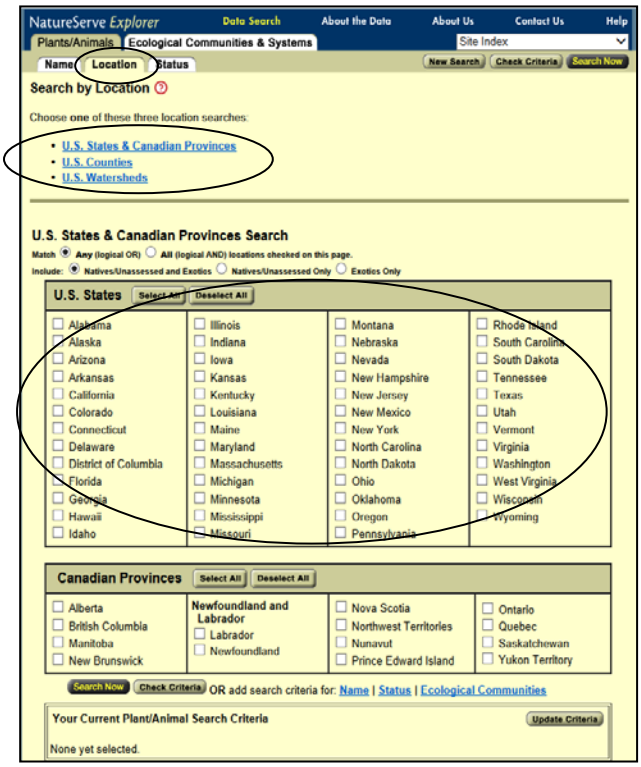
   Or, you can search by group in the **And/Or Search by Group** field. (e.g. mussel) [It is necessary to click **Find Group Below** or **Next**, when using this approach].

4. Click **Search Now** to retrieve all the elements that match the criteria [Use any button].
5. View the results.



VI. Searching by **Location**

1. In the **Plants/Animals** tab, click **Location.**
2. Click on the type of location search you want.
   - U.S. States and Canadian Provinces
   - U.S. Counties
   - U.S. Watershed



[NOTE]: You can only choose one type of location search in each search.

Searching by U.S. States and Canadian Provinces

1. Click all the states and provinces where you want to find plants and animals.
2. Click **Search Now** to retrieve all the elements that match the criteria.
3. View the results.

[NOTE]: If you choose more than one state/province, you will need to choose ANY (logical OR).

Searching by U.S. Counties

Selecting U.S. Counties using the dropdown menu

1. Select a **State** from the dropdown menu.
   - The county dropdown will be populated with counties in the selected state.
2. Select a **County** from the dropdown menu.
3. Click **Search Now** to retrieve all the elements that match the criteria.
4. View the results.

Selecting U.S. Counties using the map

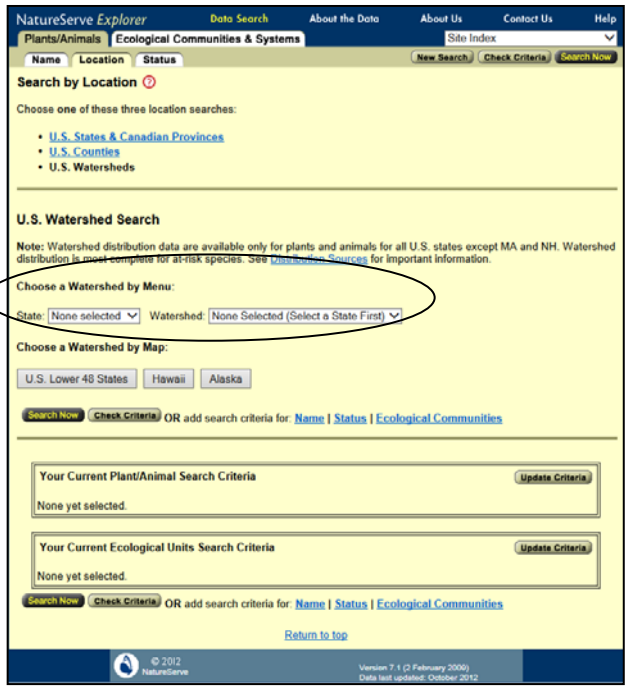
1. On the U.S. Counties search page, click the map you want to use to select a county.
   - U.S. Lower 48 states
   - Hawaii
   - Alaska
2. From the selected sub-area on the map, click on the county you want to add to your search criteria. You may need to navigate the map to find the county you want.
   - You will be returned to the U.S. Counties search page, and the dropdowns will be populated with the county you chose.
3. Click **Search Now** to retrieve all the elements that match the criteria.
4. View the results.

[NOTE]: You can only search by one county at a time. This feature only works with Internet Explorer.

Searching by U.S. Watersheds

Selecting U.S. Watersheds using the dropdown menu

1. Select a **State** from the dropdown menu.
    - The watershed dropdown will be populated with watersheds in the selected state.
2. Select a **Watershed** from the dropdown menu.
3. Click **Search Now** to retrieve all the elements that match the criteria.
4. View the results.



Selecting U.S. Watersheds using the map

1. On the U.S. Watersheds search page, click the map you want to use to select a watershed.
    - U.S. Lower 48 states
    - Hawaii
    - Alaska
2. From the selected sub-area on the map, click on the watershed you want to add to your search criteria. You may need to navigate the map to find the watershed you want.
    - You will be returned to the U.S. Watersheds search page, and the dropdowns will be populated with the watershed you chose.
3. Click **Search Now** to retrieve all the elements that match the criteria.
4. View the results.

[NOTE]: You can only search by one watershed at a time. This feature only works with Internet Explorer.

Tips for Navigating the County & Watershed Search Maps

- **Detail and overview map**
  Detail map: displays all U.S. counties or U.S. watersheds in the chosen area. Only displays part of the whole area.
  Overview map: displays the overview themes. Displays the whole area. A red rectangle shows where the area displayed in the detail map is located.
- **Navigate using overview map** Navigate to a certain area in the detail map by clicking in the overview map.
- **Zoom in, zoom out and pan**
  You can zoom in, zoom out and pan in the detail map.
- **Hotlink**
  Choose Counties or Watersheds.

VII. Example of Mussel Query for Franklin County, Ohio

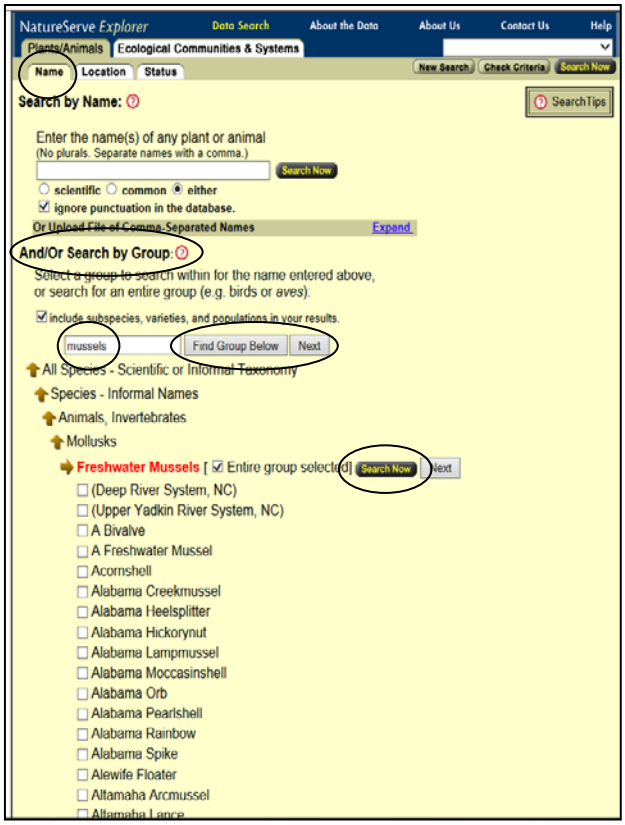
Goal: Determine the possible mussel species present in Franklin County, Ohio and the watershed(s) within the county.

1. Perform searches at the county level and watershed level.
2. Download results to find additional information not available on the website.

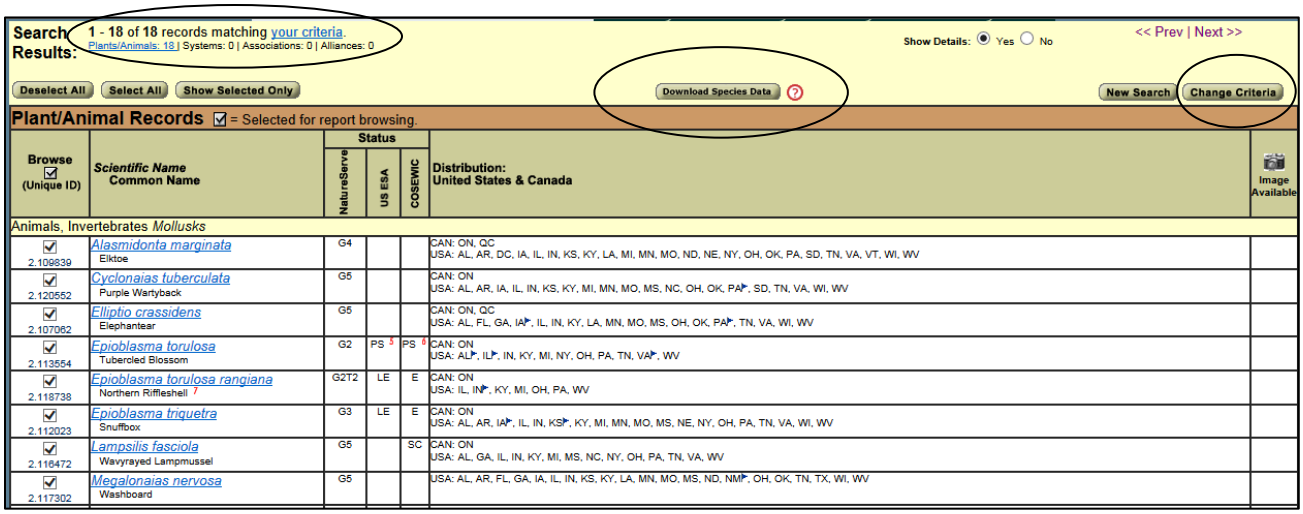
County Level Search

1. Click **Location** tab.
2. Click **U.S. Counties** from the three choices.
3. Select a **State** (*Ohio*) from the dropdown menu.
   - Now the county dropdown will be populated with counties in the selected state.
4. Select a **County** (*Franklin*) from the dropdown menu.
5. Click **Update Criteria** because you are choosing multiple filter elements (**Name and Location**) [use the button in the "Your Current Plant/Animal Search Criteria" box].
6. Click **Name** tab.
7. In the **And/Or Search by Group** field enter "mussel."
8. Click **Next** (either button works).
9. Click **Search Now** to retrieve all the elements that match the criteria.
10. View the results.

[NOTE]: The Chrome browser does not allow multiple search criteria to be entered (i.e., **Location** and **Name**), but from the search results, click **Change Criteria** to enter additional filter elements.



Results Screen for this Query (cropped)



Search Results: 1 - 18 of 18 records matching your criteria.
Plants/Animals: 18 | Systems: 0 | Associations: 0 | Alliances: 0

Show Details: Yes No << Prev | Next >>

Deselect All | Select All | Show Selected Only | Download Species Data | New Search | Change Criteria

Plant/Animal Records = Selected for report browsing.

Browse (Unique ID)	Scientific Name / Common Name	Status: NatureServe	Status: US ESA	Status: COSEWIC	Distribution: United States & Canada	Image Available
Animals, Invertebrates *Mollusks*						
2.109839	*Alasmidonta marginata* Elktoe	G4			CAN: ON, QC USA: AL, AR, DC, IA, IL, IN, KS, KY, LA, MI, MN, MO, ND, NE, NY, OH, OK, PA, SD, TN, VA, VT, WI, WV	
2.120552	*Cyclonaias tuberculata* Purple Wartyback	G5			CAN: ON USA: AL, AR, IA, IL, IN, KS, KY, MI, MN, MO, MS, NC, OH, OK, PA, SD, TN, VA, WI, WV	
2.107062	*Elliptio crassidens* Elephantear	G5			CAN: ON, QC USA: AL, FL, GA, IA, IL, IN, KY, LA, MN, MO, MS, OH, OK, PA, TN, VA, WI, WV	
2.113554	*Epioblasma torulosa* Tuberculed Blossom	G2	PS [5]	PS [6]	CAN: ON USA: AL, IL, IN, KY, MI, NY, OH, PA, TN, VA, WV	
2.118738	*Epioblasma torulosa rangiana* Northern Riffleshell [7]	G2T2	LE	E	CAN: ON USA: IL, IN, KY, MI, OH, PA, WV	
2.112023	*Epioblasma triquetra* Snuffbox	G3	LE	E	CAN: ON USA: AL, AR, IA, IL, IN, KS, KY, MI, MN, MO, MS, NE, NY, OH, PA, TN, VA, WI, WV	
2.116472	*Lampsilis fasciola* Wavyrayed Lampmussel	G5		SC	CAN: ON USA: AL, GA, IL, IN, KY, MI, MS, NC, NY, OH, PA, TN, VA, WV	
2.117302	*Megalonaias nervosa* Washboard	G5			USA: AL, AR, FL, GA, IA, IL, IN, KS, KY, LA, MN, MO, MS, ND, NM, OH, OK, TN, TX, WI, WV	

Based on these results, there are 18 possible mussel species present in Franklin County with survey data that are reported at the county level.

11. Click **Download Species Data** to download the additional data.

- Three types of species data are available:
  1. **Species Summary Report (.pdf)**
     The PDF report contains a selected set of attributes that are used to describe species in NatureServe Explorer. It is designed to provide an overview of the species data resulting from your search in a viewable format.
  2. **Species Comprehensive Data File (.xml)**
     The XML file contains the complete set of attributes that are used to describe species in NatureServe Explorer. It is designed to be utilized specifically when the actual species data need to be used rather than only viewed (as in the PDF report). Uses for XML data include conversion to a web page, or upload into a spreadsheet or application for analysis.
  3. **Species Habitat Report (Excel, .xsl)**
     The Excel spreadsheet file contains a summary of selected species and their habitats. It is designed to provide relevant species habitat information in a standardized spreadsheet format.

[NOTE]: If you need to retrieve the results report/file again, it will be available on the NatureServe Explorer server for 24 hours. Report downloads are currently limited to approximately 800 species.

12. Click the desired report type/format.

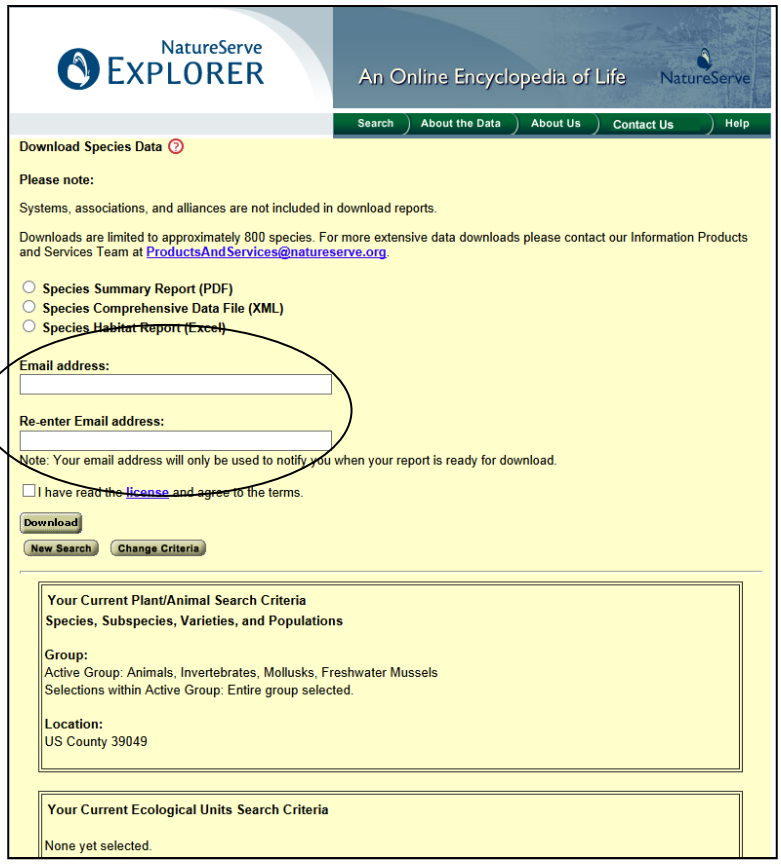
[NOTE]: Only one type of report can be conducted for each search. To download the other reports for the same query, repeat steps 1-10).

13. Enter your email address twice. (Your email address will only be used to notify you when your file is ready for download.)
14. Read the license and check the box to agree to its terms.
15. Click **Download**.

16. Wait for an email from Services_natureserve@natureserve.org. (You may want to add this email address to your spam filter safe-sender list so that it is not treated as spam.)
    - The time needed to generate the report or file is exponentially linked to the number of species that were included in the search. Data for 70 species should take a minute or two, while a report/file with thousands of species may take many hours to generate.

[NOTE]: Until a bug is fixed, even UNCHECKED species in your results list will be included in your report.

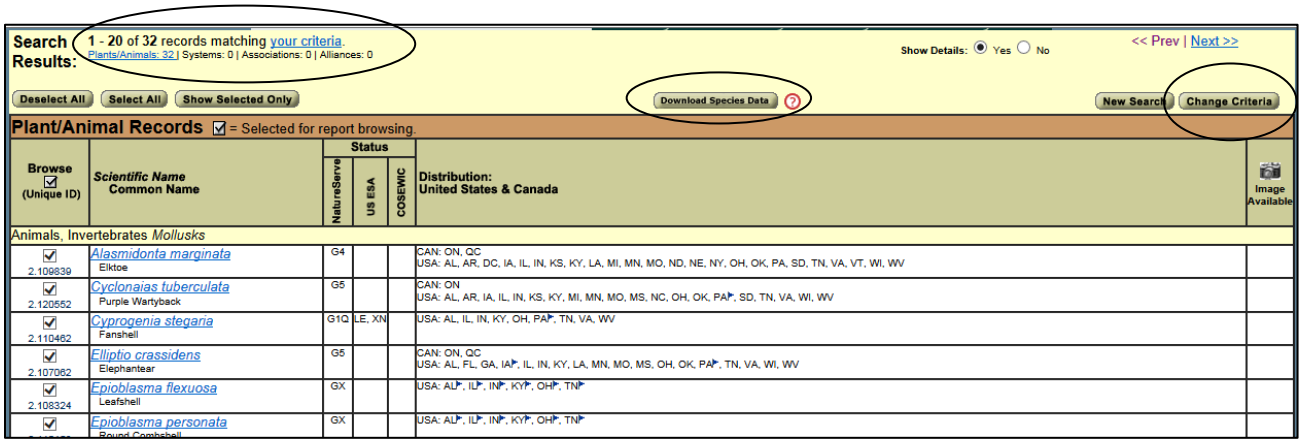
17. Accessing files
    - Once the PDF report, XML file, or Excel spreadsheet has been created, an email will be sent to the address provided:
        1. Click the link in the email to view the data in your web browser.
        2. When viewing the report or file in a web browser, you can save it to your computer by going to the File menu and choosing **Save** for Internet Explorer.
        3. Choose the location on your computer to be used for saving the .pdf, .xml, or .xsl file.



Watershed Level Search

1. Determine the watershed (Hydrologic Unit Code or HUC 8) for which a site-specific criterion is being developed.
2. Note: Franklin County, Ohio is in two watersheds (05060001 Upper Scioto; 05060002 Lower Scioto), but for this example, it is assumed that a site-specific criterion is being developed for the Upper Scioto 05060001.
3. Click **Location** tab.
4. Click **U.S. Watersheds** from the three choices.
5. Select a **State** (*Ohio*) from the dropdown menu.
   - The county dropdown will be populated with watersheds in the selected state.
6. Select a **Watershed** (*Upper Scioto, 05060001*) from the dropdown menu.
7. Click **Update Criteria** [use the button in the "Your Current Plant/Animal Search Criteria" box].
8. Click **Name** tab.
9. In the **And/Or Search by Group** field enter "mussel."
10. Click **Next.**
11. Click **Search Now** to retrieve all the elements that match the criteria.
12. View the results.
13. Repeat steps 11-17 from the *County Level Search* above to download the additional data.
14.

Results Screen for this Query (cropped)



Based on these results, there are 32 possible mussel species present in the Upper Scioto Watershed with survey data that are reported at the watershed level.

Appendix B: Additional Resources

In addition to the list of cited references above, this list of additional resources is provided for informational purposes. These lists of references and resources represent some of the information that EPA compiled during its information collection efforts. They do not represent an exhaustive list of available information on conducting mussel studies.

Sources of Available Data and Information

- Connecticut Natural Diversity Database (http://www.ct.gov/deep/cwp/view.asp?A=2702&Q=323464)
- Delaware Natural Heritage and Endangered Species Program (http://www.dnrec.delaware.gov/fw/nhesp/pages/default.aspx)
- Georgia Department of Natural Resources, State Mollusk Database (http://georgiawildlife.com/node/1284)
- Freshwater Mollusk Conservation Center at Virginia Tech (http://fishwild.vt.edu/mussel/)
- Illinois Department of Natural Resources Mussel Database (http://dnr.state.il.us/education/mussels/intro.htm)
- Illinois Natural Heritage Database (http://www.inhs.uiuc.edu/resources/dataresources.html)
- Illinois Natural History Survey Mollusk Collection Database (http://ellipse.inhs.uiuc.edu:591/INHSCollections/mollsearch.html)
- Indiana Department of Environmental Management, Macroinvertebrate Community Assessment Program (http://www.in.gov/idem/4681.htm)
- Indiana Natural Heritage Data Center (http://www.in.gov/dnr/naturepreserve/4746.htm)
- Iowater (http://www.iowater.net/)
- Kansas Department of Health and Environment (http://www.kdheks.gov/)
- Kansas Department of Wildlife, Parks and Tourism (http://www.kdwpt.state.ks.us/)
- Kentucky Department of Fish and Wildlife Resources, Center for Mollusk Conservation (http://fw.ky.gov/app2/navigation.aspx?cid=329&navpath=c741c753c755c103c325)

- Louisiana Natural Heritage Program
(http://www.wlf.louisiana.gov/wildlife/louisiana-natural-heritage-program)

- Maryland Biological Stream Survey
(http://www.dnr.state.md.us/streams/MBSS.asp)

- Maryland Natural Heritage Program
(http://dnr.maryland.gov/wildlife/plants_wildlife/nhpintro.asp)

- Missouri Stream Team
(http://www.mostreamteam.org/)

- Montana Natural Heritage Program
(http://mtnhp.org/)

- Natural Heritage New Mexico, Museum of Southwestern Biology, University of New Mexico
(http://www.msb.unm.edu/)

- NatureServe© Explorer
(http://www.natureserve.org/explorer/)

- New Jersey Endangered and Nongame Species Program, Biotics Database
(http://www.conservewildlifenj.org/protecting/mapping/)

- Ohio State University Bivalve Database
(http://www.biosci.ohio-state.edu/~molluscs/OSUM2/)

- Partnership for the Delaware Estuary
(http://www.delawareestuary.org/)

- South Dakota Natural Heritage Program Database
(http://gfp.sd.gov/wildlife/management/diversity/)

- Tennessee Valley Authority
(http://www.tva.gov/)

- The Pacific Northwest Native Freshwater Mussel Working Group (Washington, Oregon, California, Idaho and Montana)
(http://www.fws.gov/columbiariver/musselwg.htm)

- U.S. Army Corp of Engineers Website on Mussel Surveys
(http://el.erdc.usace.army.mil/mussels/index.html)

- U.S. Fish and Wildlife Service
(http://www.fws.gov/)

- Virginia Department of Game and Inland Fisheries
(http://www.dgif.virginia.gov/)

- Virginia Natural Heritage Resources Database (http://www.dcr.virginia.gov/natural_heritage/dbsearchtool.shtml)

- West Virginia Department of Environmental Protection (http://www.dep.wv.gov/Pages/default.aspx)

- West Virginia Department of Natural Resources (http://www.wvdnr.gov/)

- West Virginia Division of Highways (http://www.transportation.wv.gov/highways/Pages/default.aspx)

- Wisconsin Department of Natural Resources, Bureau of Endangered Resources Mussel Database (http://dnr.wi.gov/news/features/feature.asp?id=2&article=9)

- Wisconsin Natural Heritage Inventory (http://dnr.wi.gov/topic/NHI/)

- Wyoming Natural Diversity Database (http://www.uwyo.edu/wyndd/)

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Appendix C: Example Surveys

The following examples describe the methodologies that have been employed in waterbodies of various sizes including wadeable rivers, large areas in mid-sized streams, and large rivers.

Wadeable Rivers

Source: WDNR 2005

These guidelines provide an example of a standardized mussel sampling and reporting protocol for wadeable (i.e., less than1.2 meters (m) deep) rivers and streams as well as the wadeable portions of large rivers. This protocol was developed to answer the following study questions: are mussels present, which species are present, and what is the relationship between mussel density and habitat? Three different protocols are defined in the document and correspond to the three study objectives. The protocol highlighted below pertains only to the first objective: are mussels present or absent? This protocol is designed for cases/situations when resources (e.g., time and manpower) are limited and represents a minimum effort. The protocol was developed and funded through a joint project between the Wisconsin Department of Transportation and the Wisconsin Department of Natural Resources (WDNR). The data generated using these protocols are expected to provide a baseline of mussel distribution data in Wisconsin as part of the Wisconsin Mussel Atlas and in conjunction with WDNR basin surveys.

Before any surveying/fieldwork is performed, a record search is conducted to determine if any historical or other data exist for the particular site or basin. The information is used to develop an initial list of mussel species that may be present in the waterbody. Any endangered or threatened species or species of special concern is noted as well as their general habitat preferences. Importantly, the historical data collected are to be used only to determine possible species presence and not as an indicator of species absence. Potential sources of information include previous field surveys, natural heritage program databases, museum records, and other available literature including the results of mussel and/or benthic macroinvertebrate surveys reported in the gray literature.

The sampling design for the presence/absence determination protocol is informal. Sites (station locations) are selected on the basis that they are representative of available habitat within the sampling reach and are located reasonably far enough away from permanent structures (bridges, dams, etc.) such that the structures are not likely to affect mussel distribution (unless the study objective is to evaluate those particular sites). The number of station locations should be sufficient to give adequate longitudinal coverage of the selected stream reach, with the specific locations chosen to maximize the available stream habitat and spatial resolution of mussel distribution. Caution is advised when establishing sampling stations to avoid investigator bias toward particular habitat types because mussels can often be found in unexpected habitats. The authors note that for streams with well-developed pool riffle structures, each sampling station should be located at the base of a riffle. The authors also note that mussel species richness and density are often higher at the head and base of riffle areas and in moderate run habitat with stable mixed substrates.

Each site should be sampled for a minimum of 1 hour or until a maximum distance of 200 m is reached in streams less than 7 m wide or 300 m is reached in streams greater than 7 m wide. When tactile searches are necessary due to high turbidity, search time should be limited to one hour. When the search time limit is reached, the amount of stream distance sampled should also be noted. The authors note that the probability of detecting a mussel species during a timed search varies greatly depending upon the species, field conditions, collector experience, and length of search time. Typically the largest and most visible mussels are collected while the small species, juvenile, buried, and cryptic mussels are often overlooked.

The sampling method utilized is a relatively rapid visual search. The search team should consist of two people equipped with a mask and snorkel. Each individual should select a shoreline and search in an upstream manner quartering back and forth towards the center of the stream beginning at each station location. Visual searches should also include a tactile (hand grabbing) component, making sure to sweep hands back and forth while sifting through substrate. The authors note that the use of waders in shallow streams may limit the observers' ability to conduct additional tactile searching. In sand flats, the use of mask and snorkel may not be necessary, especially if the substrate is clearly visible. The use of the tactile search in deeper water should be conducted randomly while progressing upstream. In this particular protocol it is noted that small streams less than four m wide may be surveyed by only one person.

Information should be recorded from each survey including, but not limited to, the following:

- Location information – waterbody identification, stream name, site mile, date, collectors, county, township, GPS coordinates, nearest road/access, and site map.
- Water characteristics – time, water level, air temperature, water temperature, conductivity, turbidity, clarity, visibility, and gradient (flow).
- Sampling strategy – sampling method, search times, area searched, bank (i.e., right or left), mussel presence, and distance to live mussels.
- Habitat description – stream widths, habitat description, macrohabitat (e.g., pools, runs, riffles, rapids), substrate (e.g., detritus, clay, silt, sand), and artificial bank structures.

This protocol specifically states that surveys should only be conducted between mid-June and late September because, during this time, stream levels are near base flows, water temperatures are near maximum, and mussels are active. Additionally it is recommended that an experienced malacologist and experienced collectors design the survey and be on site at the time of sampling. Mussel sampling is strongly influenced by collector experience, and therefore, it is recommended that experienced field crews be used to collect mussel data. Experienced collectors are often able to collect a greater number of individuals and species, especially small and cryptic colored specimens, when compared to inexperienced collectors.

Large Area: Mid-sized Streams

Source: Smith et al. 2001

This document provides an example of a mussel survey conducted by the U.S. Geological Survey for biological assessments of the effects of a series of bridge replacements on the Allegheny River for two federally-listed mussel species. The protocol provides an example of

combining qualitative sampling to determine presence and quantitative sampling to determine density for rare species over large areas.

The survey protocol involves three steps: delineating areas of direct and indirect effects of construction, qualitative sampling in areas of direct and indirect effects, and quantitative sampling in areas of direct effects. Direct effects include mortality, displacement, or interference with growth or reproduction caused during or shortly after construction activities. Indirect effects include scouring, sedimentation, and pooling due to construction related changes to river flow.

The total study area was 56,250 square meters (m^2), with a direct effects area of 18,600 m^2 and an indirect effects area of 37,650 m^2. The direct effects areas extended 50 m upstream and 50 m downstream of the existing bridge, and the indirect effects areas were 50-100 m upstream and 50-200 m downstream of the bridge. These areas were based on similar projects of the same size as well as the fact that preliminary engineering plans involved constructing a causeway and dropping the existing bridge into the river and partially onto the causeway before removal.

For qualitative sampling, a timed search was performed with a target search rate of 0.5 m^2/minute. The study area was divided into 24 cells, where 18 cells were 50 m x 50 m and 6 cells were 50 m long and of variable widths. Search times were prorated for cells that were less than 50 m x 50 m. Three teams of four observers were deployed, which allowed three cells to be sampled simultaneously. Each observer spent 60 minutes searching within ¼ of the cell, so the total search time per cell was 240 minutes. Each cell was sampled by snorkeling in wadeable water (i.e., depths less than one m deep) or SCUBA (i.e., depths greater than one m) with the assumption that the search rate was equal for snorkelers and divers.

A shell midden search was also performed as part of the qualitative analysis. Both banks along the entire study area were searched for middens. All deposits were mapped, and spent valve pairs were counted and identified by species.

Quantitative sampling of the direct effects area consisted of a double sampling design using 0.25-m^2 quadrats that were systematically placed with multiple random starts. A total of 562 quadrats were sampled. Sampling also included excavation of a random subset (183) of the quadrats to a depth of approximately 10 centimeters or to hardpan. The excavated substrate was sifted through a 6.35 millimeter mesh screen. Observers recorded species counts in each quadrat, and surface and buried mussels were recorded separately. After the count, mussels were replaced in the substrate.

The qualitative timed search found 17 species in the direct and indirect effects areas and live mussels in all 24 cells. Few individuals and species were found in areas of fast current. The shell midden search found recent shell material in 11 middens and included 15 species. Quantitative sampling found 14 species with a total density of 2.810 mussels/m^2. A wide range of mussel sizes were observed including some small individuals, which indicated that the two federally-listed species reproduced recently at the study site.

Large River

Source: Clayton et al. 2013

This protocol provides basic mussel survey methodology and guidance for a consistent approach designed to document the potential presence or absence of federally-listed mussel species and protect concentrations of all native mussels within West Virginia. The goal of the document is to provide standardized protocols to address all stream types in West Virginia and the full complement of potential instream activities (e.g., dredging, bridge projects, shoreline protection, outfalls, etc). Protocols are limited to four groups of stream types where the watershed area above the impact point is 2,590 hectares (10 square miles) or larger. The information included below for this example is specific to the protocol for Group 4 streams or large rivers where federally-listed threatened or endangered species are expected. These include the Ohio River downstream of Hannibal lock and dam, Little Kanawha River (slackwater section adjoining the Ohio River), and the Kanawha River navigation pools. This protocol is an update of ORVET 2004.

The approach to the survey consists of two phases: a Phase 1 survey using transect or cell searches and, depending on the outcome, a Phase 2 quantitative or intensive qualitative survey, if required.

The objective of a Phase 1 survey is to determine if a diverse mussel community is present and to delineate the area with a mussel concentration. The survey design consists of a visual search of transects, 1 m in width, spaced no more than 100 m apart, placed perpendicular to stream flow or cells not to exceed 10 m by 10 m in size. A visual search includes moving cobble and woody debris; hand sweeping away silt, sand and/or small detritus; and disturbing/probing the upper five centimeters (two inches) of substrate in order to better view the mussels which may be there. A minimum of one minute/m^2 of visual searching is expended in each segment of heterogeneous substrate. To develop a species richness curve, additional searches in 5- to 10-minute increments are conducted in areas with mussel concentrations until at least 6 samples are collected without an addition of new species. Any potential federally-listed species should be brought to the surface for identification.

This protocol also advocates the need for including buffer zones when mussel populations are found. In the case of disturbances from outfalls, the recommended upstream buffer zone is 10 m, the downstream buffer zone is 100 m beyond the mixing zone, and there is a 10 m lateral buffer zone. Data are compiled from transects established in each of three distinct areas separately. Data are recorded by 10 m segments along the transect or by cell position. Mussels observed along the transect or within a cell are recorded as occurring in a particular segment or cell. Appropriate information describing the depth and habitat conditions along each transect and within each cell (e.g., depositional areas, silt, mud, detritus, hard-pan, sand, and scoured areas where mussels cannot burrow, gravel, cobble, etc.) is recorded for each segment or cell. If a trigger is met and avoidance is not an option, then a Phase 2 survey is required. Survey results that trigger avoidance of a Phase 2 survey include the following:

- Five individuals per 10 m segment in any area of the survey.

- Presence of at least three species that are not among the nine in Group 4 streams that can be excluded in defining a diverse mussel concentration along any one transect or within a qualitative survey conducted between transects.

The objective of Phase 2 is to collect sufficient data to determine if federally-listed mussel species are likely to be present within the mussel concentration defined in Phase 1. The Phase 2 survey within a Group 4 stream consists of more intensive qualitative surveys as described by Smith (2006). This requires an additional percentage of area to be surveyed, which is accomplished by adding additional transects between and around transects meeting trigger(s). The area meeting the trigger criteria is delineated, and the amount of additional survey effort is calculated using the criteria and formula from Smith (2006). Criteria to be used in calculations are as follows:

- Expected density 0.01.
- Ohio River downstream of Willow Island Dam and Kanawha River upstream of Elk River, minimum 90 % probability of detection.
- Ohio River, Willow Island Pool, Kanawha River downstream of Elk River, and Little Kanawha River slack water, 75 % probability of detection.
- Search efficiency 0.4.

There are a number of other considerations noted in the protocol. Surveying can only occur from May 1 to October 1. Any other time frame must be pre-approved and may require another survey protocol. A minimum visibility requirement is also in place; visibility must be at least 0.5 m (approximately 20 inches) with or without lights at the depth of the survey. The survey must note the actual visibility on the day of the survey rather than just indicating that the minimum requirement was met. If the visibility does not meet the requirement, the survey is either rescheduled or performed using a different sampling method in consultation with the appropriate state or federal agencies.

Appendix D: Examples of Typical Survey Data

The types of data collected during a mussel survey are dependent on the study objective(s) and the sampling approach, design, and method. Some of the data generally collected and useful for developing site-specific criteria using the Recalculation Procedure may include the following items:

- Taxonomic identification (e.g., Order, Family, Genus and Species).
- Date of survey.
- Number of live individuals and number of spent shells by condition category (shells often are sorted into subfossil, weathered, and recent categories).
- Threatened or endangered status.
- Length of the segment survey, type, GPS coordinates.
- Time spent on the search.
- Water quality/physical parameters (e.g., dissolved oxygen, pH, temperature, water current speed, turbidity/visibility, water depth, weather conditions, etc.).
- Habitat type and substrate.
- Catch per unit effort/search effort.
- Estimated viability/threats.

In line with some of the above items, the following presents example field survey forms from Carlson et al. 2008 and Barbour et al. 1999 that surveyors might use to characterize the site conditions on the day of the survey including location of the site, water quality parameters, physical habitat features, and the sampling method used (e.g., tactile only, tactile with snorkel, tactile with SCUBA):

Site Number: Field Number:		Time Beg:	Date:
Watershed/Drainage:		End:	State:
Waterbody:		Latitude:	Long:
Location:		Stream Order:	Stream Type:
Gage Station:		Surveyor(s):	

Determining PSA	Distance upstream: Distance downstream:	**Survey Technique**	Tactile Only ☐ Tactile With Snorkel ☐ Tactile With SCUBA ☐

Instream Features Quantitative

Please specify all units of measurement

% Canopy Cover: _____ **Wetted Width:**_____

Surface Velocity (at thalweg):_____

Water Depth (at thalweg):_____

Bank Height (rt/lt*):_____ **Bank Angle(rt/lt*):**_____

Instream Features Qualitative

Channel Alteration: ☐ No ☐ Yes

Describe:

Shoring Structures: ☐ None ☐ Limerock ☐ Gabion

☐ Concrete ☐ Rip-rap ☐ Other:_____ **Extent:**

Substrate composition (% est.): Gravel___ Silt ____ Clay_____

Clay Marl____ Fine sand____ Coarse s. _____ Medium s. _____

Boulder ___ Bedrock ___ Cobble ___

Water Quality

Water Temp: ____ ℃

Dissolved Oxygen: ____mg/L

Conductivity _____

pH ____ **Other:**

303d Listed: ☐ yes ☐ no

Designated Use:

Violated Criteria:

Water Clarity

☐ Clear
☐ Slightly turbid
☐ Turbid
☐ Opaque

Heavy Rain in past 7 days: Yes ☐ No ☐

Air Temperature: _____ Est. ☐ Act. ☐

Survey Weather Conditions: Scattered showers ☐

Heavy rain ☐ Clear/sunny ☐

Steady rain ☐ % Cloud cover____

Channel Stability (Check one box for each column):

	Deposition/Aggradation	Incision/Degradation
Excellent	Large, fresh deposits *absent* High number of deep pools ☐	No mass-wasting or significant erosion of banks Channel slightly entrenched High number of deep pools ☐
Good	Large, fresh deposits *uncommon* Moderate number of deep pools ☐	Some bank erosion apparent, no mass wasting Channel slightly-moderately entrenched Moderate number of deep pools ☐
Fair	Large, fresh deposits *common* Low-moderate number of deep pools ☐	Active bank erosion, potential mass-wasting Channel moderately-highly entrenched Low-moderate number of deep pools ☐
Poor	Large, fresh deposits *very common* Few, if any, deep pools ☐	Active bank erosion, frequent mass-wasting Channel moderately-highly entrenched Few, if any, deep pools ☐

Impoundments:

☐ None ☐ yes (Describe):

Fish Passage: Blocked?

☐ yes
☐ no

Describe:

Fish Presence:

☐ Absent
☐ Rare
☐ Common
☐ Abundant

Woody Material:

☐ None/infreq.
☐ Moderate
☐ Extensive

Riparian Features Quantitative

Rt* Buffer width(ft):

☐ 10-25
☐ 25-75
☐ 78-150
☐ 150+

Lt* Buffer width(ft):

☐ 10-25
☐ 25-75
☐ 78-150
☐ 150+

Landuse Characterization:

(100 feet to either side of the stream)

	Rt Bk	Lt Bk	
Natural Forest			%
Silviculture			%
Pasture			%
Agricultural			%
Residential			%
Commercial			%
Industrial			%

Site Road Crossing

Road Type: ☐ Paved ☐ Unpaved

Name (if known): _____

Crossing Type: ☐ Pipe culvert ☐ Box culvert ☐ Bridge ☐ Paved box culvert

Riparian Features Qual.

Local Non-Point Source Pollution Potential:

☐ No evidence ☐ Slight
☐ Moderate potential ☐ Obvious sources
☐ Livestock access

Describe:

Floodplain Access:

	Rt*	Lt*
None	☐	☐
Partial	☐	☐
Full	☐	☐

Bank Erosion:

☐ Non-eroding
☐ Active Erosion
☐ Mass-wasting

Notes

Source: Carlson et al. 2008

BENTHIC MACROINVERTEBRATE FIELD DATA SHEET

STREAM NAME	LOCATION	
STATION #_____ RIVERMILE_____	STREAM CLASS	
LAT _____ LONG _____	RIVER BASIN	
STORET #	AGENCY	
INVESTIGATORS		LOT NUMBER
FORM COMPLETED BY	DATE _____ TIME _____ AM PM	REASON FOR SURVEY

HABITAT TYPES	**Indicate the percentage of each habitat type present** ❑ Cobble_____% ❑ Snags_____% ❑ Vegetated Banks_____% ❑ Sand_____% ❑ Submerged Macrophytes_____% ❑ Other ( )_____%
SAMPLE COLLECTION	**Gear used** ❑ D-frame ❑ kick-net ❑ Other _____ **How were the samples collected?** ❑ wading ❑ from bank ❑ from boat **Indicate the number of jabs/kicks taken in each habitat type.** ❑ Cobble_____ ❑ Snags_____ ❑ Vegetated Banks_____ ❑ Sand_____ ❑ Submerged Macrophytes_____ ❑ Other ( )_____
GENERAL COMMENTS	

QUALITATIVE LISTING OF AQUATIC BIOTA
Indicate estimated abundance: 0 = Absent/Not Observed, 1 = Rare, 2 = Common, 3= Abundant, 4 = Dominant

Periphyton	0	1	2	3	4	Slimes	0	1	2	3	4
Filamentous Algae	0	1	2	3	4	Macroinvertebrates	0	1	2	3	4
Macrophytes	0	1	2	3	4	Fish	0	1	2	3	4

FIELD OBSERVATIONS OF MACROBENTHOS
Indicate estimated abundance: 0 = Absent/Not Observed, 1 = Rare (1-3 organisms), 2 = Common (3-9 organisms), 3= Abundant (>10 organisms), 4 = Dominant (>50 organisms)

Porifera	0	1	2	3	4	Anisoptera	0	1	2	3	4	Chironomidae	0	1	2	3	4
Hydrozoa	0	1	2	3	4	Zygoptera	0	1	2	3	4	Ephemeroptera	0	1	2	3	4
Platyhelminthes	0	1	2	3	4	Hemiptera	0	1	2	3	4	Trichoptera	0	1	2	3	4
Turbellaria	0	1	2	3	4	Coleoptera	0	1	2	3	4	Other	0	1	2	3	4
Hirudinea	0	1	2	3	4	Lepidoptera	0	1	2	3	4						
Oligochaeta	0	1	2	3	4	Sialidae	0	1	2	3	4						
Isopoda	0	1	2	3	4	Corydalidae	0	1	2	3	4						
Amphipoda	0	1	2	3	4	Tipulidae	0	1	2	3	4						
Decapoda	0	1	2	3	4	Empididae	0	1	2	3	4						
Gastropoda	0	1	2	3	4	Simuliidae	0	1	2	3	4						
Bivalvia	0	1	2	3	4	Tabinidae	0	1	2	3	4						
						Culcidae	0	1	2	3	4						

Source: Barbour et al. 1999