APPENDIX L

SURVEY AND OTHER PROTOCOLS

TABLE OF CONTENTS

Attachment L-1

Reserved

Attachment L-2

Indiana Bat Habitat Assessment Protocols

Attachment L-3

Determination of Potential Winter Habitat for Indiana Bats

Attachment L-4

Indiana Bat Survey Protocols

Attachment L-5

Service Guidelines for Bog Turtle Surveys April 2006

Attachment L-6

Bog Turtle Pre-construction Survey Protocol

Attachment L-7

Bog Turtle Recovery Plan - Appendix A April 18, 2001

Attachment L-8

Karst Monitoring Protocols

Attachment L-9

Figure 6.2.3.3_2 Sinkhole Mitigation Procedures

Attachment L-10

Figure 6.2.3.3_3 Sinkhole Mitigation Procedures

Attachment L-11

Figure 6.2.3.3_4 Sinkhole Mitigation Procedures

Attachment L-12

Figure 6.2.3.3_5 Sinkhole Mitigation Procedures

Attachment L-13

WV Sinkhole Mitigation Guidance

Attachment L-14

Herbicide List for use in Madison Cave Isopod Habitat

Attachment L-15

Mussel Survey Protocols

Attachment L-16

Mussel Relocation Mark/Recapture Protocols

Attachment L-17

Sediment Transport Estimation Method

Attachment L-18

Disinfection Techniques

Attachment L-19

Riparian Restoration Standard

Attachment L-20

NRCS Standards for Water Quality
and
Riparian Corridors

Attachment L-21

Nashville Crayfish Survey Protocols

Attachment L-22

American Burying Beetle Survey Guidance
May 20, 2009

Attachment L-23

American Burying Beetle Baiting Away Guidance
May 20, 2009

Attachment L-24

American Burying Beetle Trapping And Relocating Guidance May 20, 2009

Appendix L-2

Indiana Bat Habitat Assessment Protocols

These protocols are currently being prepared and will be included in this MSHCP when available from the Service.

Appendix L-3

Indiana Bat Winter Habitat Assessment Protocols

NiSource personnel or its consultants will determine whether potentially-suitable winter habitat exists within the project area by conducting "Winter Habitat Assessments" as described below. The results of these assessments will be recorded and documented in NiSource's annual compliance report. Results will be valid for two years and can be completed anytime of year. The Winter Habitat Assessment Protocols are:

i. Examine identified potential winter habitat for the following characteristics:

1. The openings should be at least one foot in diameter or larger.
2. The passage should continue beyond the dark zone and not have an obvious end within 40 feet of entrance (Note: This may not be verifiable by surveyor due to safety concerns).
3. Entrances that are flooded or prone to flooding (i.e., debris on ceiling), collapsed, or otherwise inaccessible to bats will be excluded.
4. Openings that have occurred recently (i.e., within the past 12 months) due to creation or subsidence will be excluded. However, a written description and photographs of the opening must be included in the pre-survey report.

Appendix L-4

Indiana Bat Survey Protocols

The current “Indiana Bat Mist Netting Guidelines” provided in Appendix 5 of the 2007 Indiana Bat draft Revised Recovery Plan or future versions of superseding Service-approved guidelines will be applied.

GUIDELINES FOR BOG TURTLE SURVEYS[1]

(revised April 2006)

RATIONALE

A bog turtle survey (when conducted according to these guidelines) is an attempt to determine presence or probable absence of the species; it does not provide sufficient data to determine population size or structure. Following these guidelines will standardize survey procedures. It will help maximize the potential for detection of bog turtles at previously undocumented sites at a minimum acceptable level of effort. Although the detection of bog turtles confirms their presence, failure to detect them does not absolutely confirm their absence (likewise, bog turtles do not occur in all appropriate habitats and many seemingly suitable sites are devoid of the species). Surveys as extensive as outlined below are usually sufficient to detect bog turtles; however, there have been instances in which additional effort was necessary to detect bog turtles, especially when habitat was less than optimum, survey conditions were less than ideal, or turtle densities were low.

PRIOR TO CONDUCTING ANY SURVEYS

If a project is proposed to occur in a county of known bog turtle occurrence (see attachment 1), contact the U.S. Fish and Wildlife Service (Service) and/or the appropriate State wildlife agency (see attachment 2). They will determine whether or not any known bog turtle sites occur in or near the project area, and will determine the need for surveys.

< If a wetland in or near the project area is *known* to support bog turtles, measures must be taken to avoid impacts to the species. The Service and State wildlife agency will work with federal, state and local regulatory agencies, permit applicants, and project proponents to ensure that adverse effects to bog turtles are avoided or minimized.

< If wetlands in or adjacent to the project area are *not* known bog turtle habitat, conduct a bog turtle habitat survey (Phase 1 survey) if:

1. The wetland(s) have an emergent and/or scrub-shrub wetland component, or are forested with suitable soils and hydrology (see below), *and*

2. Direct and indirect adverse effects to the wetland(s) cannot be avoided.

   See *Bog Turtle Conservation Zones*[2] for guidance regarding activities that may affect bog turtles and their habitat. In addition, consult with the Fish and Wildlife Service and/or appropriate State wildlife agency to definitively determine whether or not a Phase 1 survey will be necessary.

---

[1] These guidelines are a modification of those found in the final "Bog Turtle (*Clemmys muhlenbergii*), Northern Population, Recovery Plan" (dated May 15, 2001). Several minor revisions were made to facilitate survey efforts and increase searcher effectiveness. As additional information becomes available regarding survey techniques and effectiveness, these survey guidelines may be updated and revised. Contact the Fish and Wildlife Service or one of the state agencies listed in Attachment 1 for the most recent version of these guidelines.

[2] See Appendix A of the "Bog Turtle (*Clemmys muhlenbergii*), Northern Population, Recovery Plan" (dated May 15, 2001).

BOG TURTLE HABITAT SURVEY (= Phase 1 survey)

The purpose of this survey is to determine whether or not the wetland(s) are *potential* bog turtle habitat. These surveys are performed by a recognized, qualified bog turtle surveyor (contact the Service or the appropriate State wildlife agency to receive a list of recognized, qualified bog turtle surveyors). The following conditions and information apply to habitat surveys.

< Surveys can be performed any month of the year (except when significant snow and/or ice cover is present). This flexibility in conducting Phase 1 surveys allows efforts during the Phase 2 survey window to be spent on wetlands most likely to support bog turtles (*i.e.*, those that meet the criteria below).

< Potential bog turtle habitat is recognized by three criteria *(not all of which may occur in the same portion of a particular wetland*):

1. **Suitable hydrology**. Bog turtle wetlands are typically spring-fed with shallow surface water or saturated soils present year-round, although in summer the wet area(s) may be restricted to near spring head(s). Typically these wetlands are interspersed with dry and wet pockets. There is often subsurface flow. In addition, shallow rivulets (less than 4 inches deep) or pseudo-rivulets are often present.

2. **Suitable soils**. Usually a bottom substrate of permanently saturated organic or mineral soils. These are often soft, mucky-like soils (this does not refer to a technical soil type); you will usually sink to your ankles (3-5 inches) or deeper in muck, although in degraded wetlands or summers of dry years this may be limited to areas near spring heads or drainage ditches. In some portions of the species' range, the soft substrate consists of scattered pockets of peat instead of muck.

3. **Suitable vegetation**. Dominant vegetation of low grasses and sedges (in emergent wetlands), often with a scrub-shrub wetland component. Common emergent vegetation includes, but is not limited to: tussock sedge (*Carex stricta*), soft rush (*Juncus effusus*), rice cut grass (*Leersia oryzoides*), sensitive fern (*Onoclea sensibilis*), tearthumbs (*Polygonum* spp.), jewelweeds (*Impatiens* spp.), arrowheads (*Saggitaria* spp.), skunk cabbage (*Symplocarpus foetidus*), panic grasses (*Panicum* spp.), other sedges (*Carex* spp.), spike rushes (*Eleocharis* spp.), grass-of-Parnassus (*Parnassia glauca*), shrubby cinquefoil (*Dasiphora fruticosa*), sweet-flag (*Acorus calamus*), and in disturbed sites, reed canary grass (*Phalaris arundinacea*) or purple loosestrife (*Lythrum salicaria*). Common scrub-shrub species include alder (*Alnus spp*.), red maple (*Acer rubrum*), willow (*Salix spp*.), tamarack (*Larix laricina*), and in disturbed sites, multiflora rose (*Rosa multiflora*). Some forested wetland habitats are suitable given hydrology, soils and/or historic land use. These forested wetlands include red maple, tamarack, and cedar swamps.

Suitable hydrology and soils are the critical criteria (*i.e.*, the primary determinants of potentially suitable habitat).

< Suitable hydrology, soils and vegetation are necessary to provide the critical wintering sites (soft muck, peat, burrows, root systems of woody vegetation) and nesting habitats (open

areas with tussocky or hummocky vegetation) for this species. It is very important to note, however, that one or more of these criteria may be absent from portions of a wetland or wetland complex supporting bog turtles. Absence of one or more criteria does not preclude bog turtle use of these areas to meet important life functions, including foraging, shelter and dispersal.

< If these criteria (suitable soils, vegetation and hydrology) are present in the *wetland*, then the *wetland* is considered to be potential bog turtle habitat, regardless of whether or not that portion of the wetland occurring within the project boundaries contains all three criteria. If the *wetland* is determined to be potential habitat and the project will directly or indirectly impact *any portion* of the wetland (see *Bog Turtle Conservation Zones*), then either:

    < Completely avoid all direct and indirect effects to the wetland, in consultation with the Service and appropriate State wildlife agency, OR

    < Conduct a Phase 2 survey to determine the presence of bog turtles.

< The Service and appropriate State wildlife agency (see list) should be sent a copy of survey results for review and comment including: a USGS topographic map indicating location of site; project design map, including location of wetlands and stream and delineation of wetland type (PEM, PSS, PFO, POW) and “designated survey areas”[3]; color photographs of the site; surveyor's name; date of visit; opinion on potential/not potential habitat; a description of the hydrology, soils, and vegetation. A phase 1 report template and field form are available from the States and Service.

BOG TURTLE SURVEY (= Phase 2 survey)

If the wetland(s) are identified as potential bog turtle habitat (see Phase 1 survey), and direct and indirect adverse effects cannot be avoided, conduct a bog turtle survey in accordance with the specifications below. Note that this is *not* a survey to estimate population size or structure; a long-term mark/recapture study would be required for that.

Prior to conducting the survey, contact the appropriate State agency (see attached list) to determine whether or not a scientific collector's permit valid for the location and period of the survey will be required.

The Phase 2 survey will focus on the areas of the wetland that meet the soils, hydrology and vegetation criteria, as defined under the Phase 1 survey guidelines. Those areas that meet the criteria are referred to as “designated survey areas” for Phase 2 and Phase 3 survey purposes.

1. Surveys should only be performed during the period from April 15-June 15. For the Lake Plain Recovery Unit (see Recovery Plan), surveys should only be performed during the period from May 1 to June 30. This coincides with the period of greatest annual turtle activity (spring emergence and breeding) and before vegetation gets too dense to accurately survey. While turtles may be found outside of these dates, a result of no turtles would be

[3] “Designated survey areas” are those areas of the wetland that meet the soils, hydrology and vegetation criteria for potential bog turtle habitat. These areas may occur within the emergent, scrub-shrub or forested parts of the wetland.

considered inconclusive. Surveys beyond June also have a higher likelihood of disruption or destruction of nests or newly hatched young.

2. Ambient air temperature at the surface in the shade should be ≥ 55° F.

3. Surveys should be done during the day, at least one hour after sunrise and no later than one hour before sunset.

4. Surveys may be done when it is sunny or cloudy. In addition, surveys may be conducted during and after light rain, provided air temperatures are ≥ 65° F.

5. At least one surveyor must be a recognized qualified bog turtle surveyor[4], and the others should have some previous experience successfully conducting bog turtle surveys or herpetological surveys in wetlands. To maintain survey effort consistency and increase the probability of encountering turtles, the same surveyors should be used for each wetland.

6. A minimum of four (4) surveys per wetland site are needed to adequately assess the site for presence of bog turtles. <u>At least two of these surveys must be performed in May</u>. From April 15 to April 30, surveys should be separated by six or more days. From May 1 to June 15, surveys should be separated by three or more days. The shorter period between surveys during May and June is needed to ensure that surveys are carried out during the optimum window of time (*i.e*., before wetland vegetation becomes too thick).

   Note that bog turtles are more likely to be encountered by spreading the surveys out over a longer period. For example, erroneous survey results could be obtained if surveys were conducted on four successive days in late April due to possible late spring emergence, or during periods of extreme weather because turtles may be buried in mud and difficult to find.

   Because this is solely a presence/absence survey, survey efforts at a particular wetland may cease once a bog turtle has been found.

7. Survey time should be at least four (4) to six (6) person-hours per acre of designated survey area per visit. Additional survey time may be warranted in wetlands that are difficult to survey or that have high quality potential habitat. The designated survey area includes all areas of the wetland where soft, mucky-like soils are present, regardless of vegetative cover type. This includes emergent, scrub-shrub, and forested areas of the wetland.

   If the cover is too thick to effectively survey using Phase 2 survey techniques alone (*e.g.*, dominated by multiflora rose, reed canary grass, *Phragmites*), contact the Service and State wildlife agency for guidance on Phase 3 survey techniques (trapping) to supplement the Phase 2 effort. In addition, Phase 3 (trapping) surveys may also be warranted if the site is in

[4] Searching for bog turtles and recognizing their habitat is a skill that can take many months or years of field work to develop. This level of expertise is necessary when conducting searches in order to ensure that surveys are effective and turtles are not harmed during the survey (*e.g.*, by stepping on nests). Many individuals that have been recognized as qualified to conduct bog turtle surveys obtained their experience through graduate degree research or employment by a state wildlife agency. Others have spent many years actively surveying for bog turtles as amateur herpetologists or consultants.

the Lake Plain-Prairie Peninsula Recovery Unit. Check with the Service or State wildlife agency for further guidance.

8. Walk quietly through the wetland. Bog turtles will bask on herbaceous vegetation and bare ground, or be half-buried in shallow water or rivulets. Walking noisily through the wetland will often cause the turtles to submerge before they can be observed. Be sure to search areas where turtles may not be visible, including under mats of dead vegetation, shallow pools, underground springs, open mud areas, vole runways and under tussocks. Do not step on the tops of tussocks or hummocks because turtle nests, eggs and nesting microhabitat may be destroyed. Both random opportunistic searching and transect surveys should be used at each wetland.

   The following survey sequence is recommended to optimize detection of bog turtles:

   - Semi-rapid walk through the designated survey area using visual encounter techniques.

   - If no bog turtles are found during visual survey, while walking through site identify highest quality habitat patches. Within these highest quality patches, begin looking under live and dead vegetation using muddling and probing techniques.

   - If still no bog turtles are found, the rest of the designated survey area should be surveyed using visual encounter surveys, muddling and probing techniques.

9. Photo-documentation of each bog turtle located will be required; a macro lens is highly recommended. The photos should be in color and of sufficient detail and clarity to identify the bog turtle to species and individual. Therefore, photographs of the carapace, plastron, and face/neck markings should be taken of each individual turtle. Do not harass the turtle in an attempt to get photos of the face/neck markings; if gently placed on the ground, most turtles will slowly extend their necks if not harassed. If shell notching is conducted, do the photo-documentation after the notching is done.

10. The following information should be collected for each bog turtle: sex, carapace length-straight line and maximum length, carapace width, weight, and details about scars/injuries. Maximum plastron length information should also be collected to differentiate juveniles from adults as well as to obtain additional information on recruitment, growth, and demography.

11. Each bog turtle should be marked (*e.g.*, notched, PIT tagged) in a manner consistent with the requirements of the appropriate State agency and/or Service. Contact the appropriate State wildlife agency prior to conducting the survey to determine what type of marking system, if any, should be used.

12. All bog turtles must be returned to the point of capture as soon as possible on the same day as capture. They should only be held long enough to identify, measure, weigh, and photograph them, during which time their exposure to high temperatures must be avoided. No bog turtles may be removed from the wetland without permission from the Service and appropriate State agency.

13. The Fish and Wildlife Service and appropriate State agency should be sent a copy of survey results for review and concurrence, including the following: dates of site visits; time spent per designated survey area per wetland per visit; names of surveyors; a site map including wetlands and delineations of designated survey areas; a table indicating the size of each wetland, the designated survey area within each wetland, and the survey effort per visit; a description of the wetlands within the project area (*e.g.*, acreage, vegetation, soils, hydrology); an explanation of which wetlands or portions of wetlands were or were not surveyed, and why; survey methodology; weather per visit at beginning and end of survey (air temperature, wind, and precipitation); presence or absence of bog turtles, including number of turtles found and date, and information and measurements specified in item 10 above; and other reptile and amphibian species found and date.

ADDITIONAL SURVEYS / STUDIES

Proper implementation of the Phase 2 survey protocol is usually adequate to determine species presence or probable absence, especially in small wetlands lacking invasive plant species. Additional surveys, however, may be necessary to determine whether or not bog turtles are using a particular wetland, especially if the Phase 2 survey results are negative but the quality and quantity of habitat are good and in a watershed of known occurrence. In this case, additional surveys (Phase 2 and/or Phase 3 (trapping) surveys), possibly extending into the following field season, may be recommended by the Service or appropriate State agency.

If bog turtles are documented to occur at a site, additional surveys/studies may be necessary to characterize the population (*e.g.*, number, density, population structure, recruitment), identify nesting and hibernating areas, and/or identify and assess adverse impacts to the species and its habitat, particularly if project activities are proposed to occur in, or within 300 feet of, wetlands occupied by the species.

Attachment 1

CONTACT AGENCIES - BY STATE

(April 2006)

STATE	FISH AND WILDLIFE SERVICE	STATE AGENCY
Connecticut	U.S. Fish and Wildlife Service New England Field Office 22 Bridge Street, Unit #1 Concord, NH 03301	Department of Environmental Protection Env. & Geographic Information Center 79 Elm Street, Store Floor, Hartford, CT 06106 *(info about presence of bog turtles in or near a project area)* Department of Environmental Protection Wildlife Division, Sixth Floor 79 Elm Street, Store Floor, Hartford, CT 06106 *(to get a Scientific Collectors Permit or determine what type of marking system to use)*
Delaware	U.S. Fish and Wildlife Service Chesapeake Bay Field Office 177 Admiral Cochrane Drive Annapolis, MD 21401	Nongame & Endangered Species Program Delaware Division of Fish and Wildlife 4876 Hay Point Landing Road Smyrna, DE 19977
Maryland	U.S. Fish and Wildlife Service Chesapeake Bay Field Office 177 Admiral Cochrane Drive Annapolis, MD 21401	Maryland Department of Natural Resources Wildlife & Heritage Division PO Box 68, Main Street Wye Mills, MD 21679
Massachusetts	U.S. Fish and Wildlife Service New England Field Office 22 Bridge Street, Unit #1 Concord, NH 03301	Division of Fisheries and Wildlife Dept. Fisheries, Wildlife and Env Law Enforcement Rt. 135 Westboro, MA 01581
New Jersey	U.S. Fish and Wildlife Service New Jersey Field Office 927 North Main Street, Bldg. D-1 Pleasantville, NJ 08232	New Jersey Division of Fish and Wildlife Endangered and Nongame Species Program 143 Van Syckels Road Hampton, NJ 08827
New York	U.S. Fish and Wildlife Service 3817 Luker Road Cortland, NY 13045	New York Natural Heritage Program 625 Broadway, 5th Floor Albany, NY 12233-4757 Phone: (518) 402-8935 *(info about presence of bog turtles in or near a project area)* NYS Department of Environmental Conservation Division of Fish, Wildlife, and Marine Resources Special Licenses Unit 600 Broadway, 5th Floor Albany, NY 12233-4752 *(for endangered species permit applications)*
Pennsylvania	U.S. Fish and Wildlife Service Pennsylvania Field Office 315 South Allen Street, Suite 322 State College, PA 16801	Natural Diversity Section Pennsylvania Fish and Boat Commission 450 Robinson Lane Bellefonte, PA 16823

Attachment 2

BOG TURTLE COUNTIES OF OCCURRENCE OR LIKELY OCCURRENCE[1]

(April 2006)

STATE	COUNTY	
Connecticut	Fairfield	Litchfield
Delaware	New Castle	
Maryland	Baltimore Carroll	Cecil Harford
Massachusetts	Berkshire	
New Jersey	Burlington Gloucester Hunterdon Middlesex Monmouth Morris	Ocean Salem Somerset Sussex Union Warren
New York	Albany Columbia Dutchess Genesee Orange Oswego Putnam	Seneca Sullivan Ulster Wayne Westchester
Pennsylvania	Adams Berks Bucks Chester Cumberland Delaware Franklin	Lancaster Lebanon Lehigh Monroe Montgomery Northampton Schuylkill York

[1] *This list is valid for one year from the date indicated. It may, however, be revised more frequently if new counties of occurrence are documented. Updates to this list are available from the Service upon request.*

APPENDIX L-6

NiSource HCP
Bog Turtle Pre-Construction Survey Protocol
(revised April 1, 2009)

The purpose of a pre-construction survey is to find bog turtles within a proposed work area and move them to a safer location before work begins. Because bog turtles are small, cryptic and shy, it is not likely that all bog turtles will be found within a search area, unless the search area is relatively small and is thoroughly searched. Pre-construction surveys will be most effective in non-mucky[1] areas of the wetland, since turtles cannot escape into firm soils. However, they may still evade surveyors by hiding under dense wetland vegetation or by moving back into areas that have already been searched. While pre-construction surveys are not always effective in avoiding take, they are considering a viable option to reduce take, especially in non-mucky portions of the wetland or in small, isolated mucky areas[2].

Pre-construction surveys will be done by qualified bog turtle surveyors. Lists of qualified surveyors are available from the Fish and Wildlife Service Field Office in the State in which the survey is proposed.

Pre-construction surveys will be done between April 15 and September 15, which approximates the bog turtle active season. Surveys are likely to be most effective between April 15 and May 30, when bog turtles are quite active and wetland vegetation is not as dense as it is later in the growing season.

Survey Methods

1. Clearly mark the full extent of the proposed disturbance area[3].

2. Where wetland vegetation is dense and difficult to search, consider cutting wetland vegetation to a height of approximately 6-8 inches using a mower or weed-eater and raking away this vegetation.

3. Thoroughly search the proposed disturbance area. Visual pre-construction surveys will take anywhere from several hours to a few days, depending upon the size of the area to be searched.

4. If a bog turtle is found in the proposed disturbance area, collect and record appropriate turtle data in accordance with Service and State requirements. Hold the turtle in an appropriate container with 1-2 inches of water in a safe, shaded location on-site until the pre-construction survey is complete for that day.

[1] "Non-mucky" refers to soils that only be probed (e.g., with a blunt tool handle) to a depth of less than 3 inches.
[2] "Mucky" refers to soft, saturated soils that can be probed (e.g., with a blunt tool handle) to a depth of at least 3 inches. In this case, it does NOT refer to a specific wetland soil type(s) or classification.
[3] The disturbance area includes all areas that will be affected by any type of construction or heavy equipment use, including, but not limited to, temporary roads, staging areas, construction areas, temporary fill or stockpiling areas, areas where timber mats will be placed, etc.

5. Immediately following the first pre-construction survey (*i.e.*, the same day), install silt fencing to isolate the work area from the remainder of the wetland (see AMM #). The purpose of this fencing is two-fold – to keep bog turtles from entering the disturbance area, and to keep sediment from entering the undisturbed portion of the wetland.

6. Once silt fencing has been installed, release any bog turtles that were found during the pre-construction survey into appropriate habitat in the same wetland, outside the fenced area.

7. Before beginning any work activities within the fenced area, conduct a second pre-construction survey within the next 1-2 days and move any bog turtles to wetland habitat outside the fenced area.

8. If 2500 ft^2 (*e.g.*, 50 x 50 ft) or more of the proposed disturbance area is “mucky”, conduct at least 10 days of trapping in the fenced area using a trapping density of at least 20 traps/acre to locate bog turtles and remove them from the disturbance area. Trapping will be done by Service-approved surveyors with appropriate permits and authorizations.

9. Once the second pre-construction survey is complete (#7) and the trapping survey is complete (#8), work activity within the fenced area may begin.

10. If the silt fencing is breached during the bog turtle active season (between April 15 and September 15), immediately repair the breach and conduct another pre-construction survey within the fenced area before resuming any work.

11. Within 30 days, provide the Service and appropriate State wildlife agency with copies of all field forms and data sheets documenting bog turtle captures.

APPENDIX A

BOG TURTLE CONSERVATION ZONES

(revised April 18, 2001)

Projects in and adjacent to bog turtle habitat can cause habitat destruction, degradation, and fragmentation. Of critical importance is evaluating the potential direct and indirect effects of activities that occur in or are proposed for upland areas adjacent to bog turtle habitat. Even if the wetland impacts from an activity are avoided (i.e., the activity does not result in encroachment into the wetland), activities in adjacent upland areas can seriously compromise wetland habitat quality, fragment travel corridors, and alter wetland hydrology, thereby adversely affecting bog turtles.

The following bog turtle conservation zones have been designated with the intent of protecting and recovering known bog turtle populations within the northern range of this species. The conservation suggestions for each zone are meant to guide the evaluation of activities that may affect high-potential bog turtle habitat, potential travel corridors, and adjacent upland habitat that may serve to buffer bog turtles from indirect effects. *Nevertheless, it is important to recognize that consultations and project reviews will continue to be conducted on a case-by-case basis, taking into account site- and project-specific characteristics.*

Zone 1

This zone includes the wetland and visible spring seeps occupied by bog turtles. Bog turtles rely upon different portions of the wetland at different times of year to fulfill various needs; therefore, this zone includes the entire wetland (the delineation of which will be scientifically based), not just those portions that have been identified as, or appear to be, optimal for nesting, basking or hibernating. In this zone, bog turtles and their habitat are most vulnerable to disturbance, therefore, the greatest degree of protection is necessary.

Within this zone, the following activities are likely to result in habitat destruction or degradation and should be avoided. These activities (not in priority order) include:

- development (e.g., roads, sewer lines, utility lines, storm water or sedimentation basins, residences, driveways, parking lots, and other structures)
- wetland draining, ditching, tiling, filling, excavation, stream diversion and construction of impoundments
- heavy grazing
- herbicide, pesticide or fertilizer application[1]
- mowing or cutting of vegetation[1]
- mining
- delineation of lot lines (e.g., for development, even if the proposed building or structure will not be in the wetland)

Some activities within this zone may be compatible with bog turtle conservation but warrant careful evaluation on a case-by-case basis:

- light to moderate grazing
- non-motorized recreational use (e.g., hiking, hunting, fishing)

Zone 2

The boundary of this zone extends *at least 300 feet* from the edge of Zone 1 and includes upland areas adjacent to Zone 1. Activities in this zone could indirectly destroy or degrade wetland habitat over the short or long-term, thereby adversely affecting bog turtles. In addition, activities in this zone have the potential to cut off travel corridors between wetlands occupied or likely to be occupied by bog turtles, thereby isolating or dividing populations and increasing the risk of turtles being killed while attempting to disperse. Some of the indirect effects to wetlands resulting from activities in the adjacent uplands include: changes in hydrology (e.g., from roads, detention basins, irrigation, increases in impervious surfaces, sand and gravel mining); degradation of water quality (e.g., due to herbicides, pesticides, oil and salt from various sources including roads, agricultural fields, parking lots and residential developments); acceleration of succession (e.g., from fertilizer runoff); and introduction of exotic plants (e.g., due to soil disturbance and roads). This zone acts as a filter and buffer, preventing or minimizing the effects of land-use activities on bog turtles and their habitat. This zone is also likely to include at least a portion of the groundwater recharge/supply area for the wetland.

Activities that should be avoided in this zone due to their potential for adverse effects to bog turtles and their habitat include:

- development (e.g., roads, sewer lines, utility lines, storm water or sedimentation basins, residences, driveways, parking lots, and other structures)
- mining
- herbicide application[1]
- pesticide or fertilizer application
- farming (with the exception of light to moderate grazing - see below)
- certain types of stream-bank stabilization techniques (e.g., rip-rapping)
- delineation of lot lines (e.g., for development, even if the proposed building or structure will not be in the wetland)

Careful evaluation of proposed activities on a case-by-case basis will reveal the manner in which, and degree to which activities in this zone would affect bog turtles and their habitat. Assuming impacts within Zone 1 have been avoided, evaluation of proposed activities within Zone 2 will often require an assessment of anticipated impacts on wetland hydrology, water quality, and habitat continuity.

Activities that are likely to be compatible with bog turtle conservation but that should be evaluated on a case-by-case basis within this zone include:

- light to moderate grazing
- non-motorized recreational use (e.g., hiking, hunting, fishing)
- mowing or cutting of vegetation

Zone 3

This zone includes upland, wetland, and riparian areas extending either to the geomorphic edge of the drainage basin or at least one-half mile beyond the boundary of Zone 2. Despite the distance from Zone 1, activities in these areas have the potential to adversely affect bog turtles and their habitat. This particularly applies to activities affecting wetlands or streams connected to or contiguous with Zone 1, because these areas may support undocumented occurrences of bog turtles and/or provide travel corridors. In addition, some activities (e.g., roads, groundwater withdrawal, water/stream diversions, mining, impoundments, dams, "pump-and-treat" activities) far beyond Zone 1 have the potential to alter the hydrology of bog turtle habitat, therefore, another purpose of Zone 3 is to protect the ground and surface water recharge zones for bog turtle wetlands. Where the integrity of Zone 2 has been compromised (e.g., through increases in impervious surfaces, heavy grazing, channelization of stormwater runoff), there is also a higher risk of activities in Zone 3 altering the water chemistry of bog turtle wetlands (e.g., via nutrient loading, sedimentation, and contaminants).

Activities occurring in this zone should be carefully assessed in consultation with the Fish and Wildlife Service and/or appropriate State wildlife agency to determine their potential for adverse effects to bog turtles and their habitat. Prior to conducting activities that may directly or indirectly affect wetlands, bog turtles and/or bog turtle habitat surveys should be conducted in accordance with accepted survey guidelines.

---

[1] Except when conducted as part of a bog turtle habitat management plan approved by the Fish and Wildlife Service or State wildlife agency

KARST FEATURE MONITORING PROTOCOLS

The purpose of this section is to establish a standard set of monitoring protocols for karst features occurring within the range of the Madison Cave Isopod (MCI) encompassed by the NiSource Gas Transmission Pipeline Right of Way (ROW) and adjacent areas. The intent of these protocols is to minimize impact to the habitat of the MCI, by preventing the intrusion of unfiltered surface water, contaminants, and sediment into the phreatic aquifer via transport through open surface conduits located in the epikarst stratum.

I. Definitions

1. **Karst Specialist** – A Certified Professional Geologist engaged in the practice of engineering geology (or) a Virginia Registered Professional Engineer engaged in the practice of Geotechnical Engineering, with a minimum of 5 years experience in karst geology and remediation. Practice experience shall be verified by a statement of qualifications (e.g. resume, CV, project experience, etc.).
2. **Cave –** A natural hole in the ground, large enough for human entry is probably the most useful definition. This covers the enormous variety of caves that do occur, but eliminates the many artificial tunnels and galleries incorrectly named caves. The size criterion is arbitrary and subjective, but practical, as it eliminates narrow openings irrelevant to explorers but very significant hydrologically, that may be better referred to as *proto-caves*, *sub-conduits* or *fissures*. A cave may be a single, short length of accessible passage, or an extensive and complex network of tunnels as long as hundreds of kilometers.
3. **Doline; Sinkhole** – A basin- or funnel-shaped hollow or depression in limestone, ranging in diameter from a few meters up to a kilometer and in depth from a few to several hundred meters. Some dolines are gentle grassy hollows or depressions; others are rocky cliff-bounded basins. A distinction may be made by direct solution of the limestone surface zone, (solution dolines), and those formed by collapse over a cave, (collapse dolines), but it is generally not possible to establish the origin of individual examples. Generally referred to as a "sinkhole" in the United States, the term doline is more widely accepted by the international geology community.
4. **Throat –** An opening within a sinkhole leading into the subsurface, too small to qualify as a cave and often called a *proto-cave*, *sub-conduit* or *fissure*. Throats may be "open" (i.e. air-filled or water-filled), or "closed/clogged" (filled with debris including but not limited to: loose-soil; gravel; rock; dead-fall wood or brush; trash).
5. **Parapet** – The outer edge or perimeter of a doline (sinkhole).

6. **Ponor** – 1. Hole or opening in the bottom or side of a depression where a surface stream or lake flows either partially or completely underground into the karst ground-water system. 2. Hole in the bottom or side of a doline through which water passes to or from an underground channel. Also known as a swallow hole.
7. **Solution Cavity** – A natural cavity or depression formed by the dissolution of soluble bedrock, typically not large enough to allow the entry of a human being and therefore not classified as a cave.
8. **Breccia** - Angular fragments of rock commonly, but not inevitably, cemented by finer-grained materials including silica, iron minerals, and calcite to form a new rock. Many fault planes are marked by zones of broken rock, either loose or re-cemented, forming a fault breccia.
9. **Non-Karst Closed Depression** – A natural or non-natural topographic depression that is not formed by karst processes and is not floored by bedrock. Examples include (but are limited to) construction-related soil subsidence, silage pits, farm ponds, scour pools, animal wallows, large animal burrows, and pits created by removal of tree stumps.

II. Inspection Protocols

Pre-Construction Inspection – Prior to the commencement of any earth disturbance activity, the area of the pipeline that will be affected by the planned activities shall be inspected by the karst specialist (KS) as follows:

a. The KS will walk the entire section of the pipeline ROW in the designated work area, and note any suspect karst features including sinkholes, caves, areas of soil subsidence or closed depressions.
b. The locations of any observed features shall be noted on site drawings and flagged for surveying and/or recorded using sub-meter accuracy GPS instrumentation.
c. The KS will issue a report summarizing the findings of the inspection, with specific reference to the potential impact of any observed features to the MCI habitat. Findings shall include an inventory of feature type(s), drainages, potential impact to the feature by the planned activities, and recommendations to limit impacts if they are expected.
d. Features that are considered to have potential impact to the MCI habitat are: caves, sinkholes with throats, ponors, open solution cavities, abandoned wells, and sinking streams. (Note – If a sinkhole throat is filled, the type of fill, i.e. rock, soil, flood debris, etc. shall be described in detail).
e. Features that are not considered to have an impact to the MCI habitat are: soil-bottomed (stable) sinkholes (i.e. no evidence of recent soil raveling or tension cracks along the parapet), karst springs, or non-karst closed depressions.

f. The pre-construction inspection will have a “shelf-life” of 1 year from the day of the inspection. If work does not commence within 1 year, a new inspection must be completed prior to any earth disturbing activities.

g. The pre-construction inspection report shall be delivered to NiSource and the USFWS no later than 1-month after the completion of field survey.

Monitoring of Pre-Identified Features During Construction – Features identified during the pre-construction inspection shall be monitored as follows:

a. If an identified feature with potential impact to the MCI habitat falls within the area designated for earth disturbing activities, the feature will be documented by field location and with photographs, and then assessed for pre-construction remediation by the NiSource Engineering staff with input and guidance to be provided by the KS.

b. If a feature that has potential impact to the MCI falls within the ROW but is not intercepted by the work, that feature shall be monitored during the work by NiSource staff for any changes such as:

   1. soil subsidence
   2. rock collapse
   3. sedimentation
   4. increased surface water infiltration
   5. flooding
   6. clogging

   Or any other changes in morphology or function that might indicate potential impact to the epikarst stratum caused by the work.

c. All features, whether remediated or left in an undisturbed natural state, shall be monitored by NiSource staff for any changes in appearance, drainage, siltation, etc. at 1 year, 2 years, and 5 year intervals after the completion of the earth disturbing activities. If any changes are observed, NiSource staff will report the condition to the KS, who will provide consult on potential impacts to the MCI habitat and remedial actions, accordingly.

Monitoring of Features that are intercepted during Construction – Features that are intercepted during construction shall be monitored as follows:

<u>Level 1 Inspection of Features intercepted during construction</u> – If any feature is intercepted during work activities including drilling, blasting, and excavation or trenching, the NiSource staff will conduct an initial assessment of the feature to determine if further inspection (Level 2) by the KS will be required. Suspect features shall include:

1. Bedrock enclosed conduits or voids;
2. Solution pockets that extend beyond visual examination range (and therefore may be open);
3. Areas of soft soils;
4. Soil voids;
5. Highly fractured bedrock;
6. Areas of breccia enclosed within the surrounding bedrock.

Level 2 Inspection of Features intercepted during construction **–** If any of the aforementioned features are observed during the Level 1 inspection, work will stop within a 100-foot radius of the feature, and then the KS will conduct a Level 2 Inspection as follows:

a. The KS will examine the feature and determine if it has potential impact to the MCI habitat based on potential connectivity with the phreatic aquifer via the epikarst stratum. The choice of characterization methods shall be determined by the KS, and shall include any combination of (but not be limited to):
    1. visual assessment;
    2. electrical resistivity survey;
    3. track drill probes;
    4. infiltration testing;
    5. or other techniques utilized to facilitate subsurface characterization of karst features.
b. If the feature is determined to have potential impact to the MCI habitat, the KS will advise the NiSource Engineering staff regarding appropriate remedial actions.
c. If the feature is determined to not have potential impact to the MCI habitat, work will resume as planned.
d. All features that are intercepted during construction and subsequently remediated shall be located by the NiSource engineering staff surveyors exclusively, and monitored by NiSource staff for any changes in appearance, drainage, siltation, etc. at 1 year, 2 years, and 5 year intervals after the completion of the earth disturbing activities. If any changes are observed, the KS will provide consult on potential impact to the MCI habitat and remedial actions, if necessary.
e. All Level 2 inspections, findings and remedial activity shall be summarized in a report by the KS, to be delivered to NiSource and the USFWS after the completion of the field work.

Monitoring of Features that form during Construction – Features that form during construction shall be monitored as follows:

Level 1 Inspection of Features that form during construction – If any feature forms during work activities including hydro-testing, drilling, blasting, and excavation or trenching, the NiSource staff will conduct an initial assessment of the feature to determine if further inspection (Level 2) by the KS will be required. Suspect features shall include:

1. Sinkholes;
2. Soil subsidence areas;
3. Rock collapses.

This shall apply to any of the above features that may form either within the work area, whether located along the proposed disturbance section or anywhere within the covered lands within a 100-yard radius the work area.

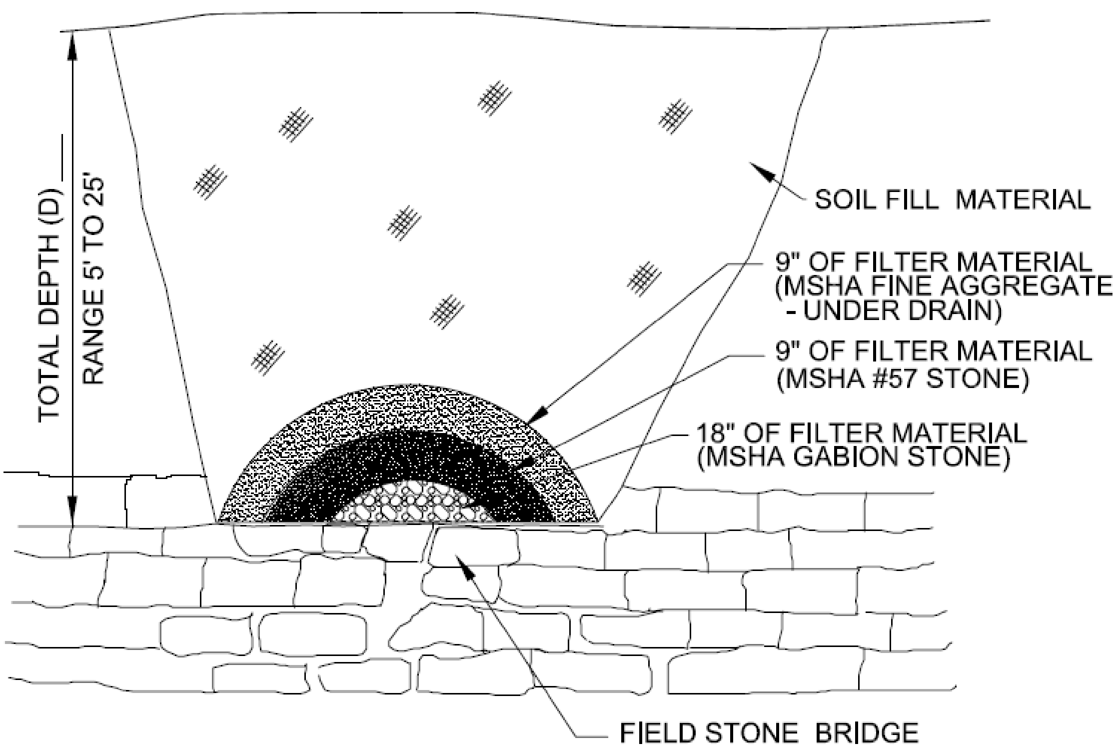
Level 2 Inspection of Features that form during construction **–** If any of the aforementioned features are observed during the Level 1 inspection, work will stop in the area of the feature, and then the KS will conduct a Level 2 Inspection as follows:

a. The KS will examine the feature and determine if it has potential impact to the MCI habitat based on potential hydraulic connectivity with the phreatic aquifer via the epikarst stratum.
b. The choice of characterization methods shall be determined by the KS, and shall include any combination of (but not be limited to):
    1. visual assessment;
    2. electrical resistivity survey;
    3. track drill probes;
    4. infiltration testing;
    5. or other techniques utilized to perform subsurface characterization of karst features.
c. If the feature is determined to have potential impact to the MCI habitat, the KS will consult with the NiSource Engineering staff regarding appropriate remedial actions.
d. If the feature is determined to not have potential impact to the MCI habitat, work will commence as planned.
e. All features that form during construction, whether remediated or left in an undisturbed natural state, shall be located on the site plans by the NiSource engineering staff surveyors, and shall be monitored by NiSource staff for any changes in appearance, drainage, siltation, etc. at 1 year, 2 years, and 5 year intervals after the completion of the earth disturbing activities. If any changes are observed, the KS will provide consult on potential impact to the MCI habitat and remedial actions, if necessary. This

monitoring shall be carried out on all features that form during work activities, regardless of whether they have a potential impact to the MCI habitat or not.[1]

[1]The monitoring of any feature that forms during work is critical, in that there may be no opening to the subsurface (i.e. open throat) when it first forms, but subsequently an opening may appear that will require remedial actions.

MARYLAND STANDARDS FOR AGRICULTURAL BMPS
DETAIL 725-A - SINKHOLE TREATMENT



NOTE:
A NONWOVEN GEOTEXTILE MEETING MSHA CLASS SE MAY BE SUBSTITUTED FOR THE MSHA #57 STONE AND MSHA FINE AGGREGATE.

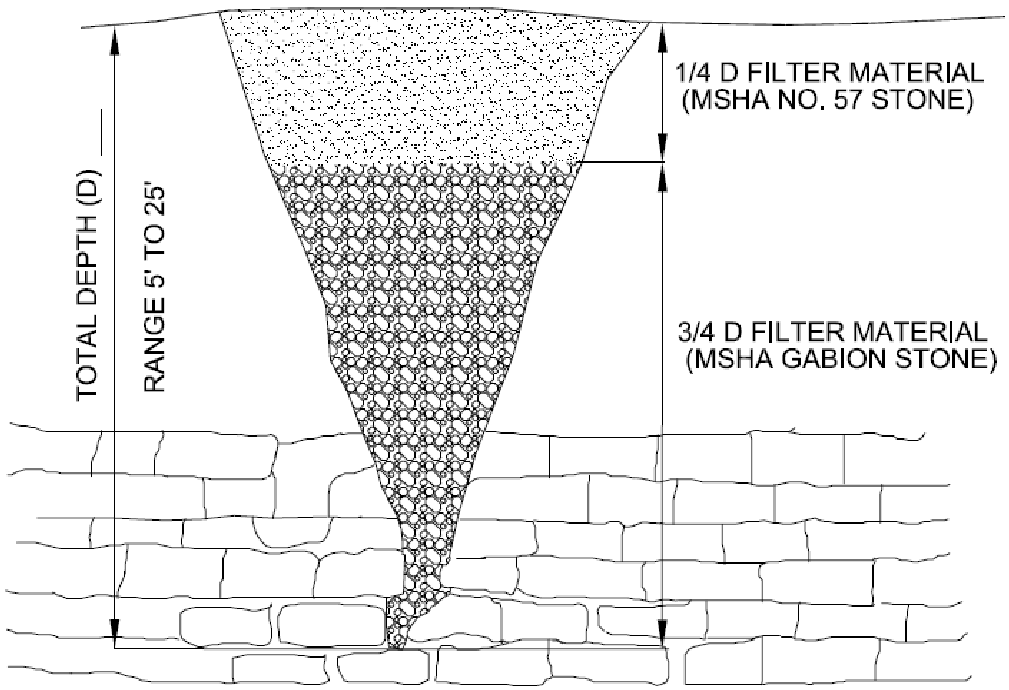
SINKHOLE TREATMENT

(DRAINAGE AREA LESS THAN 5 ACRES)
725-A-INV FILTER.DWG
NTS

U.S.DEPARTMENT OF AGRICULTURE NATURAL RESOURCES CONSERVATION SERVICE MARYLAND	725-A-INV FILTER.DWG 6/03	MARYLAND DEPARTMENT OF AGRICULTURE MARYLAND SOIL CONSERVATION DISTRICTS

Figure 6.2.3.3-2 Sinkhole Mitigation Procedures

MARYLAND STANDARDS FOR AGRICULTURAL BMPS
DETAIL 725-B - SINKHOLE TREATMENT



SINKHOLE TREATMENT

(DRAINAGE AREA 5-15 ACRES)

725-B - INV FILTER.DWG

NTS

U.S.DEPARTMENT OF AGRICULTURE NATURAL RESOURCES CONSERVATION SERVICE MARYLAND	725-B-INV FILTER.DWG 6/03	MARYLAND DEPARTMENT OF AGRICULTURE MARYLAND SOIL CONSERVATION DISTRICTS

Figure 6.2.3.3-3 Sinkhole Mitigation Procedures

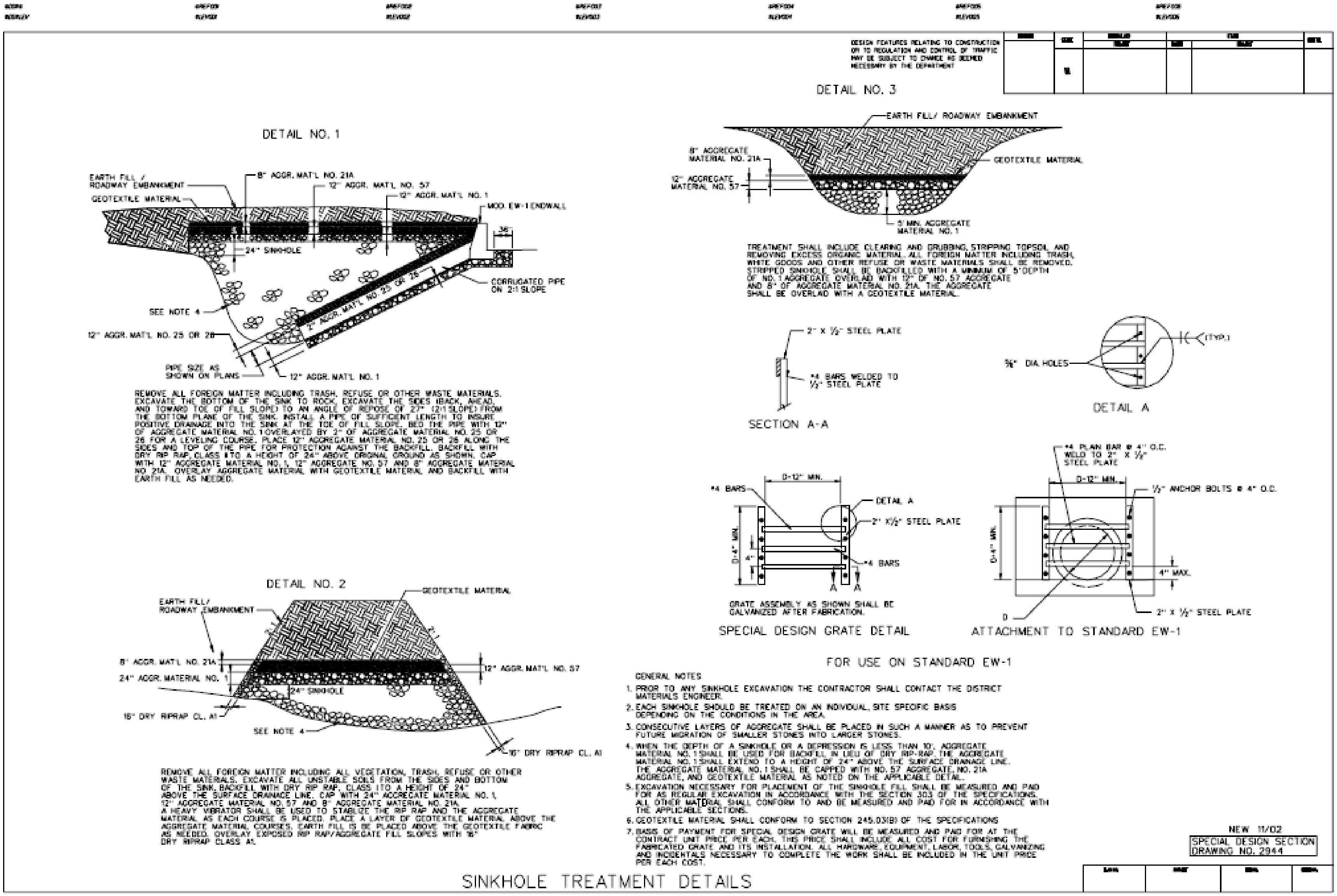


Figure 6.2.3.3-4 Sinkhole Mitigation Procedures

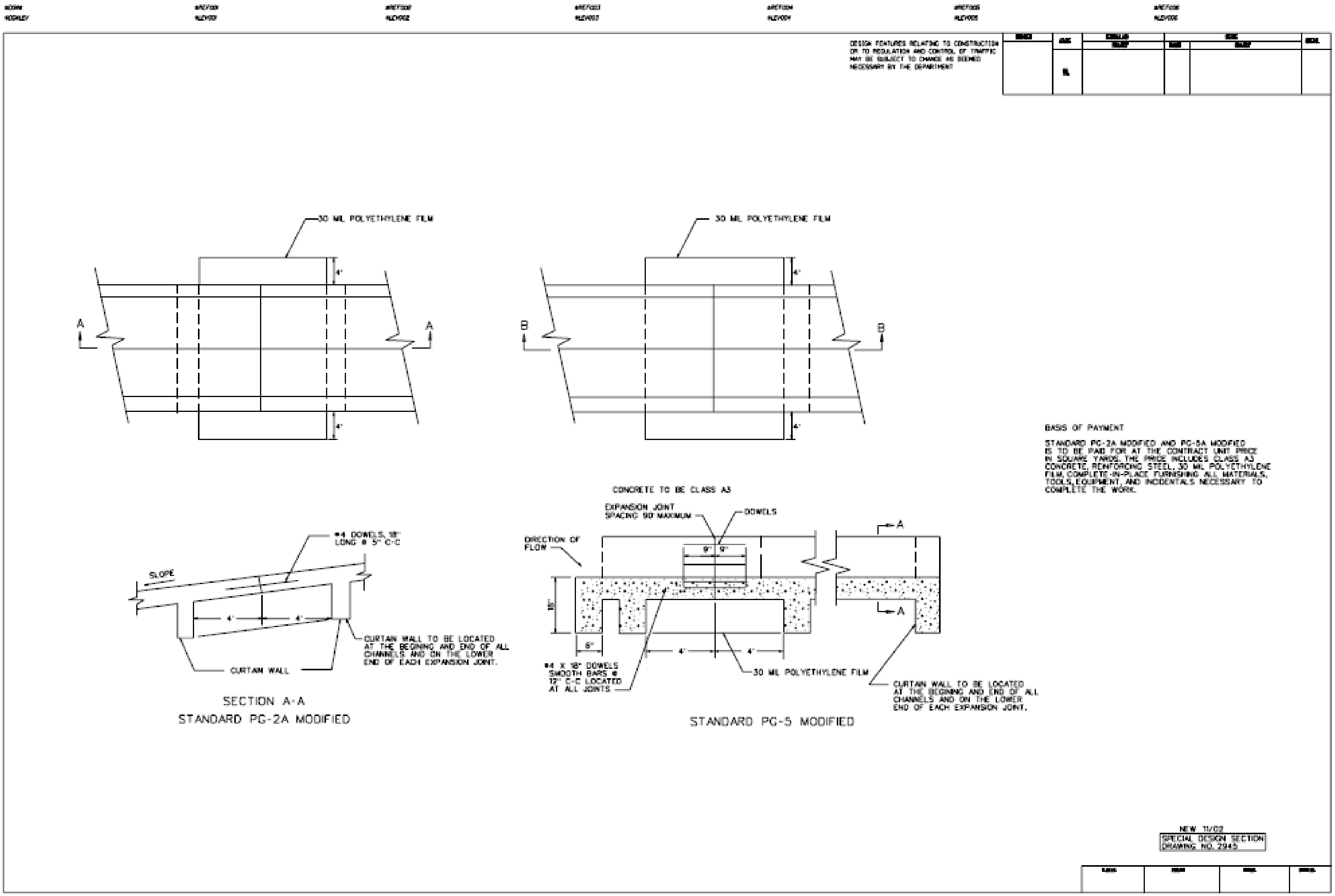


Figure 6.2.3.3-5 Sinkhole Mitigation Procedures

West Virginia Department of Environmental Protection
Division of Water and Waste Management
Groundwater Protection Program

Sinkhole Mitigation Guidance

August 8, 2005

Purpose:

These sinkhole mitigation designs serve to allow the filling of sinkholes while maintaining recharge to the aquifer, reducing potential contamination threats to groundwater, and eliminating safety hazards at sinkhole entries.

General:

Consideration should be given to the method used for removing contaminated materials from sinkholes and reducing or eliminating direct inflow of surface water into sinkholes. Land treatment methods that improve the filtration and infiltration of surface water before it enters the sinkhole should be used along with the mitigation of the sinkhole.

Before selecting a treatment option the following should be considered:

- Land use
- Existing and planned land treatment
- Sinkhole drainage area
- Dimensions of the sinkhole opening
- Safe outlet for diverted surface water
- Environmentally safe disposal of sinkhole "clean out" material
- Availability and quality of filter material
- Safety of equipment and operators and laborers during installation

Treatment selection should be based on the dimensions of the sinkhole drainage area and include direct sinkhole treatment with surface water control measures and filter strips. Whichever treatment option is chosen, it should avoid surface water ponding or the creation of high soil moisture conditions in excess of 72 hours.

Treatment designs apply to sinkholes with excavated depths of 5 to 25 feet and with drainage areas up to 15 acres. Excavations up to 5 feet are sufficient for most sinkholes. Sinkholes with excavation depths of greater than 25 feet or with uncontrolled drainage areas greater than 15 acres may require adjustments to the treatment measure(s) and/or surface water control measure(s). In these cases, geologic and engineering assistance must be obtained and a site-specific treatment design prepared.

Treatment for Sinkholes with Drainage Areas Less than 5 Acres

Treat the sinkhole using the mitigation design in Figure 1 of this guidance document. The treatment site should be inspected after periods of heavy precipitation because some material may run into adjacent sinkhole voids causing a surface depression. In this case, maintenance will include adding soil material at the surface. The existing land use or practice may continue over the treated sinkhole as long as the treatment is maintained.

Treatment for Sinkholes with Drainage Areas of 5 Acres or More and Having a Safe Outlet

The following additional treatment criteria are applicable to sinkholes with drainage areas of 5 acres or more where a safe outlet can be provided to divert surface water away from the sinkhole. A safe outlet is one that does not erode, divert surface water to another sinkhole or injection well, or cause flood damage to crops, property, buildings, or highways/roads.

Surface water control measures should be situated to reduce the internal drainage area around the sinkhole to less than 5 acres. The choice of surface water control measures is generally based on site-specific conditions.

Treatment for Sinkholes with Drainage Areas of 5 to 15 acres and Having No Safe Outlet

Treat the sinkhole using the mitigation design in Figure 2 of this guidance document. The site should be inspected after periods of heavy precipitation because some material may run into adjacent sinkhole voids causing a surface depression. In this case, maintenance will include adding soil material at the surface. The sinkhole should remain as unused land.

Vegetated Buffer Area

A vegetated buffer area should be installed around the sinkhole to improve runoff water quality by filtration and adsorption of contaminants. The vegetated buffer area should be installed within the sinkhole drainage area and should begin at the treated sinkhole.

The minimum width (in feet) of the vegetated buffer area is determined by multiplying the sinkhole drainage area (in acres) by seven. This width should provide beneficial filtering for some distance outside the sinkhole because surface water runoff may be temporarily held before reaching the treated sinkhole.

Appropriate vegetation should be used for the buffer area. Use native vegetation as much as possible. **DO NOT** use noxious plants or weeds. It is recommended that a plant nursery be consulted for the appropriate vegetation.

Acceptable Materials

Engineering fabric - must meet the applicable requirements of AASHTO M-288.

Aggregates – fine aggregates, gravel, or rock rip rap that conforms to the West Virginia Department of Highways, Standard Specifications for Roads and Bridges, Sections 702, 703, and 704.

Specifications

Use the following guidance for installing a mitigation design for sinkholes and sinkhole areas with drainage areas of less than 5 acres:

1. Remove and properly dispose of materials dumped in and around the sinkhole in accordance with applicable federal, state, and local laws.

2. Excavate loose material from the sinkhole and try to expose the solution void(s) in the bottom. Enlarge the sinkhole, as necessary, to allow for installation of the filter material.

3. Select stone that is approximately 1.5 times larger than the solution void(s). Place the stone into the void(s) forming a competent bridge. Stone used for the bridge should have rock strength equal to, at least, moderately hard (*e.g.,* resistant to abrasion or cutting by a knife blade but can be easily dented or broken by light blows with a hammer). Shale or similar soft and non-durable rock is not acceptable.

4. Place a layer of filter material over the bridge to a minimum thickness of 24 inches. Approximately 35 percent of the material should be larger than the opening between the bridge and the void(s). There should be no discernable large openings around the bridge. The material should be either gabion stone, stone for rip rap, or stone for special rock fill that conforms to West Virginia Department of Highways, *Standard Specification Roads and Bridges*, Section 704.

5. Place a layer of smaller size filter material over the previous layer to a minimum thickness of 10 inches. The size of the material should be ¼ to ½ the size of that used in the previous layer. The material should be No. 57 aggregate, which conforms to West Virginia Department of Highways, *Standard Specifications Roads and Bridges*, Sections 703.1.1, 703.1.2, 703.1.3, 704.1.4, and 703.2.1. Unacceptable filter material consists of pea gravel or slags (steel, electromagnetic, or power plant).

6. Place a layer of sand-sized filter material over the previous layer ~~at~~ to a minimum thickness of 10 inches. The sand must be compatible in size with the previous layer to prevent piping. The material should be fine aggregate that conforms to West Virginia Department of Highways, *Standard Specification Roads and Bridges*, Sections 702.1.1, 702.1.2, and 702.1.3.

7. Engineering fabric conforming to AASHTO M 288 may be substituted for the stone and sand filter materials discussed in 5 and 6.

8. Backfill over the top filter layer or engineering fabric with soil material to the surface. This should be mineral soil with at least 12 percent fines. Reuse soil material excavated from the sinkhole as much as possible and place any available topsoil over the backfill. Overfill by about 5 percent to allow for settling.

9. Establish vegetation on the mitigated sinkhole and other disturbed areas of the site.

Use the following guidance for installing a mitigation design for sinkholes and sinkhole areas with drainage areas of 5 to 15 acres:

1. Remove and properly dispose of materials dumped in and around the sinkhole.

2. Excavate loose material from the sinkhole.

3. Place a layer of filter material into the sinkhole, allowing the stone to fill the void(s) below the bottom of excavated sinkhole. The size should be ¼ to ½ the size of the void(s). This material can be WVDOH gabion stone, rip rap stone, or special rock fill stone.

4. Place a layer of the same size filter material to a thickness of about ¾ TD (TD = total depth) above the sinkhole bottom.

5. Place a layer of smaller size filter material over the previous layer to a thickness of about ¼ D. Bring this layer to surface level. The size should be ¼ to ½ the size of the previous layer. The material should be No. 57 aggregate, which conforms to West Virginia Department of Highways, *Standard Specification Roads and Bridges*, Sections 703.1.1, 703.1.2, 703.1.3, 703.2.1, and 704.1.4. Unacceptable stone consists of pea gravel or slags (steel, electrometallurgical, or power plant).

6. Shale or similar soft and non-durable rock is not acceptable.

7. Establish vegetation on the mitigated sinkhole and disturbed areas of the site.

Engineering Fabric Requirements for Subsurface Drainage

Engineering fabric used in the mitigation of sinkholes should meet the applicable requirements of AASTHO M 288, Section 7.2

Engineering Fabric Installation

Proper construction and installation techniques are essential to ensure that the intended function of the engineering fabric is fulfilled.

When sewn seams are necessary, the seam strength must be equal to or greater than 90 percent of the specified grab strength, as measured in accordance with ASTM D 4632.

When sewn seams are used for the seaming of the engineering fabric, the thread must be high strength polypropylene, or polyester. Nylon thread is unacceptable.

For Sinkhole Mitigation Design A, place the engineering fabric loosely, with no wrinkles or folds, and with no void spaces between the fabric and the bridge. Overlap successive sheets of engineering fabric a minimum of 12 inches, with the upstream sheet overlapping the downstream sheet.

Prior to covering, the engineering fabric should be inspected to ensure that it has not been damaged (*e.g.* holes, tears, rips) during installation. An engineer or the engineer's designated representative should conduct the inspection. The designated representative should be a certified field inspector.

Damaged fabric must be repaired immediately. Cover the damaged area with an engineered fabric patch that overlaps to 12 inches beyond the damaged area.

Any damaged engineering fabric that cannot be repaired shall be replaced as directed by the engineer.

Place material over the engineering fabric in such a manner as to avoid stretching and subsequently tearing the fabric. Do not drop stone and soil placement from a height greater then one meter. Do not allow stone with a mass of more than 100 kg to roll down the slope of the sinkhole.

Grading the sinkhole slope is not permitted if the grading will result in the movement of the stone directly above the engineering fabric.

Operation and Maintenance

The owner/operator is responsible for maintaining the mitigated sinkhole and sinkhole area. At a minimum, the following maintenance practices should be performed:

1. Mow grass and plantings as necessary to promote vigorous growth.

2. Inspect mitigation measures at least twice a year and after all major rain events. Repairs to the sinkhole mitigation measures should be made promptly were warranted.

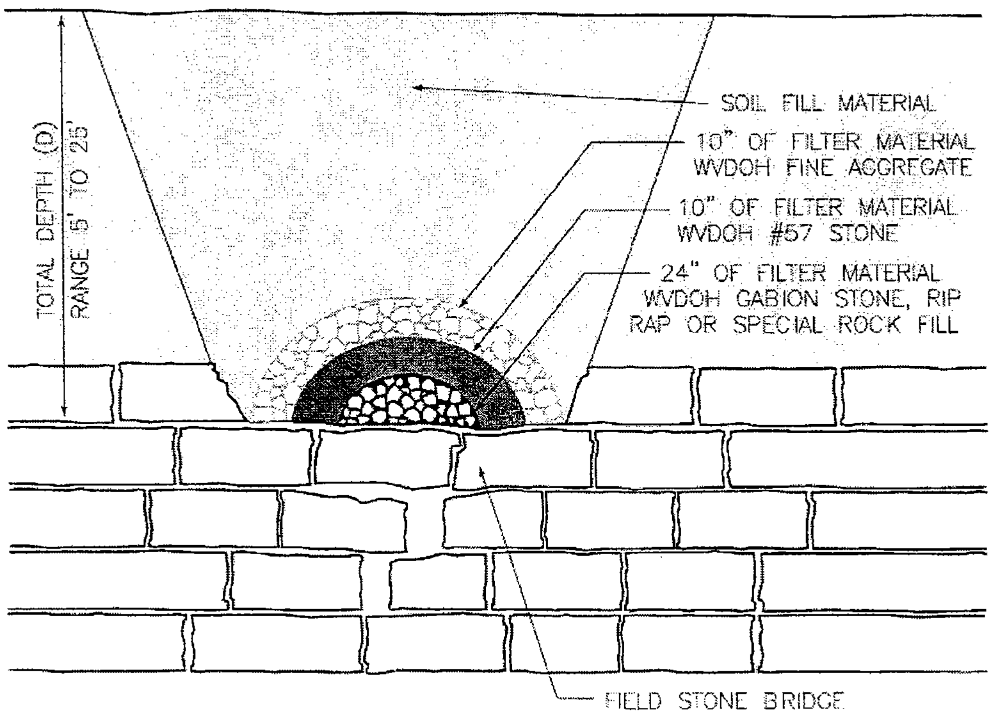
References:

USDA Natural Resources Conservation Center, January 2004. *Maryland Conservation Practice Standard, Sinkhole and Sinkhole Area Treatment, Code 725.*

West Virginia Department of *Highways, Standard Specifications Roads and Bridges*, 2000, Section 702, "Fine Aggregates", Section 703, "Coarse Aggregates", Section 704, "Stone and Crushed Aggregate", Section 715, "Miscellaneous Materials".

WEST VIRGINIA DEPARTMENT OF ENVIRONMENTAL PROTECTION
SINKHOLE MITIGATION GUIDANCE

FIGURE 1



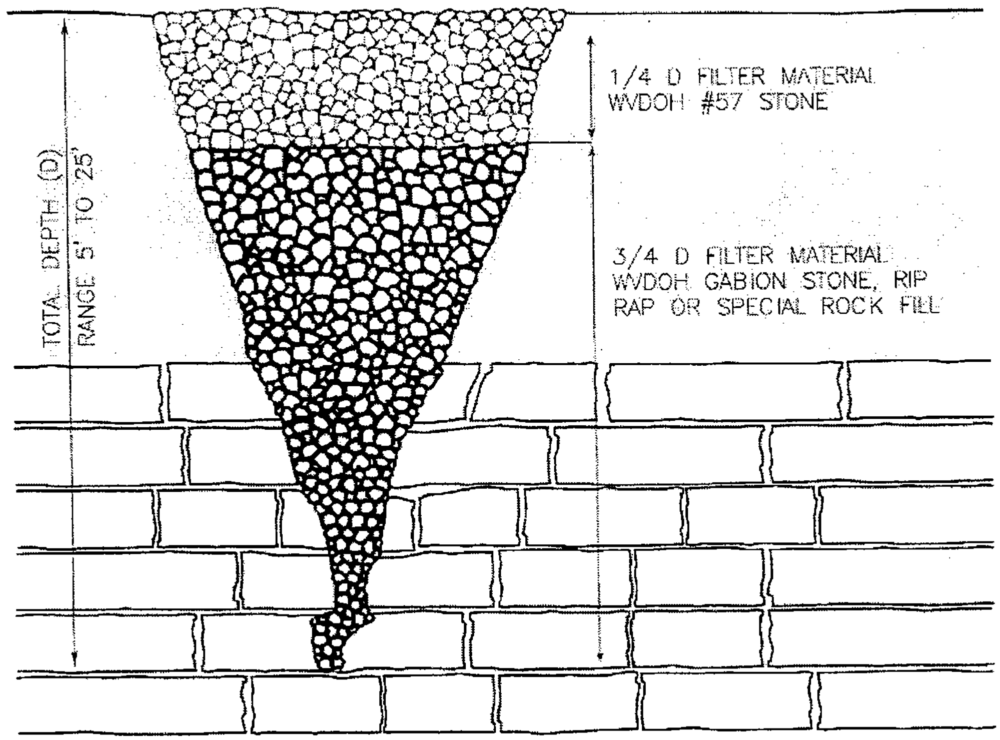
NOTE:
A NONWOVEN GEOTEXTILE MEETING AASHTO M288, SECTIONS 7.1-7.2 MAY BE SUBSTITUTED FOR THE WVDOH #57 STONE AND WVDOH FINE AGGREGATE

SINKHOLE MITIGATION

(DRAINAGE AREA LESS THAN 5 ACRES)

WEST VIRGINIA DEPARTMENT OF ENVIRONMENTAL PROTECTION
SINKHOLE MITIGATION GUIDANCE

FIGURE 2



SINKHOLE MITIGATION

(DRAINAGE AREA 5 TO 15 ACRES)

Appendix L-14

Herbicide List for use in Madison Cave Isopod Habitat

These protocols are currently being prepared and will be included in this MSHCP when available from the Service.

Appendix L-15

Mussel Survey Protocols

These protocols are currently being prepared and will be included in this MSHCP when available from the Service. These protocols will be based, in part, on the specifications provided in Smith 2006, *Survey design for detecting rare freshwater mussels* (attached).

J. N. Am. Benthol. Soc., 2006, 25(3):701–711

Survey design for detecting rare freshwater mussels

David R. Smith[1]

Aquatic Ecology Laboratory, Leetown Science Center, US Geological Survey, 11649 Leetown Road, Kearneysville, West Virginia 25430 USA

Abstract. A common objective when surveying freshwater mussels is to detect the presence of rare populations. In certain situations, such as when endangered or threatened species are potentially in the area of a proposed impact, the survey should be designed to ensure a high probability of detecting species presence. Linking survey design to probability of detecting species presence has been done for quantitative surveys, but commonly applied designs that are based on timed searches have not made that connection. I propose a semiquantitative survey design that links search area and search efficiency to probability of detecting species presence. The survey can be designed to protect against failing to detect populations above a threshold abundance (or density). I illustrate the design for surveys to detect clubshell (*Pluerobema clava*) and northern riffleshell (*Epioblasma torulosa rangiana*) in the Allegheny River. Monte Carlo simulation indicated that the proposed survey design performs well under a range of spatial distributions and low densities (<0.05 m^2) where search area is sufficient to ensure that the probability of detecting species presence is predicted to be ≥ 0.85.

Key words: unionid, probability of species detection, detectability, qualitative sampling, rare populations, species presence, timed search, occupancy.

A common objective of surveys of freshwater mussels is to detect the presence of rare populations, e.g., when assessing site-specific impacts on endangered or threatened species (Wilcox et al. 1993, Smith et al. 2001a) or when delineating the range of a rare species (Strayer et al. 1996). An important application of this objective is determining the presence of an endangered or threatened species in an area of a proposed impact. In that case, confirmation of species presence would halt or influence the activity that would cause the impact, whereas failure to detect a species when it was in fact present (analogous to a Type II error) could permit an adverse impact to occur. Thus, a survey designed to achieve this objective should ensure a high probability of detecting species presence.

Intuition tells us that the probability of detecting species presence is related to species abundance and spatial distribution, sampling effort, search efficiency within the area sampled (i.e., detectability), and the distribution of sampling effort within a study site. McArdle (1990) and Green and Young (1993) related detection of rare species to the number of sampling units taken in a quantitative, quadrat-based survey assuming perfect search efficiency. Near-perfect search efficiency would be achieved in a freshwater mussel survey by sediment excavation (Hornbach and Deneka 1996, Smith et al. 2001b). Green and Young (1993) provided guidelines for designing a quantitative survey that would ensure a high probability of detecting rare species. However, their guidelines have not been widely adopted for freshwater mussel surveys, in part because quantitative sampling is perceived as time-consuming and expensive (Obermeyer 1998), and timed-search surveys result in more species detections per unit time than quadrat-based surveys (Strayer et al. 1997, Vaughn et al. 1997, Obermeyer 1998).

Timed searches are qualitatively more efficient than quadrat-based surveys, but an explicit method to relate search time to the probability of detecting species presence does not appear to exist. Strayer et al. (1997) calculated probability of detection for timed searches for *Elliptio complanata*, but cited high variance of catch per unit effort statistics as a limitation on the generality of a timed-search-based detection curve. Metcalfe-Smith et al. (2000) found that $>50\%$ of species present are missed when typical search times are used and that increased search time resulted in more species detections. However, the essential question of how much search time is enough to ensure a high

[1] E-mail address: drsmith@usgs.gov

probability of detecting a rare species remains unanswered.

I propose an alternative survey design that is intermediate between a timed search and Green and Young's (1993) quadrat-based sampling. The design relates probability of detecting species presence to search area and search efficiency. The semiquantitative approach does not require sediment excavation, but does require a priori information on search efficiency. Search effort is constrained to defined areas (i.e., sampling units), so the survey design can be linked to probability of detecting species presence. I describe an example survey designed to detect clubshell (*Pluerobema clava*) and northern riffleshell (*Epioblasma torulosa rangiana*) in the Allegheny River. Last, I evaluate the design using a Monte Carlo simulation that includes spatially clustered populations because the survey design relies on assumptions about spatial distribution of rare populations.

Survey Design

I developed the survey design by specifying the survey objective and applying a model to link the objective to elements of the design. In particular, I considered factors that affect search efficiency (e.g., detectability) because it is an important element in mussel survey design. I also considered relevant statistical principles that could guide how best to distribute the area to be searched within a site.

Survey objective

Clear, specific, and quantifiable objectives are central to successful survey design (Strayer and Smith 2003, McDonald 2004). The primary objective of our survey was to detect the presence of a rare population, but a survey objective should be defined further and stated quantitatively to allow for evaluating whether a proposed design will meet the objective. For example, the objective might be stated quantitatively: "To detect the presence of any of the endangered or candidate species in a site with probability $\geq$0.85 given that species abundance is $\geq$100 individuals." This statement has 2 important elements: 1) the minimum threshold for the probability of detecting presence of a species, and 2) a species abundance or density that is deemed biologically meaningful. I used an abundance of 100 individuals only as an example. The determination of a biologically meaningful threshold should involve multiple considerations including legal mandates, life history, population viability, and comparisons of densities throughout a local watershed, region, or range.

Modeling the sampling process

A model of the sampling process is needed to relate the proposed objective to the survey design. The model represents the expected survey results (counts of mussels) as a function of the controlling factors—mussel abundance, search area, and search efficiency. Search efficiency, which is also termed detectability, is the probability of detecting an individual mussel given that it is within the search area.

The expected number of individuals counted in a survey of a site can be represented as

$$E(C) = \alpha\beta T \qquad [1]$$

where C is the count of individuals, E(C) is the expected count based on a repeatable sampling process, α is the fraction of the site that is searched, β is the probability of detecting an individual given that it is in the search area, and T is the total number of individuals in the site (Williams et al. 2002:244). The expected number of individuals in the search area is $\alpha\, T = a\mu$ where a is the search area and μ is species density. Note that the fraction of the site that is searched is $\alpha = a/A$ where A denotes the area of the site. The search area is the sum of the areas of each unit in the sample, i.e., $a = \sum_{i=1}^{n} a_i$ where n is the sample size and a_i is the area of the i^{th} sampling unit (typically a_i is the same or nearly the same for all sampling units).

Search efficiency, which refers to the probability of detecting an individual given that it is in the search area, is a function of search rate (time per unit area) and search area (Fig. 1). In eq. 1, search efficiency is denoted by β. In theory, if one spends enough time and effort searching an area, all individuals that are present within the search area will be detected, in which case $\beta = 1$. However, in actual sampling situations, search time and effort are restricted so that not all individuals in the sample area are detected and $\beta < 1$.

Mussel sampling techniques have been classified as quantitative, semiquantitative, or qualitative (Strayer and Smith 2003). This classification can be related to the parameters in eq. 1 (Table 1). Quantitative and semiquantitative sampling are distinguished from qualitative sampling by α. α is known when sampling is quantitative or semiquantitative, but α is not known when sampling is qualitative. Quantitative and semiquantitative sampling are distinguished by β. Quantitative sampling is the case where $\beta = 1$ or $\beta < 1$ and is estimated. In either case, β can be accounted for in eq. 1. Semiquantitative sampling is the case where β is unknown. Unbiased estimation of abundance or density is possible only when α and β are known or

TABLE 1. Contrast of sampling techniques (classified as qualitative, semiquantitative, or quantitative) based on fraction of site searched (α), search efficiency or detectability (β), and which parameter(s) are known or estimated. C = count of mussels in a sample at a site, T = total number of individuals in the population at a site, $\hat{T}$ = estimated total number of individuals at a site, $\hat{\beta}$ = estimated probability of detecting an individual given that it is within the search area.

Sampling technique	α	β	Survey result
Qualitative	Unknown	Unknown	Incomplete count
Semiquantitative	Known	Unknown	Incomplete count within searched area
Quantitative	Known	Known or estimated	Abundance estimate: $\hat{T} = C/\left(\alpha\hat{\beta}\right)$

estimated, i.e., $\hat{T} = C/(\alpha\hat{\beta})$ where $\hat{T}$ is the estimated total number of individuals in the study site (i.e., the abundance estimate) and $\hat{\beta}$ is an unbiased estimate of the probability of detecting an individual given that it is in the search area.

Detecting the presence of a rare species within a site is equivalent to detecting at least one individual of that species, and it follows from eq. 1 that this event is a function of α, β, and T. That is:

$$\begin{aligned}&\text{Prob(detecting at least one individual)}\\&= \text{Prob}(C > 0) = f(\alpha\beta T).\end{aligned} \qquad [2]$$

Green and Young (1993) considered sampling rare populations of freshwater mussels in quadrats and derived a formula for the probability of detecting the presence of a low-density population (i.e., $\mu < 0.10/\text{m}^2$) using a Poisson probability distribution:

$$\text{Prob(detecting at least one individual)} = 1 - e^{-mn} \qquad [3]$$

where m is the number of individuals within a sampling unit and n is the number of random sampling units searched. The Poisson assumption implies that mussels at very low density have a spatially random distribution. This assumption does not imply an absence of underlying ecological relationships, such as habitat associations and dispersal mechanisms, which affect distribution (Downing and Downing 1991). Rather, it indicates that when mussels are geographically rare at a site (i.e., $\mu < 0.1/\text{m}^2$), their low density masks underlying ecological relationships and their spatial distribution is random from a statistical perspective. Green and Young (1993) presented empirical data to support this contention. In addition, Smith et al. (2003) found that low-density mussels on the Cacapon River, West Virginia, had random distributions as evidenced by variance-to-mean ratios. A variance-to-mean ratio of 1 indicates a Poisson distribution (Elliott 1977). Downing and Downing (1991) presented a formula for variance as a function of the mean number of individuals collected that was developed empirically from surveys in lentic and lotic habitats. The Downing and Downing (1991) formula indicates that the variance-to-mean ratio approaches 1 (spatial randomness) as the mean approaches 0.10, the threshold for rarity used by Green and Young (1993). I used data from Smith et al. (2001b) and found variance-to-mean ratios for 60 species/site combinations (31 species at 14 sites) that indicated mussel distributions were statistically spatially random for $\mu \leq 0.10/\text{m}^2$. The same relationship between density and spatial distribution has been found in other populations (McArdle 1990, Welsh et al. 1996). Therefore, I propose eq. 3 as a useful approximation for guiding survey design, and I evaluate its use in a simulation that includes spatially clustered populations and sampling units other than quadrats (see below).

Equation 3 can be revised to account for search efficiency by including the parameter β, thereby making a connection to the sampling-process model in eq. 1. The expected number of individuals detected is $\beta nm = \beta\alpha T$. Thus:

$$\begin{aligned}&\text{Prob(detecting at least one individual)}\\&= 1 - e^{-\beta\alpha T} = 1 - e^{-\beta a T/A} = 1 - e^{-\beta a \mu}.\end{aligned} \qquad [4]$$

Equation 4 can be used to examine the effect of search efficiency (β), search area (a), and density (μ) on the probability of detecting at least one individual or, analogously, the probability of detecting species presence. Figure 2 shows the probability of detecting species presence for $\mu = 0.01$, 0.05, and $0.10/\text{m}^2$, $\beta =$ 0.2, 0.4, 0.6, and 0.8, and $a = 100$ to $1000\ \text{m}^2$. Equation 4 also could be used to examine the effect of abundance (T) for a given study site area (A) instead of μ. Table 2 shows probability of detecting species presence for $T =$ 100 to 500 and $A = 16{,}000$ and $32{,}000\ \text{m}^2$.

Factors that affect search efficiency

Search efficiency is a function of search area and search time (Fig. 1). The exact form of that relationship is not known and will vary over time and area. For a given search area, the more time spent searching, the higher the search efficiency. It is likely that search efficiency will increase quickly as search time is

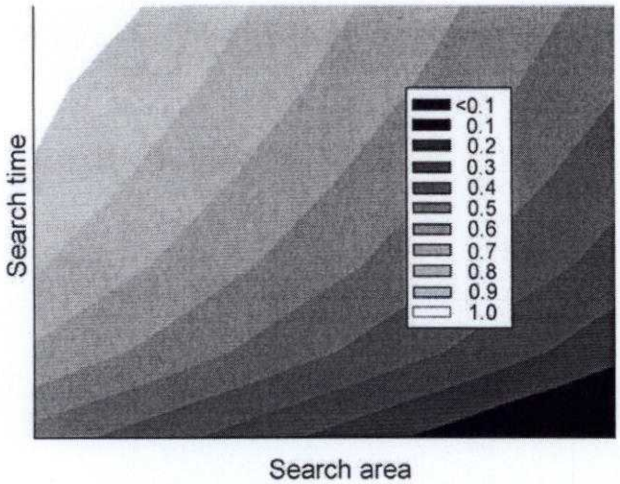


FIG. 1. Search efficiency (β; legend) as a function of search time and search area (a). The axes are not labeled because the exact form of the relationship is determined by a variety of factors involving mussel biology, physical environment, and observer capabilities.

increased from low to moderate levels and the rate of increase in search efficiency will slow as it approaches complete detection, exhibiting a point-of-diminishing-returns-type phenomenon. These relationships between search time and search efficiency also have been shown empirically (Metcalfe-Smith et al. 2000).

The exact form of the relationship between search efficiency and search time will depend on a number of factors (Strayer et al. 1997), some of which are inherent to the biology and natural history of the mussel species. For example, some species are more cryptic than others by virtue of their size, coloration, or reproductive behavior (Miller and Payne 1993, Obermeyer 1998, Haag and Warren 2000). Mussels exhibit seasonal patterns in vertical migration associated with day length and water temperatures (Amyot and Downing 1991, Watters et al. 2001, Perles et al. 2003). Other biological factors include gender and demographics. For example, female northern riffleshell (*Epioblasma torulosa rangiana*) are more visible than males (Smith et al. 2001a), and small mussels are difficult to detect (Miller and Payne 1988, Hornbach and Deneka 1996, Richardson and Yokley 1996, Smith et al. 2001b). Other factors, such as turbidity, hydrologic variability, substrate, and vegetative cover, are associated with the physical environment (Di Maio and Corkum 1997, Smith et al. 2001b). Last, some factors, such as observer experience, visual acuity, and fatigue, are associated with the observer (Strayer et al. 1997).

Only those mussels that are epibenthic or not buried can be found in a search restricted to the substrate surface (Amyot and Downing 1991). If an area is searched thoroughly so that all mussels on the substrate surface have been found, then search efficiency will be capped at the proportion of mussels that are on the surface. Beyond that level of effort, excavation would be required to increase search efficiency to the point that all or nearly all mussels within the searched area are found (Smith et al. 2001b).

Impact of search efficiency on survey design

Because search efficiency directly affects the probability of detecting species presence, it should be considered when designing a survey. Two approaches could be used to incorporate search efficiency in survey design. First, one could be conservative and assume that search efficiency (β) was low. Then the relationship from eq. 4 (Table 2, Fig. 2A–D) could be used as a guide to find the search area (a) that would ensure that the probability of detecting species presence is sufficiently high (Fig. 2A–D). For example, if β were assumed to be ≤ 0.2, then a would have to be >1000 m^2 to have a probability of detecting at least one individual = 0.85 for $\mu = 0.01/\text{m}^2$ (Fig. 2A). This a would be equivalent to ten 1-m-wide × 100-m-long transects (distribution of search effort throughout the site is discussed below). The assumed β could be based on life-history traits, such as likelihood that an individual would be endobenthic (Amyot and Downing 1991). This approach would be precautionary.

Second, β could be estimated at another time and place where the rare species was numerous or by a pilot survey based on a related, but more common, species. For example, β could be estimated by searching the surface of quadrats before excavating sediment (cf. Haukioja and Hakala 1974, Smith et al. 2001b). In this case, the estimate of β and eq. 4 could be used to predict the a that would result in the desired probability of detecting species presence. For example, if β for a search rate of 2 min/m^2 were estimated as 0.4, then a = 500 m^2 would ensure a probability of detecting species presence = 0.85 for $\mu = 0.01/\text{m}^2$ (Fig. 2B), and 1000 min (16.67 h) of search time would be required. The shortcoming of using an estimate from another time and place is that β would be estimated under one set of conditions and applied under a similar, but not identical, set of conditions. If an overestimate of β were used in survey design, then the probability of detecting species presence also would be overestimated, and the design would not be precautionary. The number of quadrats needed to estimate β would depend on μ at the site and the

TABLE 2. Probability of detecting species presence given the study site area (A), search efficiency (β), abundance (T), and search area (a). Bold font indicates probability of species detection $\geq$0.85.

			a (m^2)								
A (m^2)	β	T	100	200	300	400	500	600	700	800	900
16,000	0.2	100	0.12	0.22	0.31	0.39	0.46	0.53	0.58	0.63	0.68
		200	0.22	0.39	0.53	0.63	0.71	0.78	0.83	**0.86**	**0.89**
		300	0.31	0.53	0.68	0.78	**0.85**	**0.89**	**0.93**	**0.95**	**0.97**
		400	0.39	0.63	0.78	**0.86**	**0.92**	**0.95**	**0.97**	**0.98**	**0.99**
		500	0.46	0.71	**0.85**	**0.92**	**0.96**	**0.98**	**0.99**	**0.99**	**1.00**
	0.4	100	0.22	0.39	0.53	0.63	0.71	0.78	0.83	**0.86**	**0.89**
		200	0.39	0.63	0.78	**0.86**	**0.92**	**0.95**	**0.97**	**0.98**	**0.99**
		300	0.53	0.78	**0.89**	**0.95**	**0.98**	**0.99**	**0.99**	**1.00**	**1.00**
		400	0.63	**0.86**	**0.95**	**0.98**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**
		500	0.71	**0.92**	**0.98**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	0.6	100	0.31	0.53	0.68	0.78	**0.85**	**0.89**	**0.93**	**0.95**	**0.97**
		200	0.53	0.78	**0.89**	**0.95**	**0.98**	**0.99**	**0.99**	**1.00**	**1.00**
		300	0.68	**0.89**	**0.97**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
		400	0.78	**0.95**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
		500	**0.85**	**0.98**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
32,000	0.2	100	0.06	0.12	0.17	0.22	0.27	0.31	0.35	0.39	0.43
		200	0.12	0.22	0.31	0.39	0.46	0.53	0.58	0.63	0.68
		300	0.17	0.31	0.43	0.53	0.61	0.68	0.73	0.78	0.82
		400	0.22	0.39	0.53	0.63	0.71	0.78	0.83	**0.86**	**0.89**
		500	0.27	0.46	0.61	0.71	0.79	**0.85**	**0.89**	**0.92**	**0.94**
	0.4	100	0.12	0.22	0.31	0.39	0.46	0.53	0.58	0.63	0.68
		200	0.22	0.39	0.53	0.63	0.71	0.78	0.83	**0.86**	**0.89**
		300	0.31	0.53	0.68	0.78	**0.85**	**0.89**	**0.93**	**0.95**	**0.97**
		400	0.39	0.63	0.78	**0.86**	**0.92**	**0.95**	**0.97**	**0.98**	**0.99**
		500	0.46	0.71	**0.85**	**0.92**	**0.96**	**0.98**	**0.99**	**0.99**	**1.00**
	0.6	100	0.17	0.31	0.43	0.53	0.61	0.68	0.73	0.78	0.82
		200	0.31	0.53	0.68	0.78	**0.85**	**0.89**	**0.93**	**0.95**	**0.97**
		300	0.43	0.68	0.82	**0.89**	**0.94**	**0.97**	**0.98**	**0.99**	**0.99**
		400	0.53	0.78	**0.89**	**0.95**	**0.98**	**0.99**	**0.99**	**1.00**	**1.00**
		500	0.61	**0.85**	**0.94**	**0.98**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**

proportion of individuals on the substrate surface (Smith et al. 2001b, Strayer and Smith 2003). Therefore, the environmental conditions in the pilot survey should be as close as possible to the conditions likely to be encountered at the site where species presence will be determined. Information on species-specific densities and search efficiencies are available in the literature in some cases (e.g., Smith et al. 2001a), and unpublished agency surveys are likely to provide relevant data.

Statistical principles guiding the distribution of search effort within the site

Two statistical principles, in particular, are useful for guiding distribution of search effort. First, spatially balanced sampling has been recognized as efficient for sampling natural resources (Christman 2000, Stevens and Olsen 2004). A spatially balanced sample is one that is distributed throughout a site or population. Various systematic or grid sampling methods qualify as spatially balanced. Second, it is generally more efficient (reduces sampling error) to distribute effort among many small units than a few large units. This principle is particularly relevant when the population is spatially clustered (Elliott 1977). The mitigating factor is the effort required to move among units. Many small units require more between-unit travel than few large units. Thus, the challenge is to find a sampling-unit size that represents a compromise between cost and sampling error. These principles can be combined with stratification to allocate effort efficiently and to ensure that sampling is done in all habitats. For example, a site can be stratified by macrohabitat (e.g., riffle, run, pool) and search area can be allocated proportionately or according to anticipated habitat value (i.e., more effort in better habitat). On the other hand, the survey could be conducted in phases as suggested by Kovalak et al. (1986) and implemented recently by Villella and Smith (2005). During the 1st phase, an informal search or surveillance can be conducted to delineate mussel beds or

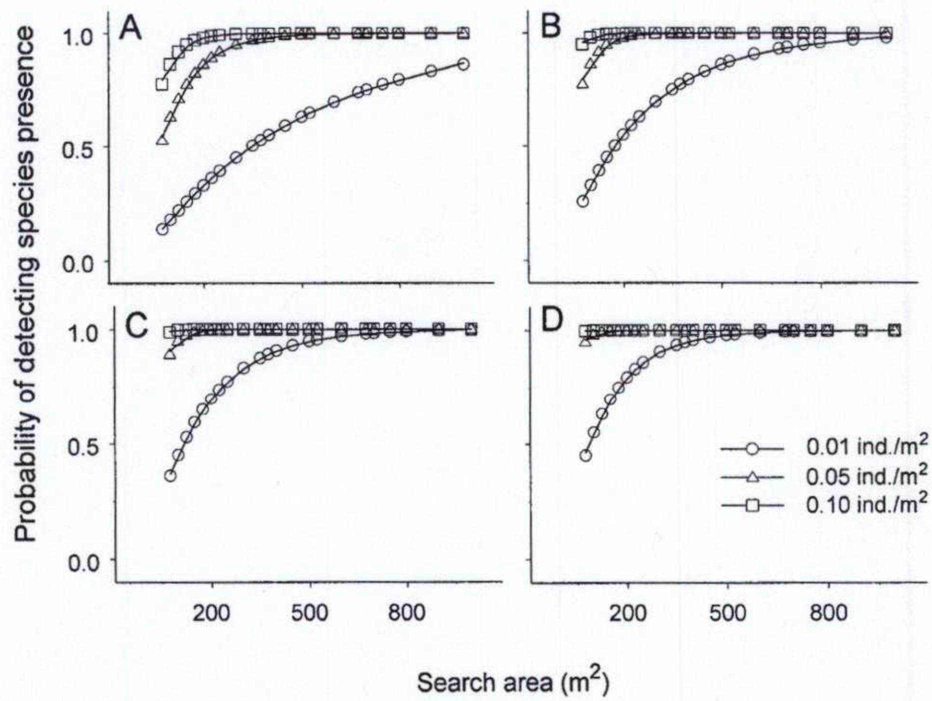


FIG. 2. Probability of detecting species presence as a function of search area (a) and density (0.01, 0.05, 0.10 individual/m^2) of mussels when search efficiency (β) was 0.2 (A), 0.4 (B), 0.6 (C), and 0.8 (D).

habitat. During the 2nd phase, the semiquantitative approach can be applied after the search area (a) has been determined to ensure a sufficiently high probability of detecting species presence. The predetermined a should be allocated so that most, but not all, of the area occurs within the bed or habitat identified during the 1st phase.

A cautionary note is warranted regarding the distribution of sampling effort according to an explicit or implied habitat model. If the habitat model is a good approximation, then it can be helpful in distributing search area. Depth and hydrological variability are useful predictors of mussel density (Haukioja and Hakala 1974, Strayer and Ralley 1993, Di Maio and Corkum 1995). However, if the model is a poor approximation, as Strayer and Ralley (1993) found for microhabitat variables, then model-based distribution can be inefficient at best and misleading at worst. A poor habitat model could lead to omission of the actual habitat from the area searched.

Detection of Clubshell (*Pleurobema clava*) and Northern Riffleshell (*Epioblasma torulosa rangiana*) in the Allegheny River

I used data from the clubshell and northern riffleshell in the Allegheny River to illustrate the design of a survey to detect their presence. In previous surveys on the Allegheny River, Smith et al. (2001a) reported that a thorough search of the substrate surface required 2 min/m^2 of search time. At the West Hickory bridge site, ~30% and 50% of clubshell and northern riffleshell were found at the substrate surface, respectively.

Suppose the goal was to protect a site against adverse impact if either species was present at $\mu \geq 0.01/\text{m}^2$ with a probability of detecting species presence $\geq$ 0.85. (Tolerance for risk is a subjective decision that often would be set during the regulatory process.) To protect either species, the β corresponding to the least detectable species, the clubshell, would be used. In this case, we assume that the substrate surface within a will be searched thoroughly so that β is the proportion of mussels on the substrate surface. Given this information, we can design a survey using eq. 4:

$$0.85 = 1 - e^{-0.30a0.01}$$

and solve for a:

$$a = \frac{\ln(1 - 0.85)}{-0.003}$$
$$= 632\text{m}^2.$$

Based on the principle of spatially balanced sampling, at least 632 m^2 of search area should be distributed throughout the site. A reasonable design would be to search within transects oriented perpendicular to shoreline or the thalweg. Following the rule that more small units are better, use of 0.5-m-wide transects would allow greater spatial dispersion of sampling effort; however, logistics and tradition might favor 1-m-wide transects, especially at sites where SCUBA is required. Transect length would depend on site dimensions. For example, if the site was 100 m across the river, then seven 1-m-wide transects would be required. Good spatial balance and coverage would be achieved by selecting a random start and placing transects at equal intervals. An improvement on that plan would include 2 random starts. To increase probability of detecting species presence to 0.95, ten 1×100 m transects would be required.

After a has been determined based on $\hat{\beta}$ and a μ that is to be protected, the time required to conduct the survey can be calculated. Based on 2 min/m^2 to search the surface substrate thoroughly, searching seven $1 \times$ 100-m transects would require ~23 h, which could be divided among multiple observers. The survey could be accomplished in ~1 d with a crew of 4. This time and effort does not seem to be an unreasonable survey cost when the objective is to detect a rare or endangered species before an adverse impact occurs. Budgets for construction projects, for example, can amount to hundreds of thousands to millions of dollars. The cost to conduct a rigorous mussel survey is trivial by comparison.

TABLE 3. Abundance (T), search efficiency (β), and cluster size for the populations used to simulate the proposed survey design. The study site was 16,000 m^2 (160 m $\times$ 100 m). Variance-to-mean ratios were calculated for individuals within 1 m $\times$ 100 m transects.

			Variance-to-mean ratio	
T	β	Cluster size	Entire population	Detectable portion of the population
100	0.2	1	1.19	0.91
		3	2.03	1.11
		5	2.33	1.04
	0.4	1	1.21	1.17
		3	1.93	1.40
		5	2.17	1.29
	0.6	1	1.05	1.04
		3	1.91	1.58
		5	2.84	1.77
300	0.2	1	0.87	0.85
		3	2.44	1.08
		5	2.53	1.20
	0.4	1	0.83	1.03
		3	1.85	1.39
		5	2.11	1.36
	0.6	1	0.94	1.08
		3	1.77	1.31
		5	2.62	1.92
500	0.2	1	1.13	1.15
		3	1.84	1.19
		5	2.91	1.44
	0.4	1	1.03	1.04
		3	1.61	1.11
		5	2.19	1.39
	0.6	1	1.03	0.80
		3	2.01	1.45
		5	2.81	2.04

Monte Carlo Simulation

To evaluate the proposed survey design, a computer program was used to generate locations for individual mussels within a site of 16,000 m^2 (100 m $\times$ 160 m), apply search efficiencies so that different proportions of the mussels were detectable, and count detectable mussels within systematically placed 1-m transects. Abundance at the site was a Poisson random variable with means of 100, 300, and 500 mussels representing population densities of 0.006, 0.02, and 0.03 (individuals/m^2). Individual mussels were in clusters with mean sizes of 1, 3, or 5 individuals (a cluster size of 1 represented complete spatial randomness). The location of the cluster center was random within the site, and individuals were distributed from the cluster center at a uniform random angle and exponential random distance, with mean distance of 1 m. Search efficiencies of 0.2, 0.4, or 0.6 were applied to determine whether each individual in the population was detectable. Detectable individuals were counted within 1-m transects oriented across the short axis of the site (100 m). Areas searched were 400, 600, 800, and 1000 m^2. The probability of detecting species presence was calculated as the proportion of 1000 replications where at least one individual was counted. Computations were done in SAS (version 9.1 SAS Institute, Cary, North Carolina).

The populations showed differing degrees of spatial clustering (Table 3, Fig. 3). Variance-to-mean ratios increased with cluster size were lower when calculated using detectable individuals only. Thus, the detectable portion of the population appears less spatially clustered than the actual population.

Simulated probabilities of detecting species presence generally tracked the probabilities predicted from eq. 4 (Table 4). Variability in the simulated probabilities was caused by variability in abundance, search efficiency, cluster size, and sample selection. This result is relevant because abundance, search efficiency, and spatial distribution would not be known exactly when using eq. 4 for survey design. The simulations indicated that eq. 4 is a useful guide under a range of conditions. Most important, the survey design

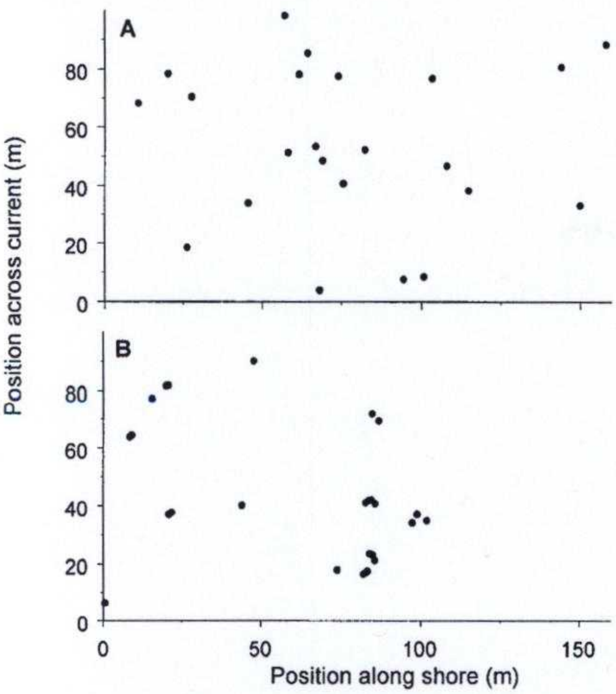


FIG. 3. Example spatial distributions of detectable mussels used to evaluate the survey design when simulated abundance was 100, search efficiency was 0.2, and cluster sizes were 1 (A) and 5 (B). Detectable mussels were a random subset of the abundance determined by the search efficiency. There were 23 detectable mussels in A and 29 in B.

performed well when *a* was predicted to result in a high probability of detecting species presence. Simulated probabilities of detecting species presence were $\geq$0.85 in 92% (77 of 84) of cases where eq. 4 predicted the probabilities would be $\geq$0.85 (Table 4).

Discussion

Clear, specific, and quantitative objectives are prerequisites to a successful survey design (McDonald 2004). For example, the objective for a pre-dredging survey could be to detect the presence of any endangered or candidate species with probability $\geq$0.85 given that species density is $\geq 0.01/\text{m}^2$. An important question to ask when designing a survey is whether the proposed design will meet the stated objective (Strayer and Smith 2003). The survey design described here provides a method for answering that question by linking survey elements, i.e., search area and search efficiency, to the probability of detecting species presence.

The proposed survey design, which is intermediate between timed search and quadrat methods, requires that the search area be constrained within sampling units, but excavation is not required because search efficiency is assumed to be less than perfect. Distribution of the search area within the site is flexible within guidelines. Based on well-established principles of sampling natural resources, it is best to distribute sampling effort throughout a study site in relatively small sampling units. The size of the sampling units is mitigated by logistic considerations with transects recommended in some cases because of ease of field application. A Monte Carlo simulation confirmed that use of systematically placed transects is a good approach for the objective of species detection. However, use of transects would not be a good approach when the objective is to estimate abundance or density because some amount of excavation would be required and, therefore, quadrats would be required (Smith et al. 2001b, Strayer and Smith 2003). Information on habitat or mussel beds can be used to stratify the site and to allocate the search area within strata either proportionately or with more of the search effort allocated to better habitats. More complex sample-selection procedures, such as unequal probability sampling, could be applied. However, ease of application should be an overarching concern, and simple selection procedures, such as systematic sampling, would be preferable.

Some population abundances or densities are unlikely to be detected without substantial sampling effort by increasing search efficiency or search area (Table 2). This constraint is unavoidable in any protocol. The proposed survey design incorporates sampling techniques (i.e., transect-based, semiquantitative sampling) that are part of many existing protocols. However, the user of the proposed design can be fully aware of population sizes that are likely to be detected by explicitly stating the probability of detecting species presence for given population size and sampling effort. As one reviewer noted, a main advantage of the proposed design is that the user has an answer to the question: "How much sampling effort is enough?"

A reasonable concern with the proposed design is the cost to survey a site. The recommended sampling effort is likely to exceed the costs associated with currently applied protocols. Few protocols for rare species detection have been published; however, Young et al. (2001) recommended at least 2 person-hours of search time in optimal habitat before concluding that a species was absent if no individuals were detected. At a search rate of 2 min/m^2, a 2-h search would be equivalent to $<100\ \text{m}^2$ of search area, which appears to be an insufficient effort for detecting rare species. A search area of 100 m^2 resulted in a probability of detecting species presence as low as 0.12

TABLE 4. Probabilities of detecting species presence observed from a computer simulation and predicted by eq. 4. Abundance (*T*), search efficiency (β), and cluster size are mean values used in the simulation, but were random variables in the simulation. Cluster locations were random within a 16,000-m^2 study site. Searches were conducted within 1 m × 100 m transects. The search area (*a*) was the sum of the transect areas. Bold font indicates combinations with predicted probabilities ≥0.85.

			a (m^2)							
			400		600		800		1000	
T	β	Cluster size	Simulated	Predicted	Simulated	Predicted	Simulated	Predicted	Simulated	Predicted
100	0.2	1	0.45	0.39	0.78	0.53	0.55	0.63	0.51	0.71
		3	0.36	0.39	0.73	0.53	0.61	0.63	0.89	0.71
		5	0.43	0.39	0.57	0.53	0.70	0.63	0.75	0.71
	0.4	1	0.76	0.63	0.95	0.78	**0.82**	**0.86**	**0.91**	**0.92**
		3	0.63	0.63	0.89	0.78	**0.79**	**0.86**	**1.00**	**0.92**
		5	0.49	0.63	0.65	0.78	**0.82**	**0.86**	**0.92**	**0.92**
	0.6	1	0.89	0.78	**0.97**	**0.89**	**1.00**	**0.95**	**0.95**	**0.98**
		3	0.67	0.78	**0.87**	**0.89**	**0.96**	**0.95**	**0.94**	**0.98**
		5	0.72	0.78	**0.86**	**0.89**	**0.95**	**0.95**	**0.73**	**0.98**
300	0.2	1	0.75	0.78	**0.97**	**0.89**	**0.95**	**0.95**	**1.00**	**0.98**
		3	0.73	0.78	**0.78**	**0.89**	**1.00**	**0.95**	**1.00**	**0.98**
		5	0.79	0.78	**0.69**	**0.89**	**0.97**	**0.95**	**0.96**	**0.98**
	0.4	1	**0.92**	**0.95**	**1.00**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**
		3	**0.83**	**0.95**	**1.00**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**
		5	**0.94**	**0.95**	**1.00**	**0.99**	**0.93**	**1.00**	**1.00**	**1.00**
	0.6	1	**1.00**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
		3	**0.88**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
		5	**0.95**	**0.99**	**0.97**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
500	0.2	1	**0.92**	**0.92**	**1.00**	**0.98**	**1.00**	**0.99**	**1.00**	**1.00**
		3	**0.90**	**0.92**	**0.92**	**0.98**	**1.00**	**0.99**	**1.00**	**1.00**
		5	**0.95**	**0.92**	**0.96**	**0.98**	**1.00**	**0.99**	**1.00**	**1.00**
	0.4	1	**1.00**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
		3	**0.96**	**0.99**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
		5	**1.00**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	0.6	1	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
		3	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
		5	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**

and <0.85 for all but one combination of abundance and search efficiency in Table 2. If this result is any indication, using the proposed survey design would lead to increased sampling effort and higher survey costs than currently practiced. A legitimate and reasonable question is whether the added cost is worthwhile and affordable. Ultimately, that question will have to be answered on a case-by-case basis by the organizations that are funding the survey. One counterbalancing consideration is the cost of failing to detect the presence of a rare population within the area of a pending adverse impact. Cost would be reduced if searching stopped as soon as one individual of the rare species was detected; however, that practice would limit the utility of the survey. There certainly are circumstances when designing a survey to achieve a high probability of detecting species presence will be worthwhile. Surveys of federally endangered species in areas of proposed adverse impacts would probably be one of those circumstances.

Acknowledgements

This paper was motivated by my participation in a review of survey protocols for detecting rare populations in the Allegheny River prior to sand and gravel dredging. The review was organized by Bob Carline and sponsored by the Pennsylvania Fish and Boat Commission. I appreciate the informative and thoughtful discussions on mussel sampling with the review panel members: Bob Carline, Drew Miller, Dick Neves, and Gerald Dinkins. I thank Paul Geissler, Jim Nichols, Penelope Pooler, Jennifer Brown, Rita Villella, Caryn Vaughn, and 3 anonymous referees for helpful comments on the paper.

Literature Cited

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

Christman, M. C. 2000. A review of quadrat-based sampling of rare, geographically clustered populations. Journal of Agricultural, Biological, and Environmental Statistics 5: 168–201.

Di Maio, J., and L. D. Corkum. 1995. Relationship between the spatial distribution of freshwater mussels (Bivalvia: Unionidae) and the hydrological variability of rivers. Canadian Journal of Zoology 73:663–671.

Di Maio, J., and L. D. Corkum. 1997. Patterns of orientation in unionids as a function of rivers with differing hydrological variability. Journal of Molluscan Studies 63:531–539.

Downing, J. A., and W. L. Downing. 1991. Spatial aggregation, precision, and power in surveys of freshwater mussel populations. Canadian Journal of Fisheries and Aquatic Sciences 49:985–991.

Elliott, J. M. 1977. Some methods for the statistical analysis of samples of benthic invertebrates. Freshwater Biological Association Scientific Publication 25. Freshwater Biological Association, Ambleside, UK.

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

Haag, W. R., and M. L. Warren. 2000. Effects of light and presence of fish on lure display and larval release behaviours in two species of freshwater mussels. Animal Behaviour 60:879–886.

Haukioja, E., and T. Hakala. 1974. Vertical distribution of freshwater mussels (Pelecypoda, Unionidae) in southwestern Finland. Annales Zoologici Fennici 11:127–130.

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

Kovalak, W. P., S. D. Dennis, and J. M. Bates. 1986. Sampling effort required to find rare species of freshwater mussels. Pages 34–45 *in* B. G. Isom (editor). Rationale for sampling and interpretation of ecological data in the assessment of freshwater ecosystems. ATSM STP 894. American Society for Testing and Materials, Philadelphia, Pennsylvania.

McArdle, B. H. 1990. When are rare species not there? Oikos 57:276–277.

McDonald, L. L. 2004. Sampling rare populations. Pages 11–42 *in* W. L. Thompson (editor). Sampling rare or elusive species: concepts, designs, and techniques for estimating population parameters. Island Press, Covelo, California.

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

Miller, A. C., and B. S. Payne. 1988. The need for quantitative sampling to characterize size demography and density of freshwater mussel communities. American Malacological Bulletin 6:49–54.

Miller, A. C., and B. S. Payne. 1993. Qualitative versus quantitative sampling to evaluate population and community characteristics at a large-river mussel bed. American Midland Naturalist 130:133–145.

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

Perles, S. J., A. D. Christian, and D. J. Berg. 2003. Vertical migration, orientation, aggregation, and fecundity of the freshwater mussel *Lampsilis siliquoidia*. Ohio Journal of Science 103:73–78.

Richardson, T. D., and P. Yokley. 1996. A note on sampling technique and evidence of recruitment in freshwater mussels (Unionidae). Archiv für Hydrobiologie 137:135–140.

Smith, D. R., R. F. Villella, and D. P. Lemarié. 2001a. Survey protocol for assessment of endangered freshwater mussels in the Allegheny River. Journal of the North American Benthological Society 20:118–132.

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

Smith, D. R., R. F. Villella, D. P. Lemarié, and S. von Oettingen. 2001b. How much excavation is needed to monitor freshwater mussels? Pages 203–218 *in* R. A. Tankersley, D. I. Warmolts, G. T. Watters, B. J. Armitage, P. D. Johnson, and R. S. Butler (editors). Freshwater Mollusk Symposium Proceedings. Ohio Biological Survey, Columbus, Ohio.

Stevens, D. L., and A. R. Olsen. 2004. Spatially balanced sampling of natural resources. Journal of the American Statistical Association 99:262–278.

Strayer, D. L., S. Claypool, and S. J. Sprague. 1997. Assessing unionid populations with quadrats and timed searches. Pages 163–169 *in* K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo (editors). Conservation and management of freshwater mussels II. Initiatives for the future. Upper Mississippi River Conservation Committee, Rock Island, Illinois. (Available from: Illinois Natural History Survey, 607 East Peabody Drive, Champaign, Illinois 61820 USA.)

Strayer, D. L., and J. Ralley. 1993. Microhabitat use by an assemblage of stream-dwelling unionaceans (Bivalvia), including two rare species of *Alasmidonta*. Journal of the North American Benthological Society 12:247–258.

Strayer, D. L., and D. R. Smith. 2003. A guide to sampling freshwater mussel populations. American Fisheries Society Monograph No. 8. American Fisheries Society, Bethesda, Maryland.

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

Vaughn, C. C., C. M. Taylor, and K. J. Eberhard. 1997. A comparison of the effectiveness of timed searches vs. quadrat sampling in mussel surveys. Pages 157–162 *in* K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo (editors). Conservation and management of freshwater mussels II. Initiatives for the future. Upper Mississippi River Conservation Committee, Rock Island, Illinois. (Available from: Illinois Natural History Survey, 607 East Peabody Drive, Champaign, Illinois 61820 USA.)

VILLELLA, R. F., AND D. R. SMITH. 2005. Two-phase sampling to estimate river-wide populations of freshwater mussels. Journal of the North American Benthological Society 24: 357–368.

WATTERS, G. T., S. H. O'DEE, AND S. CHORDAS. 2001. Patterns of vertical migration in freshwater mussels (Bivalvia: Unionida). Journal of Freshwater Ecology 16:541–550.

WELSH, A. H., R. B. CUNNINGHAM, C. F. DONNELLY, AND D. B. LINDENMAYER. 1996. Modelling the abundance of rare species: statistical models for counts with extra zeros. Ecological Modelling 88:297–308.

WILCOX, D. B., D. D. ANDERSON, AND A. C. MILLER. 1993. Survey protocol and decision criteria for estimating the likelihood that *Lampsilis higginsi* is present in areas within the Upper Mississippi River System. Pages 163–167 *in* K. S. Cummings, A. C. Buchanan, and L. M. Koch (editors). Conservation and management of freshwater mussels. Upper Mississippi River Conservation Committee, Rock Island, Illinois. (Available from: Illinois Natural History Survey, 607 East Peabody Drive, Champaign, Illinois 61820 USA.)

WILLIAMS, B. K., J. D. NICHOLS, AND M. J. CONROY. 2002. Analysis and management of animal populations. Academic Press, New York.

YOUNG, M. R., P. J. COSGROVE, L. C. HASTIE, AND B. HENNIGER. 2001. A standardised method for assessing the status of freshwater mussels in clear, shallow rivers. Journal of Molluscan Studies 67:395–405.

Received: 25 August 2005
Accepted: 17 March 2006

Appendix L-16

Mussel Relocation Mark/Recapture Protocols

These protocols are currently being prepared and will be included in this MSHCP when available from the Service. These protocols will be based, in part, on the specifications provided in USFWS and VDGIF 2008, *Freshwater mussel guidelines for Virginia;* Dunn and Sietman 1997, *Guidelines used in four geographically diverse unionid relocations;* and Kurth 2007, *PIT tags increase effectiveness of freshwater mussel relocations* (attached).

Guidelines Used in Four Geographically Diverse Unionid Relocations

Heidi L. Dunn and Bernard E. Sietman
Ecological Specialists, Inc., St. Peters, Missouri

Abstract. The endangered status of many unionids has prompted the use of relocations as a mitigation measure. However, current data suggest that relocations have been only minimally successful possibly due to factors such as improper site selection and handling techniques. Guidelines for relocation site selection and unionid handling were developed from reviewing literature and contacting knowledgeable researchers. These guidelines were used during unionid relocations on the Elk River, West Virginia; Meramec River, Missouri; St. Croix River, Wisconsin-Minnesota; and the Wolf River, Wisconsin. Stream characteristics, collection and relocation conditions, and species varied among relocations. Preliminary results suggest that these relocations were successful. Observed mortality 1 month and 1 year following relocations was negligible (0 to 1%), and recovery ranged from 50% to 96%. We suggest the following guidelines for future relocations: (1) use field personnel that are familiar with unionids, (2) select a relocation area with stable substrate and a similar unionid community that is near the collection area, (3) keep animals moist or in water and minimize out-of-water time, (4) avoid extreme temperatures, and (5) avoid crowding animals.

Introduction

Modifications of our rivers, such as impoundment, channelization, dredging, instream construction, and the resulting siltation and hydrological changes, are often cited as the primary reasons for the decline of unionid species (Stansbery 1970, 1971; Stein 1972; Yokley 1976; Suloway et al. 1981; Miller et al. 1984; Williams et al. 1992; Parmalee and Hughes 1993; Hartfield 1993). A high percentage of North American unionids are presumed extinct, threatened, endangered, or in need of conservation (see Neves 1993). Currently, Section 7(a)(2) of the Endangered Species Act as well as equivalent legislation within some states requires that impacts to these species be minimized, and if impacts are unavoidable, that they be mitigated. Relocating unionids from instream construction, impoundment, and channelization areas has often been used to mitigate impacts to unionids (Oblad 1980; Harris 1984, 1986, 1989; Harris et al. 1992; Jenkinson 1985, 1989; Dunn 1993). However, monitoring studies suggest that in most cases unionid recovery and/or survival may be less than ideal (Sheehan et al. 1989; Burke 1991; Aquatic Resources Center 1993; Dunn 1993; Koch 1993; Layzer and Gordon 1993).

Dunn (1994) and Cope and Waller (1995) reviewed literature and Dunn (1994) contacted knowledgeable researchers on previous relocation studies and found that habitat stability in the relocation area (Sheehan et al. 1989; Dunn 1993; Layzer and Gordon 1993) and handling methods (Ahlstedt, pers. comm.; Harris, pers. comm.; Neves, pers. comm.) were consistently noted as possible reasons for relocation success or failure. Unionid survival and/or recovery following relocation also varied among species within most studies (Oblad 1980; Sheehan et al. 1989; Dunn 1993).

Habitat stability in the relocation area seems to be a key factor. Low recovery was attributed to changes in substrate or habitat in some relocations (Sheehan et al. 1989; Hubbs et al. 1991; Dunn 1993; Layzer and Gordon 1993), and Dunn (1993) recovered fewer relocated unionids from areas with less stable substrate in the Ohio River. Handling methods, such as overcrowding, prolonged periods out of water, exposure to extreme temperature, and improper placement in the substrate, have frequently been speculated as possible causes of low recovery and survival. However, researchers disagree on unionid sensitivity to handling and few studies have tested these effects (Waller et al. 1995).

Recommendations outlined by Dunn (1994) were incorporated into four unionid relocations that varied in geographic location, riverine characteristics, and unionid species: St. Croix River (Minnesota and Wisconsin), Wolf River (Wisconsin), Meramec River (Missouri), and Elk River (West Virginia) (Figure 1). Each relocation was monitored to determine protected species survival. The Wolf, St. Croix, and Elk River monitoring also included other relocated species and the St. Croix and Wolf River

relocations included tests to substantiate handling and placement techniques. Other monitoring objectives included determining if mortality was immediate or long term (St. Croix, Wolf, and Meramec rivers), determining nonrelocated unionid mortality (all relocations), determining adequacy of buffer zones (St. Croix River), and determining if removal areas were recolonized (Meramec and Elk rivers). Monitoring for most of these relocations is not complete; therefore, results in this paper will be limited to recovery and observed mortality of relocated unionids. This paper compares preliminary monitoring results and offers guidelines for future relocations.

Study Area

In May 1994, 4,514 unionids were relocated from construction areas of the I-55 bridge over the Meramec River near St. Louis, Missouri (Figure 1). In this area, the Meramec River (river mile 6.9) is typically a flowing gravel run, about 120 m wide and 1.5 m deep; however, the area is often pooled by the Mississippi River, resulting in little to no flow and silt deposition. Substrate is mostly loose gravel and sand, with some silt particularly near the riverbanks. Areas with boulders over gravel and silt are found under and downstream of the bridge. A total of 33 species has been collected in this area (Table 1) including *Lampsilis abrupta* (federally endangered); *Elliptio crassidens, Fusconaia ebena*, and *Leptodea leptodon* (Missouri endangered); *Arcidens confragosus* and *Plethobasus cyphyus* (Missouri rare); and *Obovaria olivaria* (Missouri watch list).

In July and August 1994, 202 unionids were relocated from a pipeline construction area in the Elk River near Clendenin, West Virginia (Figure 1). Elk River is a small (55 m wide and less than 1.2 m deep in the study area), clear, high-gradient tributary of the Kanawha River. Habitat in this area of the river consists of riffles, runs, and deeper pools with cobble, gravel, and sand substrate. A total of 20 species has been collected in the study area (Table 1) including *Epioblasma torulosa rangiana, Pleurobema clava*, and *L. abrupta* (federally endangered).

In August 1994 and August 1995, 8,996 and 14,027 unionids, respectively, were relocated from I-94 bridge construction and demolition areas in the St. Croix River near Hudson, Wisconsin (Figure 1).

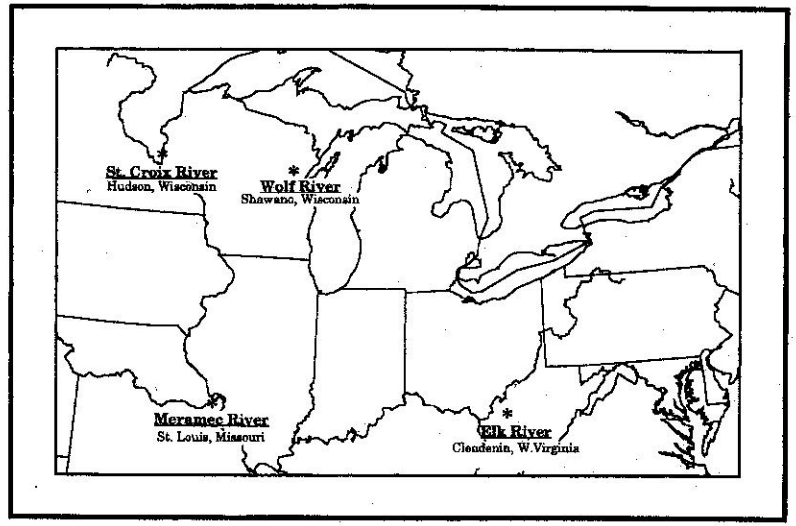


Figure 1. Distribution of unionid relocation studies.

Table 1. Unionid species recorded from each of the relocation areas.

Species[1]	Meramec River[2]	Elk River[3]	St. Croix River[4]	Wolf River[5]
Actinonaias ligamentina	X	X	X	X
Alasmidonta marginata			X	X
Amblema plicata plicata	X	X	X	X
Arcidens confragosus	X			
Cumberlandia monodonta			X	
Cyclonaias tuberculata			X	
Ellipsaria lineolata	X		X	
Elliptio crassidens	X		X	
Elliptio dilatata	X	X	X	X
Epioblasma torulosa rangiana		X		
Epioblasma triquetra		X		
Fusconaia ebena	X		X	
Fusconaia flava	X	X	X	X
Fusconaia subrotunda		X		
Lampsilis abrupta	X	X		
Lampsilis cardium	X	X	X	X
Lampsilis fasciola		X		
Lampsilis higginsii			X	
Lampsilis ovata		X		
Lampsilis siliquoidea	X	X	X	X
Lampsilis teres	X			
Lasmigona complanata complanata	X		X	
Lasmigona costata		X	X	X
Leptodea fragilis	X		X	X
Leptodea leptodon	X			
Ligumia recta	X	X	X	X
Megalonaias nervosa	X		X	
Obliquaria reflexa	X		X	X
Obovaria olivaria	X		X	X
Obovaria subrotunda		X		
Plethobasus cyphyus	X			
Pleurobema clava		X		
Pleurobema coccineum	X		X	X
Potamilus alatus	X	X	X	X
Potamilus ohiensis	X		X	
Ptychobranchus fasciolaris		X		
Pyganodon grandis	X		X	X
Quadrula metanevra	X		X	
Quadrula pustulosa pustulosa	X	X	X	X
Quadrula quadrula	X		X	X
Simpsonaias ambigua			X	
Strophitus undulatus undulatus	X	X	X	X
Toxolasma parvus	X		X	
Tritogonia verrucosa	X		X	X
Truncilla donaciformis	X		X	X
Truncilla truncata	X		X	X
Utterbackia imbecillis	X		X	
No. of species	33	20	34	22

[1]Nomenclature follows Turgeon et al. (1988) and Hoeh (1990).
[2]ESI (1995a)
[3]ESI (1993), USFWS (unpubl. data)
[4]Heath and Rasmussen (1990), ESI (1995b)
[5]Miller (1993), ESI (unpubl. data)

The St. Croix River at this point (approximately river mile 16.2) is a wide, riverine lake, which is pooled by Mississippi River Lock and Dam 3. The navigation channel follows the Minnesota bank and current is mostly restricted to the channel. At the I-94 bridge, the river is approximately 950 m wide and 4 to 6 m deep. The water is fairly clear and substrate is primarily sand with cobble and gravel in areas with flow. The reach between river mile 17.6 and 16.2 is listed as Essential Habitat for *Lampsilis higginsii* (USFWS 1983). A total of 34 species has been collected in the area (Table 1) including *L. higginsii* (federally endangered); *Cumberlandia monodonta, Cyclonaias tuberculata, Ellipsaria lineolata,* and *Elliptio crassidens* (Wisconsin endangered); and *Quadrula metanevra, Simpsonaias ambigua,* and *Tritogonia verrucosa* (Wisconsin threatened).

In July 1995, 24,557 unionids were relocated from the U.S. Highway 20 bridge construction area in the Wolf River near Shawano, Wisconsin. The Wolf River is a small high-gradient Lake Michigan drainage stream with riffles and pools, and is 70 m wide and up to 1.5 m deep in the relocation area. Substrate is mostly cobble and gravel with areas of hard pan clay and loose sand. A total of 22 species was collected in the project area including *Epioblasma triquetra* (Wisconsin endangered), *T. verrucosa* (Wisconsin threatened), and *Alasmidonta marginata* and *Pleurobema coccineum* (Wisconsin special concern species).

Materials and Methods

Before beginning each relocation, construction and potential impact areas were delineated. An area near the construction zone, with stable substrate and an existing unionid community, was selected as the relocation area, and 0.25 m^2-quadrat samples were collected by divers to determine substrate composition and existing unionid community characteristics.

Handling methods during collection and relocation varied somewhat among study areas, but several guidelines were followed. Unionids were collected and handled by experienced people, were not relocated during extreme hot or cold weather, were kept in water most of the time, and were handled in small batches.

In general, animals were kept moist or in water throughout each relocation. However, handling during transport varied among relocations. During the St. Croix and Wolf River relocations, unionids were quickly transferred from the river to a large (1.1 m x 0.8 m x 0.8 m) flow-through holding tank in the transport boat. Water siphoned from at least 0.5 m beneath the river's surface was continually pumped into the tank and allowed to flow out through a surface drain. A rack in the bottom of the tank prevented animals from lying in accumulated debris. Water temperature and dissolved oxygen were continually monitored in the tank, which was drained and cleaned at the end of each day.

Unionids were also quickly transferred out of the tank and into the river. Individuals were out of water for brief intervals during sorting, counting, marking, and measuring.

Unionids in the Meramec River relocation were transported between the collection and relocation area in moist burlap. Animals were briefly removed from the burlap for processing. Unionids collected in the Elk River were walked upstream to the relocation area in collecting bags, but were out of water during most of the 180 m-walk.

Monitoring grids adapted from Waller et al. (1993) were established in relocation areas. A limited number of unionids were marked with a sequential number (using a Dremel tool), measured (length in mm), weighed (g), aged (external annuli count), and placed in the grids. Unionids not placed in grids were identified, counted, and distributed in a designated general area.

Relocated unionids were monitored 1 month following relocation to assess immediate mortality and 1 year after relocation to assess long-term mortality. One month following the St. Croix, Meramec, and Wolf River relocations, a diver searched grid and general relocation areas, collecting any marked and unmarked dead shells. One year following all relocations (Meramec, St. Croix, and Elk River completed to date), one half of the grid cells were sampled by excavating the cells and collecting all unionids.

Observed mortality refers to mortality estimated from recovered shells and live unionids, since the fate of nonrecovered marked shells is unknown. Recovery refers to the percentage of marked unionids recovered during monitoring.

Results and Discussion

Preliminary data for relocations conducted in different rivers with different species and different handling techniques indicate that relocation can be successful if a few simple guidelines are followed. Recovery during the first year was high for most species in all studies even though handling methods varied among sites.

Observed mortality was minimal (< 1%) 1 month following the St. Croix, Wolf, and Meramec River relocations. Almost 600 unionids were marked and placed in monitoring grids in each of the St. Croix River relocations, and only one marked shell was recovered 1 month following each relocation (Table 2). No marked shells were found in Meramec or Wolf River grids, although shells could have been carried away by flow or predators, or buried and not readily observable. All unionids placed in the St. Croix and Wolf River general relocation areas were not examined. However, many live marked individuals were found while only three dead marked shells were observed in the 1994 St. Croix River general relocation area, and only six dead marked shells were observed in the 1995 St. Croix River and in the Wolf River general relocation areas (Table 2).

Recovery was high and observed mortality was minimal 1 year following the St. Croix, Meramec, and Elk River relocations, although recovery and observed mortality did vary among species within each relocation (Table 3). Recovery ranged from 50% in the Meramec River, a dynamic area with fairly unconsolidated gravel substrate and fairly high flow, to 96% in the Elk River, a dynamic small river with high flow but very consolidated substrate. Recovery of live marked unionids was 71% in the St. Croix River, although some mortality (1%) was observed.

In the Meramec River no marked shells were recovered and recovery of *L. abrupta* and *F. ebena* was over 80% 1 year after the relocation (Table 3). The only species with a low recovery rate (29%) was *A. confragosus*, which is typically an active species (Dunn, pers. obs.). In the St. Croix River, recovery was lowest for *Truncilla truncata* (50%) and highest for *Q. metanevra* (83%). Mortality was observed for *L. higginsii* (one individual) and *T. verrucosa* (two individuals). In the Elk River recovery was 100% for all species except *Quadrula p. pustulosa* (60%) and no marked shells were found.

Factors contributing to the success of these relocations appear to be careful handling and selection of the relocation area. Guidelines we think should be followed for a successful relocation include:

1. Use field personnel familiar with unionids.

Handling errors, such as roughly removing animals from the substrate, leaving animals out of water or in stagnant water, and not replacing animals in a natural position in the substrate, are minimized by using personnel familiar with unionid biology.

2. Select a relocation area with stable substrate and a similar unionid community that is near the collection area.

We agree with Sheehan et al. (1989) and Cope and Waller (1995) that site selection is one of the key factors in successful unionid relocations. Our goal was to select areas with stable substrate and an existing unionid community at least as species rich and dense as the construction area and that was as close as possible to the construction area. Placing unionids in stable substrate should enhance relocated unionid recovery (Sheehan et al. 1989). In many cases unionids occur in an unstable substrate, such as unconsolidated sand or gravel. However, these areas probably have a high degree of substrate and therefore unionid movement (Golightly 1982; Vannote and Minshall 1982; Huehner 1987). Although this may be natural (Matteson 1955) and may not result in unionid mortality, the probability of recovering relocated unionids is greater if the animals remain in a designated area.

Selecting a relocation area with an existing unionid community near the collection area should ensure that habitat conditions are suitable for unionids and similar to those under which the animals are currently living (such as water quality and fish species) as well as minimize transport time between construction and relocation areas. The variables determining unionid distribution are complex and attempts to quantify microhabitat and

Table 2. Monitoring results 1 month after relocation.

Site	Year	No. marked unionids placed in grids	No. marked shells recovered in grids	No. marked unionids placed in general area	No. marked shells recovered in general area
St. Croix River	1994	598	1	8,398	3
Meramec River	1994	61	0	4,453	NS
Elk River	1994	100	NS	102	NS
Wolf River	1995	831	0	23,726	6
St. Croix River	1995	591	1	13,436	6

NS=Not sampled

determine variables useful in predicting unionid distribution have met with little success (Strayer 1981; Holland-Bartels 1980; Strayer and Ralley 1993). A seemingly suitable area currently devoid of unionids may support unionids; however, unknown variables may be preventing natural unionid colonization. Selecting a relocation area with an existing unionid community reduces the chance of choosing unsuitable unionid habitat.

3. Keep animals moist or in water and minimize out-of-water time.

Unionids require humid conditions for gas exchange, and keeping animals moist will enhance survival (Waller et al. 1995).

4. Avoid extreme temperatures.

Unionid mortality during relocation has been attributed to extremely cold water and air temperature (Heath, pers. comm.; Miller, pers. comm.) and an extreme difference between water and air temperature (Koch, pers. comm.). Although temperature effects on survival have not been tested (Cope and Waller 1995), stress is typically evident in unionids held out of water on hot or cold days. Unionids should be relocated under moderate air and water temperatures, and animals should not be exposed to extreme cold or heat.

5. Avoid crowding animals.

Overcrowding may have negative effects on unionids due to waste accumulation and oxygen

Table 3. Monitoring results 1 year after relocation.

Species	No. placed in sampled cells[1]	No. collected live	No. shells collected	Percent recovered live	Percent observed mortality
Meramec River					
Arcidens confragosus	14	4	0	29	0
Fusconaia ebena	1	1	0	100	0
Lampsilis abrupta	7	7[2]	0	86	0
Obovaria olivaria	0	1[2]	0		0
Total	22	13	0	50	0
St. Croix River					
Amblema plicata plicata	129	98	0	76	0
Cyclonaias tuberculata	0				
Ellipsaria lineolata	10	7	0	70	0
Elliptio crassidens	4	3	0	75	0
Fusconaia flava	48	37	0	77	0
Lampsilis higginsii	20	13	1[2]	65	7
Obliquaria reflexa	43	28	0	65	0
Quadrula metanevra	24	20[2]	0	83	0
Tritogonia verrucosa	8	4	2	50	33
Truncilla truncata	30	15	0	50	0
Total	316	225	3	71	1
Elk River					
Actinonaias ligamentina	29	29	0	100	0
Elliptio crassidens	1	1	0	100	0
Fusconaia flava	1	1	0	100	0
Fusconaia subrotunda	10	10	0	100	0
Lampsilis ovata	5	5	0	100	0
Quadrula pustulosa pustulosa	5	3	0	60	0
Total	51	49	0	96	0

[1]Only half of the cells in each grid were sampled.
[2]Animal moved from adjacent cell into sampled cell.

depletion; however, research on these effects is currently lacking. The number of unionids placed in collecting bags was limited to approximately 100 and the number transported in the flow-through holding tank was limited to approximately 500.

Acknowledgments

Numerous organizations and people contributed to protocol preparation and the field efforts for each of these relocations. Funding was provided by the Wisconsin Department of Transportation, Missouri Highway and Transportation Department, and Columbia Gas and Transmission Corporation. Assistance with protocol preparation for these relocations was provided by Paul Burke and William Tolin (USFWS); Robert Hays, Rebecca Isenring, and Helen Kitchel (Wisconsin Department of Natural Resources-Bureau of Endangered Resources); Diane Waller and Gregory Cope (National Biological Service); and Gene Gardner (Missouri Highway and Transportation Department). These people, as well as Tom Watters and Glen Miller, assisted with field efforts. Diving services were provided by Ducktrail Ecological Services, Environmental Marine Services, and Underwater Technology Systems. Ecological Specialists, Inc., personnel who assisted us with field work include Brent McClane, Mike Freeman, Melody Giorgi, Dan Kelner, John Spiak, Mary Furman, and Chuck Theiling.

Literature Cited

Aquatic Resources Center. 1993. *Lemiox rimosus* transplant site survey 1993. Prepared for Duck River Development Agency, Shelbyville, Tennessee. 13 pp.

Burke, P.J. 1991. Draft issue paper on the apparent failure of a mussel relocation project on the St. Croix River near Prescott, Wisconsin. U.S. Fish and Wildlife Service, Twin Cities Field Office, Bloomington, Minnesota. 4 pp.

Cope, W.G., and D.L. Waller. 1995. Evaluation of freshwater mussel relocation as a conservation and management strategy. Regulated Rivers: Research and Management 11(2):147-155.

Dunn, H.L. 1993. Survival of unionids four years after relocation. Pages 93-99 *in* K.S. Cummings, A.C. Buchanan, and L.M. Koch, eds. Conservation and management of freshwater mussels. Proceedings of a UMRCC symposium, 12-14 October 1992, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

Dunn, H.L. 1994. Final report: St. Croix River I-94 bridge replacement unionid relocation protocol. Prepared for Wisconsin Department of Transportation, Eau Claire, Wisconsin. 36 pp.

Ecological Specialists, Inc. 1993. Unionid investigation of a proposed pipeline crossing on the Elk River, West Virginia. Final report prepared for GAI Consultants, Inc., Monroeville, Pennsylvania. 9 pp.

Ecological Specialists, Inc. 1995a. Meramec River I-55 bridge crossing unionid relocation. Final report prepared for Missouri Highway and Transportation Department, Jefferson City. 22 pp.

Ecological Specialists, Inc. 1995b. St. Croix River I-94 bridge replacement unionid relocation and monitoring: 1994. Report prepared for EnviroScience, Eden Prairie, Minnesota. 28 pp.

Golightly, C.G., Jr. 1982. Movement and growth of Unionidae (Mollusca: Bivalvia) in the Little Brazos River, Texas. Ph.D. Dissertation, Texas A & M University, College Station. 100 pp.

Harris, J.L. 1984. Relocation of the pink mucket pearly mussel (*Lampsilis orbiculata*) in the Spring River near Ravenden, Lawrence County, Arkansas. Environmental Division, Arkansas State Highway and Transportation Department. 9 pp.

Harris, J.L. 1986. Relocation of the fat pocketbook pearly mussel, *Proptera capax* (Green), in the St. Francis River at Madison, St. Francis County, Arkansas. Environmental Division, Arkansas State Highway and Transportation Department. 14 pp.

Harris, J.L. 1989. Relocation of the Arkansas fatmucket, *Lampsilis powelli* (Lea, 1852), at the Arkansas Highway 291 bridge, Saline-Grant Counties, Arkansas. Environmental Division, Arkansas State Highway and Transportation Department, Job #R60021. 9 pp.

Harris, J.L, R.H. Doster, and J. McLean. 1992. Relocation of the Arkansas fatmucket, *Lampsilis powelli* (Lea), at the U.S. Highway 270 bridge in Mount Ida, Montgomery County, Arkansas. Environmental Division, Arkansas State Highway and Transportation Department. 15 pp.

Hartfield, P. 1993. Headcuts and their effects on freshwater mussels. Pages 131-141 *in* K.S. Cummings, A.C. Buchanan, and L.M. Koch, eds. Conservation and management of freshwater mussels. Proceedings of a UMRCC symposium, 12-14 October 1992, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

Heath, D.J., and P.W. Rasmussen. 1990. Results of baseline sampling of freshwater mussel communities for long-term monitoring of the Saint Croix National Scenic Riverway, Minnesota and Wisconsin. Wisconsin Department of Natural Resources. 45 pp.

Hoeh, W.R. 1990. Phylogenetic relationships among eastern North American *Anodonta* (Bivalvia: Unionidae). Malacological Review 23(1-2):63-82.

Holland-Bartels, L.E. 1990. Physical factors and their influence on the mussel fauna of a main channel border habitat of the Upper Mississippi River. Journal of the North American Benthological Society 9(4):327-335.

Hubbs, D., T. Hunt, and R.D. Kathman. 1991. *Lemiox rimosus* transplant site survey. Prepared for Upper Duck River Development Agency, Shelbyville, Tennessee. 11pp.

Huehner, M.K. 1987. Field and laboratory determination of substrate preferences of unionid mussels. Ohio Journal of Science 87(1):29-32.

Jenkinson, J. 1985. Freshwater mussel transplants evaluated. AMU News 16(1):3.

Jenkinson, J.L. 1989. Relocation of *Potamilus capax* from a 4-mile reach of the St. Francis floodway in Arkansas. Prepared for Memphis District, U.S. Army Corps of Engineers, Contract #PD-88-CO44. 53 pp.

Koch, L.M. 1993. Status of fat pocketbook mussels (*Potamilus capax*) three years after re-introduction to the upper Mississippi River, Missouri. Missouri Department of Conservation. 12 pp.

Layzer, J.B., and M.E. Gordon. 1993. Reintroduction of mussels into the upper Duck River, Tennessee. Pages 89-92 *in* K.S. Cummings, A.C. Buchanan, and L.M. Koch, eds. Conservation and management of freshwater mussels. Proceedings of a UMRCC symposium, 12-14 October 1992, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

Matteson, M.R. 1955. Studies on the natural history of the Unionidae. American Midland Naturalist 53(1):126-145.

Miller, A.C., L. Rhodes, and R. Tippit. 1984. Changes in the naiad fauna of the Cumberland River below Lake Cumberland in central Kentucky. Nautilus 98(3):107-110.

Miller, G.A. 1993. Threatened and endangered mollusk species survey (Mollusca: Unionidae) of the Wolf River. Prepared for Rust E & I, Inc., Madison, Wisconsin. 3 pp.

Oblad, B.R. 1980. An experiment in relocating endangered and rare naiad mollusks from a proposed bridge construction site at Sylvan Slough, Mississippi River, near Moline, Illinois. Pages 211-222 *in* J.L. Rasmussen, ed. Upper Mississippi River bivalve mollusks. Proceedings of a UMRCC symposium. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

Neves, R.J. 1993. State-of-the-unionids address. Pages 1-10 *in* K.S. Cummings, A.C. Buchanan, and L.M. Koch, eds. Conservation and management of freshwater mussels. Proceedings of a UMRCC symposium, 12-14 October 1992, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

Parmalee, P.W., and M.H. Hughes. 1993. Freshwater mussels (Mollusca: Pelecypoda: Unionidae) of Tellico Lake: twelve years after impoundment of the Little Tennessee River. Annals of the Carnegie Museum 62(1):81-93.

Sheehan, R.J., R.J. Neves, and H.E. Kitchel. 1989. Fate of freshwater mussels transplanted to formerly polluted reaches of the Clinch and North Fork Holston rivers, Virginia. Journal of Freshwater Ecology 5(2):139-149.

Stansbery. D.H. 1970. Eastern freshwater mollusk (I) The Mississippi and St. Lawrence River systems. American Malcological Union Symposium on Rare and Endangered Mollusks. Malacologia 10(1):9-22.

Stansbery, D.H. 1971. Rare and endangered mollusks of the eastern United Sates. Pages 5-18 *in* S. E. Jorgensen and R. W. Sharp, eds. Proceedings of a symposium on rare and endangered mollusks (naiads) of the U.S. U.S. Fish and Wildlife Service, Twin Cities, Minnesota. 79 pp.

Stein, C.B. 1972. Population changes in the naiad mollusk fauna of the lower Olentangy River following channelization and highway construction. Bulletin of the American Malacological Union, Inc. 1971: 47-48.

Strayer, D.L. 1981. Notes on the microhabitats of unionid mussels in some Michigan streams. American Midland Naturalist 106(2):411-415.

Strayer, D.L., and J. Ralley. 1993. Microhabitat use by an assemblage of stream-dwelling unionaceans (Bivalvia), including two rare species of *Alasmidonta*. Journal of the North American Benthological Society 12(3):247-258.

Suloway, L., J.J. Suloway, and E.E. Herricks. 1981. Changes in the freshwater mussel (Mollusca: Pelecypoda: Unionidae) fauna of the Kaskaskia River, Illinois, with emphasis on the effects of impoundment. Transactions of the Illinois Academy of Science 74(1-2):79-90.

Turgeon, D.D., A.E. Bogan, E.V. Coan, W.K. Emerson, W.G. Lyons, W.L. Pratt, C.F.E. Roper, A. Scheltema, F.G. Thompson, and J.D. Williams. 1988. Common and scientific names of aquatic invertebrates from the United States and Canada: mollusks. American Fisheries Society Special Publication 16. 277 pp. + 12 plates.

U.S. Fish and Wildlife Service. 1983. Higgins' eye mussel recovery plan. Prepared by Higgins' Eye Recovery Team for the U.S. Fish and Wildlife Service, Rockville, Maryland. 98 pp.

Vannote, R.L., and G W. Minshall. 1982. Fluvial processes and local lithology controlling abundance, structure, and composition of mussel beds. Proceedings of the National Academy of Science 79(13):4103-4107.

Waller, D.L., J.J. Rach, W.G. Cope, and J.A. Luoma. 1993. A sampling method for conducting relocation studies with freshwater mussels. Journal of Freshwater Ecology 8(4):397-399.

Waller, D.L., J.J. Rach, W.G. Cope, and G.A. Miller. 1995. Effects of handling and aerial exposure on survival of unionid mussels. Journal of Freshwater Ecology 10(3):199-207.

Williams, J.D., S.L.H. Fuller, and R. Grace. 1992. Effects of impoundments on freshwater mussels (Mollusca: Bivalvia: Unionidae) in the main channel of the Black Warrior and Tombigbee rivers in western Alabama. Alabama Museum of Natural History 13:1-10.

Yokley, P., Jr. 1976. The effect of gravel dredging on mussel production. Bulletin of the American Malacological Union, Inc. 1976:20-22.

J. N. Am. Benthol. Soc., 2007, 26(2):253–260

PIT tags increase effectiveness of freshwater mussel recaptures

Jennifer Kurth[1]

Department of Wildlife Ecology, 5755 Nutting Hall, University of Maine, Orono, Maine 04469-5755 USA

Cynthia Loftin[2] AND **Joseph Zydlewski**[3]

Maine Cooperative Fish and Wildlife Research Unit, US Geological Survey, 5755 Nutting Hall, University of Maine, Orono, Maine 04469-5755 USA

Judith Rhymer[4]

Department of Wildlife Ecology, 5755 Nutting Hall, University of Maine, Orono, Maine 04469-5755 USA

Abstract. Translocations are used increasingly to conserve populations of rare freshwater mussels. Recovery of translocated mussels is essential to accurate assessment of translocation success. We designed an experiment to evaluate the use of passive integrated transponder (PIT) tags to mark and track individual freshwater mussels. We used eastern lampmussels (*Lampsilis radiata radiata*) as a surrogate for 2 rare mussel species. We assessed internal and external PIT-tag retention in the laboratory and field. Internal tag retention was high (75–100%), and tag rejection occurred primarily during the first 3 wk after tagging. A thin layer of nacre coated internal tags 3 to 4 mo after insertion, suggesting that long-term retention is likely. We released mussels with external PIT tags at 3 field study sites and recaptured them with a PIT pack (mobile interrogation unit) 8 to 10 mo and 21 to 23 mo after release. Numbers of recaptured mussels differed among study sites; however, we found more tagged mussels with the PIT-pack searches with visual confirmation (72–80%) than with visual searches alone (30–47%) at all sites. PIT tags offer improved recapture of translocated mussels and increased accuracy of posttranslocation monitoring.

Key words: PIT tags, freshwater mussels, survival, recapture, *Lampsilis radiata radiata*, translocation.

A goal in the national strategy for the conservation of native freshwater mussels is to "develop, evaluate, and use the techniques necessary to hold and translocate large numbers of adult mussels" (National Native Mussel Conservation Committee 1997). Successful recovery of translocated mussels is essential for accurate assessment of translocation success. Previous studies of freshwater mussel translocation used visual searches to recover mussels with varied success (Layzer and Gordon 1993, Havlik 1995, Bolden and Brown 2002, Cope et al. 2003). Survival estimates of translocated mussels often are based on the number of mussels recaptured or found dead, and mussels that are not recaptured are assumed to have emigrated from the study site (Dunn and Sietman 1997, Hamilton et al. 1997, Dunn et al. 2000). A review of 33 mussel translocation studies found a mean estimated survival rate of 51% (but mortality was not reported in 27% of the studies); the average recapture rate was 43% (range: 1–97%) (Cope and Waller 1995).

Passive integrated transponder (PIT) tags may be an effective tool for tracking translocated mussels to increase accuracy of survival estimates. PIT tags are electronic glass-encased microchips that are activated by an inductive coil. They can be attached to an organism internally or externally. The tag is passive until activated by a fixed or portable reader with an antenna. When activated, the tag transmits a unique code to the reader, identifying the individual organism (Gibbons and Andrews 2004). Tag longevity is indefinite because an internal power source is not needed. In aquatic systems, PIT tags have been used extensively to study fish passage past stationary antennae or readers (Zydlewski et al. 2001). Portable PIT-tag systems are used in shallow waters to assess spatial distributions of local fish populations, fine-scale

[1] E-mail address: jennifer_kurth@umit.maine.edu
[2] To whom correspondence should be addressed. E-mail: cyndy_loftin@umenfa.maine.edu
[3] E-mail addresses: jzydlewski@usgs.gov
[4] judith_rhymer@umit.maine.edu

movements, and microhabitat preferences (Roussel et al. 2000, Hill et al. 2006). This mobile application is ideally suited to freshwater mussel translocation studies because mussel movements often occur over short distances.

Traditional mussel recapture methods depend on visual encounters and excavation to locate burrowed mussels. PIT tags may enhance mussel recapture at sites where visibility is poor (e.g., turbid water) or when mussels are burrowed in sediments. Reliability of any tagging method depends on tag retention. The tagging method selected for freshwater mussels depends on shell thickness and the type of habitat into which the tagged mussels will be placed. Internal tagging may be best for thick-shelled species, whereas external PIT-tag placement may be more appropriate for thin-shelled species. In a fast-flowing environment with a rocky substrate, an external PIT tag might be dislodged, whereas an internal PIT tag would be protected from abrasion.

We designed an experiment to evaluate the use of PIT tags to mark and track individual freshwater mussels as part of a larger study to determine the feasibility of translocations of 2 state-listed threatened mussel species (tidewater mucket [*Leptodea ochracea*] and yellow lampmussel [*Lampsilis cariosa*]) in response to an impending dam removal. The objectives of our study were to evaluate internal and external PIT-tagging methods, retention, and posttagging survival in freshwater mussels and to determine the effectiveness of PIT-tag technology for mussel recaptures. We used the relatively common eastern lampmussel (*Lampsilis radiata radiata*) as a surrogate for the listed species to develop the method. We tested internal tagging methods for future use with thick-shelled species (e.g., yellow lampmussel) and external attachment for use with thin-shelled species (e.g., tidewater mucket).

Methods

Internal PIT tagging: mantle separation

We used 2 methods to place internal PIT tags. For method 1 (mantle separation), we placed the mussels in sandy substrate, waited until they were actively siphoning and slightly gaped, and then inserted a micropipette tip between the valves to separate them by ~5 mm. We teased the mantle tissue away from the shell and inserted the PIT tag (Digital Angel, South St. Paul, Minnesota) between the mantle and shell along the midventral margin. We also marked all mussels externally with numbered bee tags (The Bee Works, Orillia, Ontario) cemented (GC Fuji I Glass Ionomer Luting Cement; Henry Schein, Melville, New York) to the posterior end of the left valve. We sealed the bee tags with Delton Light Curing Pit and Fissure Sealant (Henry Schein). Control mussels received only the numbered bee tags. We were able to tag ~20 mussels/h with this method. Most of our time was spent waiting for mussels to gape so we could insert the micropipette tip.

In October 2004, we collected eastern lampmussels (55–101 mm length, $n = 164$) from the impoundment that will be dewatered following the Fort Halifax dam removal in the Sebasticook River near Winslow, Maine. In November 2004 (24–35 d after capture), we partitioned the mussels into a control ($n = 40$) and 3 tag-type treatment groups: 23-mm tags ($n = 40$), 12-mm tags ($n = 44$), and 12-mm tags with an antimigration cap (a plastic sleeve encasing one end of the 12-mm tag to encourage tissue adherence; Biomark, Boise, Idaho; $n = 40$). Each group consisted of mussels of all sizes (control: length 55–99 mm, 23-mm tags: length 58–101 mm, 12-mm tags: length 58–99 mm, 12-mm tags with cap: length 58–96 mm).

We maintained mussels in the Aquaculture Research Center (ARC), University of Maine, Orono, Maine, in three $2.44 \times 0.61 \times 0.30$-m fiberglass tanks filled with sand (13 cm deep) and recirculating water. We divided the mussels in each group among 3 replicates (13–15 mussels/replicate) and distributed 1 replicate from each group in each tank.

We fed the mussels an algal diet (*Phaeodactylum tricornutum*, *Chaetocerus-B*., and *Nannochloropsis oculata*; Algae Spat Formula [Innovative Aquaculture Solutions, Inc., Vancouver, British Columbia]) 3 times/wk. During each feeding, we stopped water recirculation and applied 40 to 50×10^9 algal cells/tank (R. Mair, Virginia Polytechnic Institute and State University, personal communication). To simulate changes in seasonal water temperature, we gradually reduced water temperature from 18°C (October) to 10°C (December) and maintained 10°C until the following April, then gradually increased the temperature to 18°C by June. We monitored the mussels for mortality 3 times/wk and examined them for tag retention in November 2004 and in February, April, and June 2005.

Internal PIT tagging: mantle incision

We developed a 2nd internal PIT-tagging method (mantle incision) with techniques from the cultured pearl industry (H. Dan, Virginia Polytechnic Institute and State University, personal communication). We implanted PIT tags by inserting a micropipette tip between the mussel valves to separate them by ~5 mm, making an incision with a scalpel in the midventral mantle tissue, inserting the tag between the mantle and the shell through the incision, and then removing the micropipette tip. We also marked all mussels externally with bee tags on the posterior end of the left valve. Inserting the tags took little time (20 mussels/h). Most of our time was spent waiting for mussels to gape so we could insert the micropipette tip.

In June 2005, we collected 112 eastern lampmussels (43–101 mm length) from the Sebasticook River impoundment and randomly assigned the mussels into 3 groups consisting of a control ($n = 27$) and 2 tag-type treatment groups (23-mm tags: $n = 43$, 12-mm tags with cap: $n = 42$) with 3 replicates/group (9–15 mussels/replicate), being careful to include mussels of all sizes in each group. We did not test the 12-mm tags without caps because of poor retention in the mantle-separation experiment.

We maintained tagged mussels in the ARC for 21 d to ensure tag retention and then placed 1 replicate from each group in sand in each of 3 enclosures (1 × 2-m polyvinyl chloride [PVC] pipe and rebar frames covered in hardware cloth) in Unity Pond, Maine. Unity Pond is a 1039-ha lake connected to the Sebasticook River upstream of the Winslow mussel collection site. Unity Pond contains a natural population of eastern lampmussels and thus is suitable habitat for the species. Before placing the mussels in the enclosures, we reinserted rejected tags ($n = 9$). We examined the mussels to assess tag retention and survival 60 d (August 2005) and 371 d (June 2006) after tagging.

External PIT tagging

We tested the reliability of external PIT-tag attachment and determined the probability of recapturing translocated PIT-tagged mussels that were not confined to enclosures (as in the previous experiment). We placed external PIT tags on 238 eastern lampmussels (41–88 mm length) collected during September and October 2004 from various sites in Unity Pond ($n = 90$), Sandy Stream (a 1st-order, spring-fed stream that drains into Unity Pond; $n = 88$), and the Sebasticook River impoundment near Winslow ($n = 60$). We chose these water bodies because they had naturally occurring populations of eastern lampmussels and the 2 listed species, and because, based on neutral markers, Sebasticook River and Sandy Stream populations of these mussels were genetically similar (Kelly 2004).

We tagged mussels by cementing a PIT tag to the posterior end of the right valve and a numbered bee tag to the posterior end of the left valve. After the first 30 tags (at Unity Pond), we completely encapsulated the PIT tag in dental cement to increase tag retention. We placed tagged mussels in water before the cement was fully cured (~5 min after application) to avoid overdrying and cracking of the cement. We tagged ~30 mussels/h with this method. Most of our time was spent waiting for the bee-tag sealant to dry. We used 23-mm tags at all sites. We also used some 12-mm tags at Sandy Stream and Unity Pond because of a limited supply of cement.

TABLE 1. Numbers of mussels tagged with passive integrated transponder tags in each translocation treatment during September and October 2004.

Site	Tagged and replaced (site control)	Moved within water body	Translocated from Sebasticook River
Sandy Stream	30	26	32
Unity Pond	30	30	29
Sebasticook River	30	30	–

We compared survival of translocated mussels among within-water body, between-water body, and within-site (control) translocation treatments. We measured, tagged, and moved mussels to 1 × 2-m plots or replaced them where they had been found (Table 1). We marked the corners of the plots with stakes with flagging, and recorded Global Positioning System (GPS) locations for each plot and for each of the tagged mussels that were returned to their original location.

We recaptured externally PIT-tagged mussels with a mobile PIT detection unit (PIT pack). The PIT pack used Destron Fearing FS1001A DC-powered, full duplex transceivers and custom-designed portable antennas. When a PIT tag was within range of an antenna (~0.5 m), the tag emitted a 134.2-kHz (ISO standard frequency) radio frequency, which was transmitted back to the receiver for decoding. The antennas, enclosed in an airtight PVC wand and attached to the transceiver, consisted of several wraps of 12- to 18-gauge wire, with inductance values ranging from 325 to 375 μH and a set of capacitors (Hill et al. 2006). The capacitors were attached to an antenna lead cable from the transceiver, fixing the capacitance between 33 and 44 nF. The fixed capacitance was used within the transceiver in conjunction with the adjustable capacitance to tune the resonance frequency of the system to 134.2 kHz (Hill et al. 2006). We tuned the adjustable capacitor while antennas were submerged. We conducted all field experiments with the PIT pack tuned to phase 0 to 2%, signal 1 to 20%, and current 2.5 to 5.0 amps.

We searched the release sites for externally PIT-tagged mussels ~30 d after tagging (October 2004) and visually confirmed recaptures with snorkeling. If the PIT-tag reader registered a tag but no mussel was observed, we assumed the mussel had burrowed into the substrate. To minimize substrate disturbance, we did not excavate burrowed mussels preparing to overwinter. These data were not used in the calculations of recapture success because the signals may have been from detached tags.

During June and July 2005 (271–355 d after tagging) and July and August 2006 (670–750 d after tagging), we searched again for PIT-tagged mussels at the release sites, beginning at the last location recorded with GPS during October 2004. In 2005, we conducted initial searches without the PIT pack to provide recapture percentages with visual searches only. We visually searched each site for 2 d. Approximately 1 wk later, we searched the sites using PIT-pack searches with visual confirmation and excavation to confirm recaptures (3–4 d/site). In 2006, we repeated the PIT-pack searches with visual confirmation (3 d/site). Water clarity was too poor to conduct visual searches in 2006. If the PIT pack detected a tagged mussel, but we did not see the mussel, we excavated the area within 0.5 m of the signal to 15 to 45 cm deep to determine if the signal was coming from a burrowed mussel or an unattached tag. If we found no tagged mussel after excavation, we assumed the tag had become detached. We searched (with snorkeling and the PIT pack) the sites at Unity Pond and the Sebasticook River 4 times each to at least 3 m beyond the perimeter of the original study area to detect mussels that may have moved. We also searched the shorelines for valves from dead mussels. Extensive ice scouring and spring flooding substantially reconfigured the substrate at the Sandy Stream site, so in addition to searching the study area plus 3 m beyond the perimeter, we also swept the antenna bank to bank downstream of the site for 200 m over a total of 3 d. We calculated recapture rates by dividing the number of mussels recaptured at each site by the number tagged.

Data analysis

We used adjusted χ^2 for small sample sizes (Gotelli and Ellison 2004) for all analyses.

We compared long-term tag retention among tag types and mussel mortality among treatments and controls for both mantle separation and mantle incision methods. We compared the percentages of recaptures using visual searches alone with the number of recaptures using PIT-pack searches with visual confirmation.

Results

Mussel retention of internal PIT tags in the laboratory (mantle separation)

Five percent of the PIT tags were rejected within 2 wk of internal placement via mantle separation. By 100 d after tagging, rejection had increased to 10% for 12-mm tags with caps, 12.5% for 23-mm tags, and 30% for 12-mm tags without caps. High mortality with this method was more troubling than the rejection rates. By 100 d after tagging, mortality rates were 3% for the control group (no tags), 10% for the group with 12-mm tags with caps, 25% for the group with 23-mm tags, and 27% for the group with 12-mm tags without caps. This mortality may have been caused by inexperience with the tagging procedures and mussel aquaculture husbandry (mortality in control mussels was 3% 100 d after tagging and 73% 244 d after tagging), so we discontinued using the 12-mm tags without caps, switched to the mantle-incision method, and retained the tagged mussels in field enclosures.

Long-term tag retention did not differ among tag types (adjusted $\chi^2 = 5.61$, $p = 0.691$, df = 8), and mortality did not differ among the tag-type and control groups (adjusted $\chi^2 = 7.97$, $p = 0.716$, df = 11) 100 d after tagging. We examined the condition of the PIT tags in all mussels that died over winter. By 90 d after tagging, all 12-mm PIT tags with caps were coated with nacre and attached to a valve. By 120 d after tagging, 23-mm and 12-mm PIT tags without caps that had not been rejected were similarly attached.

Mussel retention of internal PIT tags in field enclosures (mantle incision)

All mussels in the control and tag-type groups (mantle incision) were still alive 60 d after tagging (40 d after transport from the ARC to the Unity Pond enclosures) (Table 2). One 23-mm tag was rejected after the mussels were placed in the enclosures; this rejected tag was not one of the tags that had been rejected and reinserted within the 2-wk posttagging observation period. By June 2006 (371 d after tagging), 2 mussels in the enclosures had died (1 control, 1 with a 23-mm tag), and one 12-mm tag with cap was rejected. Long-term tag retention did not differ among tag types (adjusted $\chi^2 = 4.26$, $p = 0.833$, df = 8), and mortality did not differ among control and tag-type groups (adjusted $\chi^2 = 3.72$, $p = 0.882$, df = 11) 371 d after tagging.

Retention of external PIT tags and recapture of mussels in the field

Overall, ~93% of the recaptured tagged mussels retained the PIT tag (Table 3). Recapture rates with PIT-pack searches with visual confirmation exceeded recaptures from visual searches alone at all study sites during June and July 2005 (adjusted $\chi^2 = 10.198$, $p = 0.0014$, df = 1; Fig. 1). During June and July 2005 and July and August 2006, we used a combination of visual searches alone and PIT-pack searches with visual

TABLE 2. Percent mortality and % tag retention (60 d and 371 d after tagging using the mantle-incision method) of eastern lampmussels with internal passive integrated transponder tags in field enclosures in Unity Pond, Maine.

	60 d after tagging		371 d after tagging	
Treatment	% mortality	% tag retention	% mortality	% tag retention[a]
23-mm tag ($n = 43$)	0	98	2.5	97.5
12-mm tag with cap ($n = 41$)	0	100	0	97.4
Control (no tag) ($n = 27$)	0	–	4.3	–

[a] Includes mussels that died with retained tags

confirmations to recapture 77% of externally tagged mussels at Unity Pond and 80% of externally tagged mussels in the Sebasticook River (combined results from 2005 and 2006 recaptures). In Sandy Stream, where ice scouring and spring flooding reconfigured the substrate, we recovered only 25% of the tagged mussels. Ninety-five percent of the mussels we did recapture were found using PIT-pack searches with visual confirmation, and only 1 mussel was found using visual searches alone. In Sandy Stream, we found 71% of recaptured mussels >100 m from their October 2004 locations, whereas we found recovered mussels in Unity Pond and the Sebasticook River <2 m from their September–October 2004 locations. Seventeen percent (Unity Pond), 17% (Sebasticook River), and 3.5% (Sandy Stream) of the recaptured mussels found with the PIT pack were completely burrowed into the substrate (Fig. 1). We found most burrowed mussels within 6 cm of the sediment surface. However, the PIT pack detected 1 tagged (23-mm tag) living mussel burrowed 45 cm into the substrate and 3 tagged dead mussels 20 to 30 cm below the substrate surface in Sandy Stream. We also found 1 dead mussel with a PIT tag during shore sweeps at the Sebasticook River site.

Discussion

Tagging methods

Low mortality (<2%), high tag retention (~97%), and evidence that tags had fused to the shell 3 to 4 mo after tagging suggest that internal PIT tagging using the mantle-incision method may be a viable method of tagging thick-shelled freshwater mussel species that can be pried open for tag insertion without damaging the shell. Long-term survival of captive freshwater mussels is low (Patterson et al. 1997, 1999, Nichols and Garling 2002), and high mortality of captive mussels in our study (73–93% 255 d after tagging) might be attributed to inadequate nutrition, winter water temperatures in the ARC that exceeded temperatures at the mussel collection sites, and physiological stresses experienced by captive mussels that were gravid when captured. The low mortality of mussels tagged with the mantle-incision method and placed in the enclosures at Unity Pond supports this assertion. We strongly recommend field trials rather than aquaculture experiments for testing methods intended for use in the field to remove uncertainty of the effects of captivity on mussel survival.

External PIT-tag retention also was high (~93%)

TABLE 3. Percent recapture, % mortality, and % tag retention of externally passive integrated transponder–tagged eastern lampmussels in translocation experiments within and among sites (~21 mo after tagging) in Maine.

Site[a]	Treatment	Number tagged	% recapture	% mortality[b]	% tag retention[c]
Unity Pond	Translocated from Sebasticook River impoundment	29	93.1	0	100
	Translocated within Unity Pond	32	74.2	0	78.3
	Site control (not moved)	30	63.3	0	89.5
Sebasticook River	Translocated within Sebasticook River impoundment	30	93.3	0	96.4
	Site control (not moved)	30	66.7	6.7	100
Total		151	78.0	1.3	93.2

[a] Sandy Stream data omitted because of winter ice scouring and spring flooding
[b] Percent mortality calculated only for recaptured mussels
[c] Retention calculated as % recaptured mussels retaining tags

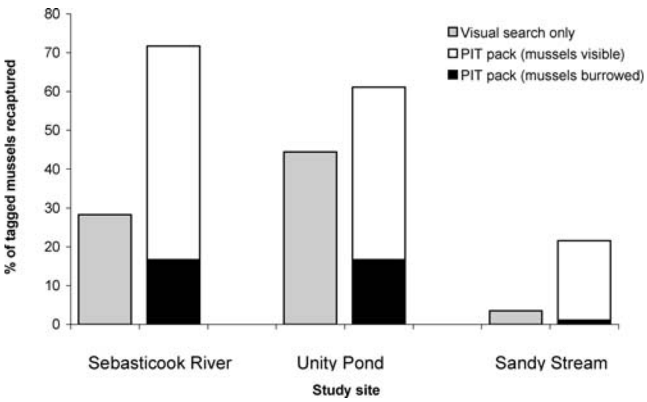


FIG. 1. Percentages of mussels externally tagged with passive integrated transponder (PIT) tags recaptured using different methods during June and July 2005.

when the PIT tag was completely encapsulated in cement and the mussel was placed in water within 5 min of cementing. However, retention was more variable with external tagging than with internal tagging methods, and ranged from ~78 to 100% at the Unity Pond site 9 mo after tagging. We attribute low retention to incomplete coverage with cement. Retention of tags completely encapsulated with cement ranged from 89.5 to 100%. We observed evidence of some cement loss from recaptured mussels; occasional reapplication of cement will ensure long-term retention of external PIT tags. Internal tag placement via mantle incision is a viable alternative to external attachment in environments where tag loss from abrasion is likely.

Previous studies assessed external freshwater mussels tagging methods with visual searches to relocate mussels marked with numbered tags (Lemarié et al. 2000) or coded wire tags inserted into mussels held in suspended pocket-nets (Layzer and Heinricher 2004). Both of these tagging methods resulted in higher tag retention than in our study, but mussels tagged using these methods can be detected only with visual searches. PIT tags provide an alternative tool for finding mussels, and this method is especially useful for long-term monitoring or where visual searches are impractical or time consuming.

Mussel recapture efficiency

The proportion of mussels visible at the substrate surface may vary by locality, time of year, species, and gender. Smith et al. (2001) detected only 31% of clubshells (*Pleurobema clava*) at the substrate surface, whereas 52% of northern riffleshells (*Epioblasma torulosa rangiana*; 80% females, 45% males) were visible. Wick (2006) observed that >90% of eastern lampmussels had burrowed to 10 to 15 cm at Sandy Stream by August, but only 26% had burrowed in the Sebasticook River impoundment at that time.

Because the water was turbid, we found burrowed mussels and mussels that would have been overlooked had the sites been searched only visually. For example, water clarity in Unity Pond was routinely poor, and only 47% of tagged mussels were recaptured visually, whereas 72% of tagged mussels were recaptured with the PIT pack and visual confirmation. In the Sebasticook River, where the visibility was compromised by silt covering the mussels, the recaptures with the PIT pack and visual confirmation (80%) were >2× those of the visual searches alone (29%). Initially, PIT tags also provided a visual cue of tagged mussels in clear water, but after several months in the water, the cement was stained or covered with algae and indistinguishable from the shell. When first applied, the white cement might provide a visual cue to predators, but only 1 shell was found in a shoreline midden in our study. Tinting the cement a dark color might eliminate this possible problem.

Low recaptures in Sandy Stream probably were caused by extensive downstream displacement of mussels in late winter and early spring when ice scour and high water flows during snowmelt reconfigured the stream bottom. The low recapture rates of PIT-tagged mussels at this site were attributed to tag loss from severe abrasion, burial in sediment beyond the detection limit, or transport beyond the regions searched.

Limitations of PIT tags in field applications

Debris on the substrate and signal interference caused by nearby iron objects (Hill et al. 2006) can affect reliability of the PIT pack. The antenna configuration we used also is limited to sites with water depth <2 m. Maximum effective depth and antenna range are not necessarily uniform among sites; these limitations should be identified at each field site so that mussel absence can be distinguished from nondetection caused by equipment limitations. Reducing the antenna size for use while snorkeling, waterproofing the PIT pack for diver use, and lengthening the antenna handle are modifications that will broaden field use of this tool. At present, PIT-tag use is limited to larger mussels (length >20 mm). However, smaller tags with greater detection ranges are in development, and eventually it should be possible to tag smaller mussels, at least externally. Although internal tags were retained, the ~3-wk captive period to ensure tag retention could limit the usefulness of internal tags. Internally tagged mussels should be held in field enclosures during the initial posttagging period when tag rejection may occur. Retaining a subset of internally tagged mussels may be a viable alternative for estimating tag retention proportions when large numbers of mussels are translocated.

The initial cost of the PIT tags and reader may exceed start-up costs for other mussel-tagging methods. The PIT pack (transceivers, batteries, antenna) we used cost ~$10,000 to construct and was designed for research on a variety of organisms such as fish, mussels, and amphibians. Smaller units can be developed for ~$2500. The PIT tags we used cost $3.50 each, but the tags work indefinitely. On the other hand, the percentage of tagged mussels recaptured using PIT tags far exceeded the percentage recaptured during visual searches. Visual searches can be time consuming and labor intensive. For long-term monitoring of individuals and populations, the added initial costs may be recouped over time, and it may be possible to share the costs with other investigators using PIT tags.

In conclusion, PIT tags permit repeated, nondestructive sampling of individuals with little disturbance, last indefinitely, and appear to have negligible effects on short-term survival of freshwater mussels. PIT tags were retained using both internal and external attachment methods. Thus, the choice of tagging method will depend on shell thickness, habitat characteristics, and ease of implementation in the field.

The need for freshwater mussel translocations to protect and conserve threatened and endangered mussel species will increase as aquatic habitat alteration continues. Superior recapture rates with PIT tags suggest that this tool is valuable for use in mussel translocations and monitoring and may improve accuracy of survival estimates for assessing translocation success. Because PIT tags have indefinite longevity, they can be used in monitoring both translocated mussels and populations at sites of concern, especially populations of endangered or threatened species. Moreover, because PIT tags provide reliable individual identification, they may be a useful tool for monitoring the growth and survival of individual mussels.

Acknowledgements

The authors thank C. Currier and R. Clark for field assistance, N. Greenberg (University of Maine Aquaculture Research Center), B. Swartz, and K. Kemper (Maine Department of Inland Fisheries and Wildlife), and R. Mair, H. Dan, and R. Neves (Virginia Polytechnic Institute and State University) for advice and assistance. We thank G. Zydlewski, M. Kinnison, B. Swartz, 2 anonymous referees, and D. Strayer for reviewing early drafts of the manuscript. Our research was funded by the Maine Department of Inland Fisheries and Wildlife through the State Wildlife Grants Program and the Endangered and Nongame Wildlife Fund, and by the US Geological Survey–State Partnership Program, the US Geological Survey Maine Cooperative Fish and Wildlife Research Unit, the Maine Outdoor Heritage Fund, and the University of Maine, Department of Wildlife Ecology. Reference to trade names and commercial products does not imply endorsement or recommendation by the US Government. This is article no. 2903 of the Maine Agricultural and Forest Experiment Station.

Literature Cited

Bolden, S. R., and K. M. Brown. 2002. Role of stream, habitat, and density in predicting translocation success in the threatened Louisiana pearlshell, *Margaritifera hembeli* (Conrad). Journal of the North American Benthological Society 21:89–96.

Cope, W. G., M. C. Hove, D. L. Waller, D. J. Hornbach, M. R. Bartsch, L. A. Cunningham, H. L. Dunn, and A. R. Kapuscinski. 2003. Evaluation of translocation of unionid mussels to *in situ* refugia. Journal of Molluscan Studies 69:27–34.

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

Dunn, H. L., and B. E. Sietman. 1997. Guidelines used in four geographically diverse unionid translocations. Pages 176–183 *in* K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo (editors). Conservation and management of freshwater mussels II: initiatives for the future. Upper Mississippi River Research Conservation Committee, Rock Island, Illinois.

Dunn, H. L., B. E. Sietman, and D. E. Kelner. 2000. Evaluation of recent unionid (Bivalvia) translocations and suggestions for future translocations and reintroductions. Pages 169–183 *in* P. D. Johnson and R. S. Butler (editors). Freshwater Mollusk Symposia Proceedings: Part II. Proceedings of the First Freshwater Mollusk Conservation Society Symposium, March 1999, Chattanooga, Tennessee. Ohio Biological Survey, Columbus, Ohio.

Gibbons, J. W., and K. M. Andrews. 2004. PIT tagging: simple technology at its best. BioScience 54:447–454.

Gotelli, N. J., and A. M. Ellison. 2004. A primer of ecological statistics. Sinauer Associates, Sunderland, Massachusetts.

Hamilton, H., J. B. Box, and R. M. Dorazio. 1997. Effects of habitat suitability on the survival of translocated freshwater mussels. Regulated Rivers: Research and Management 13:537–541.

Havlik, M. E. 1995. Are unionid mollusk translocations a viable mitigation technique? The Wolf River, Wisconsin, experience, 1992–1995. Pages 184–195 *in* K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo (editors). Conservation and management of freshwater mussels II: initiatives for the future. Upper Mississippi River Research Conservation Committee, Rock Island, Illinois.

HILL, M. S., G. B. ZYDLEWSKI, J. D. ZYDLEWSKI, AND J. D. GASVODA. 2006. Development and evaluation of portable PIT tag detection units: PITpacks. Fisheries Research 77: 102–109.

KELLY, M. W. 2004. Conservation genetics of two rare freshwater mussels: the tidewater mucket (*Leptodea ochracea*) and the yellow lampmussel (*Lampsilis cariosa*). MS Thesis, University of Maine, Orono, Maine.

LAYZER, J. B., AND M. E. GORDON. 1993. Reintroduction of mussels into the Upper Duck River, Tennessee. Pages 89–92 *in* K. S. Cummings, A. C. Buchanan, and L. M. Koch (editors). Conservation and management of freshwater mussels. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

LAYZER, J. B., AND J. R. HEINRICHER. 2004. Coded wire tag retention in ebonyshell mussels *Fusconaia ebena*. North American Journal of Fisheries Management 24:228–230.

LEMARIÉ, D. P., D. R. SMITH, R. F. VILLELLA, AND D. A. WELLER. 2000. Evaluation of tag types and adhesives for marking freshwater mussels (Mollusca: Unionidae). Journal of Shellfish Research 19:247–250.

NATIONAL NATIVE MUSSEL CONSERVATION COMMITTEE. 1997. National strategy for the conservation of native freshwater mussels. Journal of Shellfish Research 17:1419–1428.

NICHOLS, S. J., AND D. GARLING. 2002. Evaluation of substitute diets for live algae in the captive maintenance of adult and subadult Unionidae. Journal of Shellfish Research 21:875–881.

PATTERSON, M. A., B. C. PARKER, AND R. J. NEVES. 1997. Effects of quarantine times on glycogen levels of native freshwater mussels (Bivalvia: Unionidae) previously infested with zebra mussels. American Malacological Bulletin 14:75–79.

PATTERSON, M. A., B. C. PARKER, AND R. J. NEVES. 1999. Glycogen concentration in the mantle tissue of freshwater mussels (Bivalvia: Unionidae) during starvation and controlled feeding. American Malacological Bulletin 15: 47–50.

ROUSSEL, J.-M., A. HARO, AND R. A. CUNJAK. 2000. Field-test of a new method for tracking small fishes in shallow rivers using passive integrated transponder (PIT) technology. Canadian Journal of Fisheries and Aquatic Sciences 57: 1326–1329.

SMITH, D. R., R. F. VILLELLA, AND D. P. LEMARIÉ. 2001. Survey protocol for assessment of endangered freshwater mussels in the Allegheny River, Pennsylvania. Journal of the North American Benthological Society 20:118–132.

WICK, P. C. 2006. Fish host and demographics of *Lampsilis cariosa* and *Leptodea ochracea*, two threatened freshwater mussels in Maine. MS Thesis, University of Maine, Orono, Maine.

ZYDLEWSKI, G. B., A. HARO, G. WHALEN, AND S. D. MCCORMICK. 2001. Performance of stationary and portable passive transponder detection systems for monitoring of fish movements. Journal of Fish Biology 58:1471–1475.

Received: 4 April 2006
Accepted: 18 December 2006

FRESHWATER MUSSEL GUIDELINES FOR VIRGINIA

Virginia Field Office
U.S. Fish and Wildlife Service
6669 Short Lane
Gloucester, VA 23061
804-693-6694

Virginia Dept. of Game and Inland Fisheries
4010 West Broad Street
P.O. Box 11104
Richmond, VA 23230
804-367-1000

Last Updated: 3-7-08

DRAFT

LIST OF ENCLOSURES

1 - Federal and State-Listed Mussel Species in Virginia
2 - Mussel Survey and Relocation Guidelines in Virginia
3 - Surveyor List for Atlantic Slope Mussels in Virginia
4 - Surveyor List for Upper Tennessee River Basin Mussels in Virginia
5 - Time of Year Restrictions
6 - Map of Federally-Designated Critical Habitat for Mussels in Virginia

INTRODUCTION

These guidelines are for project applicants and consultants planning certain activities that will impact rivers, streams, creeks, or other waterways in Virginia. The guidelines provide recommendations for conducting freshwater mussel surveys and relocations for small construction projects of short duration involving non-point pollution sources and affecting no more than 100 linear feet of waterway. Larger projects that impact waters containing State or federally listed mussels may require additional coordination or permits from the Virginia Department of Game and Inland Fisheries (VDGIF) and/or the U.S. Fish and Wildlife Service (FWS). Coordination with these agencies should always be initiated to ensure compliance with Federal and State laws.

FWS is responsible for the conservation and management of *federally* listed freshwater mussel species. VDGIF is responsible for the conservation and management of *all* freshwater mussel species throughout Virginia. If it is known that federally listed species or critical habitat (Enclosure 6) are not present within a two-mile radius of a given site, coordination with VDGIF, but not FWS, is still necessary.

GENERAL LIFE HISTORY

Freshwater mussels are often prominent in benthic stream communities where, for the most part, they are sedentary filter-feeders consuming a major portion of the suspended particulate matter. Therefore, mussel beds act as biological filters by removing inorganic and organic material from the water column while improving water quality downstream. Individuals are typically long-lived, with particular species living for more than 50 years, while some individuals may live for more than 130 years. Because these mussels are long-lived, sedentary filter-feeders, they are prominent indicators of water quality. Freshwater mussels also serve as an important dietary component to a variety of animals, including muskrats, otters, raccoons, and some fishes.

During spawning, male mussels release sperm into the water column that females take in through their gills. The resulting larvae (known as glochidia) may be released by the female into the water column or packaged to attract fish. These larvae must attach to a fish host to survive. While attached to the gills of the fish host, development of the glochidia begins. Once metamorphosis is complete, the juvenile mussel drops off the fish host and continues to develop on the stream bottom.

Freshwater mussels are generally divided into two reproductive categories known as short-term (tachytictic) or long-term brooders (bradytictic). Short-term brooders usually spawn and release glochidia during May through July in Virginia. Long-term brooders usually spawn from August through September and release glochidia the following April through June.

SURVEYS AND RELOCATIONS

Enclosure 1 is a list of federally endangered, threatened, and candidate mussels and State endangered and threatened mussels. If a project occurs in an area that may contain suitable habitat for one of these species, FWS and/or VDGIF may recommend a survey. To determine which waterways may contain suitable habitat for State or federally-listed species, contact VDGIF for guidance (804-367-2211 or 2733). *Project applicants do not need to contact FWS if it is known that no federally-listed species or critical habitat are found within a two-mile radius of the project construction limits.* Applicants should contact FWS and VDGIF early in the planning process to determine whether federally or State-listed species or critical habitat may be impacted by the project. The effects of a project may include direct impacts from construction activities as well as downstream impacts from sedimentation and effluent discharges. If mussels were found during any previous survey/s, however old, coordination with VDGIF and FWS (where applicable) will be required. Surveys where mussels are not found (negative surveys) are typically valid for two years, after which another survey should be performed. Guidelines for freshwater mussel surveys and relocations are found in Enclosure 2. Surveyor lists are included in Enclosures 3 and 4. Since lists change frequently, visit http://www.fws.gov/northeast/virginiafield/Surveyors.html for the most current lists. If listed mussels are found in or downstream of a project area, VDGIF and/or FWS are likely to recommend time of year or other restrictions to reduce impact to the mussels. Time of year restrictions are listed in Enclosure 5. If FWS determines that the project “may affect” a federally listed species or critical habitat, consultation with FWS will be required.

LAWS AND REGULATIONS PROTECTING MUSSELS

Federal Endangered Species Act (ESA) (87 Stat. 884; 16 U.S.C. 1531 et seq.; 50 CFR Part 17) Section 7(a)(2) requires Federal agencies to ensure that any action they authorize, fund, or carry out is not likely to jeopardize the continued existence of any federally listed threatened or endangered species, or result in the destruction or adverse modification of critical habitat. The regulations implementing this Act (50 CFR 402) require the Federal agency to review its actions at the earliest possible time to determine whether its actions may affect listed species or critical habitat. If a Federal agency determines that its action "may affect" a listed threatened or endangered species or critical habitat, the agency is required to consult with FWS regarding the degree of impact and measures available to avoid or minimize the adverse effects.

Section 9 of the ESA makes it illegal for any person subject to the jurisdiction of the United States to "take" any federally listed endangered or threatened species of fish or wildlife without a special exemption. "Person" is defined under the ESA to include individuals, corporations, partnerships, trusts, associations, or any other private entity; local, State, and Federal agencies; or any other entity subject to the jurisdiction of the United States. Under the ESA, "take" means to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, collect, or to attempt to engage in any such conduct. Harm is further defined to include significant habitat modification or degradation that results in death or injury to listed species by significantly impairing essential behavior patterns such as breeding, feeding, or sheltering. Harass is defined as actions that create the likelihood of injury to listed species to such an extent as to significantly disrupt normal behavior patterns which include, but are not limited to, breeding, feeding, or sheltering.

Section 10 establishes an incidental take permit provision for private entities that includes the development of habitat conservation plans. This provision authorizes FWS, under some circumstances, to permit the taking of federally listed fish and wildlife if such taking is "incidental to, and not the purpose of carrying out otherwise lawful activities." This process is also intended to be used to reduce conflicts between listed species and private development and to provide a framework that would encourage "creative partnerships" between the private sector and local, state, and Federal agencies in the interest of endangered and threatened species and habitat conservation. When approved by FWS, this regulatory procedure results in the issuance of a permit authorizing incidental take, provided such take is mitigated by appropriate conservation measures for habitat maintenance, enhancement, and protection, coincident with development.

Virginia Endangered Species Act (29.1-563 - 29.1-570) - This law provides that VDGIF is the state regulatory authority over federally or state listed endangered or threatened fish and wildlife in the Commonwealth, defining *fish or wildlife* as "*. . . any member of the animal kingdom, vertebrate or invertebrate, except for the class Insecta, and includes any part, products, egg, or the dead body or parts thereof.*" It prohibits the taking, transportation, processing, sale, or offer for sale within the Commonwealth of any fish or wildlife listed as a federally endangered or threatened species, except as permitted by the Board of Game and Inland Fisheries for zoological, educational, scientific, or captive propagation for preservation purposes. State-listed species are provided the same protection per VDGIF Regulation 4 VAC 15-20-130.

The law further authorizes the Board of the Virginia Department of Game and Inland Fisheries to adopt the Federal list of endangered and threatened species, to declare by regulation that species not listed by the Federal government are endangered or threatened in Virginia, and to prohibit by regulation the taking, transportation, processing, sale, or offer for sale of those species. Implementing regulations pursuant to this authority (4 VAC 15-20-130 through 140) further define “take” and other terms similarly to the Federal ESA.

Federal Endangered Species Act Cooperative Agreement - Federally listed species are also protected under VDGIF jurisdiction via a cooperative agreement signed in 1976 with FWS pursuant to Section 6 of the ESA. This Cooperative Agreement recognizes VDGIF as the Virginia agency with regulatory and management authority in Virginia over federally listed or threatened animals, excluding insects, and provides for Federal/State cooperation regarding the protection and management of those species.

Enclosure 1: Federal and State Listed Mussel Species in Virginia

SCIENTIFIC NAME	COMMON NAME	STATUS		
Alasmidonta heterodon	dwarf wedgemussel	FE	SE	
Alasmidonta varicosa	brook floater		SE	
Alasmidonta viridis	slippershell mussel		SE	
Cumberlandia monodonta	spectaclecase	FC	SE	
Cyprogenia stegaria	fanshell	FE	SE	
Dromus dromas	dromedary pearlymussel	FE	SE	
Elliptio crassidens	elephantear		SE	
Epioblasma brevidens	Cumberlandian combshell	FE	SE	
Epioblasma capsaeformis	oyster mussel	FE	SE	
Epioblasma florentina walkeri	tan riffleshell	FE	SE	
Epioblasma torulosa gubernaculum	green blossom	FE	SE	EX
Epioblasma triquetra	snuffbox		SE	
Fusconaia cor	shiny pigtoe	FE	SE	
Fusconaia cuneolus	finerayed pigtoe	FE	SE	
Fusconaia masoni	Atlantic pigtoe		ST	
Hemistena lata	cracking pearlymussel	FE	SE	
Lampsilis abrupta	pink mucket	FE	SE	EX
Lasmigona holstonia	Tennessee heelsplitter		SE	
Lasmigona subviridis	green floater		ST	
Lemiox rimosus	birdwing pearlymussel	FE	SE	
Leptodea fragilis	fragile papershell		ST	
Lexingtonia dolabelloides	slabside pearlymussel	FC	ST	
Ligumia recta	black sandshell			
Pegias fabula	littlewing pearlymussel	FE	SE	
Plethobasus cyphyus	sheepnose	FC	ST	
Pleurobema collina	James spinymussel	FE	SE	
Pleurobema cordatum	Ohio pigtoe		ST	
Pleurobema plenum	rough pigtoe	FE	SE	
Pleurobema rubrum	pyramid pigtoe		SE	
Ptychobranchus subtentum	fluted kidneyshell	FC		
Quadrula cylindrica strigillata	rough rabbitsfoot	FE	SE	
Quadrula intermedia	Cumberland monkeyface	FE	SE	
Quadrula pustulosa pustulosa	pimpleback		ST	
Quadrula sparsa	Appalachian monkeyface	FE	SE	
Toxolasma lividus	purple lilliput		SE	
Tritogonia verrucosa	pistolgrip		ST	
Truncilla truncata	deertoe		SE	
Villosa fabalis	rayed bean	FC		EX
Villosa perpurpurea	purple bean	FE	SE	
Villosa trabalis	Cumberland bean	FE	SE	EX

KEY

FE - Federally Endangered　　SE - State Endangered
FT - Federally Threatened　　ST - State Threatened
FC – Candidate: FWS has enough information to list the species as threatened or endangered, but this action is precluded by other listing activities
EX - Believed to be extirpated in Virginia

Enclosure 2: Mussel Survey and Relocation Guidelines in Virginia

There are four general assessment/survey types including:

A. **Land-based review** - land-based site visit used to determine whether a water-based survey (site assessment, abbreviated, or full survey) is warranted. During a land-based review, the surveyor should look for obvious signs that would negate the need for additional, water-based surveys. For example, if it can be determined that the water body is non-perennial and/or contains no potential mussel habitat, it is unlikely that additional surveys would be needed or recommended by VDGIF or FWS. If it is determined that suitable habitat is present, the appropriate survey will be recommended. Photographs of the project site clearly showing instream habitat conditions, as well as a thorough site description, should be sent to VDGIF and FWS for review in lieu of the site assessment. If it is determined that suitable habitat is present, the appropriate survey will be recommended.

B. **Site assessment** - 20 m upstream / 80 m downstream. A site assessment is recommended to determine if suitable habitat is present at a project location and may be recommended if the presence of a listed species is questionable. If suitable habitat is present, the appropriate survey will be recommended even in the absence of mussels, since the site assessment does not serve as a substitute for a mussel survey; however, the presence of freshwater mussels should be documented during the assessment.

C. **Abbreviated survey** - 100 m upstream / 400 m downstream of project footprint.

D. **Full survey** - 200 m upstream / 800 m downstream of project footprint.

The assessment/survey type is based on the scope of the project, potential impacts, and known species distributions. Survey lengths are measured from the project footprint. *Survey distances have primarily been developed for projects where physical alteration/disturbance of the stream is the primary impact (e.g., bridge repair/replacement, utility line crossings, etc.). Potential impacts from projects involving activities such as point and non-point source discharges, water intakes, and mining may require greater survey lengths and different methods.*

Project applicants should contract with a qualified mussel surveyor. Enclosures 3 and 4 provide a list of pre-approved mussel surveyors. Since lists change frequently, visit (http://www.fws.gov/northeast/virginiafield/Surveyors.html) for the most current lists. If a pre-approved surveyor is not selected, please provide the proposed surveyor's qualifications and proposed survey design to FWS and VDGIF a minimum of 30 days prior to survey initiation. Individuals who take federally listed threatened and endangered animals must obtain a permit from VDGIF, prior to surveying. Take is defined as to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture or collect, or to attempt to engage in any such conduct. Contact information follows:

Ms. Shirl Dressler
Virginia Department of Game and Inland Fisheries
4010 W. Broad Street
P.O. Box 11104
Richmond, Virginia 23230-1104
Phone: (804) 367-6913
CollectionPermits@dgif.virginia.gov

A plan for mussel relocations, including initial surveys, must be presented to VDGIF and FWS (where applicable) for comment and approval prior to initiation of construction. Failure to provide a mussel relocation and/or survey plan may affect review and permitting of the project by VDGIF and FWS.

The recommended time of year to conduct mussel surveys and relocations is April 1 through October 31. Surveying during the cooler months is discouraged because mussels tend to be located deeper in the substrate and a greater percentage of the population is subsurface, therefore making them more difficult to find, particularly rare species. A more specific time frame may be recommended depending on the target species. A survey conducted outside this time frame requires VDGIF and Service (where applicable) approval.

Guidelines if federally-listed mussels are not present

During the initial survey, mussel species within the direct project footprint or within imminent danger from project impacts may be relocated to suitable habitat unless otherwise directed by VDGIF. Suitable habitat typically includes an area upstream of project impacts and which also harbors freshwater mussels. If such an area cannot be found, the surveyor should determine the location of most suitable habitat. The direct project footprint shall be defined as the area of potentially disturbed substrate, any zone of heavy equipment operation, plus the distance downstream that may experience significant sedimentation from construction. If not determined prior to the relocation, the surveyor is responsible for determining the most suitable relocation area. All relocated mussels must be at least partially placed in the substrate, anterior end down. Project applicants may be required to monitor relocated mussels to determine relocation success/failure.

Standard mussel relocation protocols are outlined below. These protocols may vary based on factors such as the scope of the project and the results of the initial mussel survey. If the relocation protocols vary, VDGIF will clearly outline the appropriate protocols with the project applicant. It is the project applicant's responsibility to ensure that the proper relocation protocols are used and that the contracted mussel surveyor is aware of any modifications to the standard protocols.

The reach from which mussels are to be relocated will be at least 100 m long including the project footprint. The standard protocol is as follows:

- The 1st relocation survey must occur within 30-45 days of instream construction activities and at least 7 days prior to the 2nd relocation survey.

- The 2nd relocation survey must occur within 30 days of instream construction activities and at least 7 days after the 1st relocation survey.

- All relocation surveys must include at a minimum, two passes. The target relocation percentage of the initial number of mussels collected is 80%. If on the 2nd pass, more than 20% of the initial number of mussels is collected, continued passes must be conducted until no more than 20% of the initial number of mussels is collected on the final pass. The target relocation percentage may be adjusted higher or lower depending on the species and numbers collected during the initial survey.

- If a state-listed species is found, continued passes must be conducted until no listed species are found on the final pass. If repeated passes result in continual collection of state-listed species, modification of the survey techniques may be required.

If relocation surveys are not possible due to natural conditions such as high water, contact VDGIF to arrange contingency plans.

The location of all relocated mussels must be accurately documented (preferably with geographic coordinates) and reported to VDGIF. All state-listed mussel species must be tagged and measured for potential future monitoring.

Project applicants may be required to adhere to time of year restrictions for mussel relocations as directed by VDGIF. If this is the case, for the long-term brooders, relocations can occur from June 16 though August 14 and October 1 through October 31. For short-term brooders, relocations can occur from April 1 through May 14 and August 1 through October 31.

All mussel survey and relocation results, including tag and measurement data, must be submitted to VDGIF for review, prior to instream construction activities. Reviews will be expedited due to the potential short timeframe between surveys and/or relocations and the start of instream work. Reports must contain, at a minimum, number of species found, number of individuals per species and their sizes, and number of individuals tagged.

Guidelines if federally-listed mussel species are present

Federally-listed mussels must *not* be relocated during the initial survey. If federally-listed mussels are found, they must remain exactly where found and all specimens should be photo documented, if possible. Coordination with FWS and VDGIF must occur to determine future actions.

If it is determined that a project may affect a federally-listed species, FWS will complete a consultation with the Federal action agency and prepare a biological opinion in accordance with the Federal Endangered Species Act. The relocation procedures for federally listed mussels will be specified in FWS's biological opinion and will be determined on a project-specific basis.

If relocation surveys are not possible due to conditions such as high water, contact FWS and VDGIF to arrange contingency plans. All listed mussels must be moved to suitable habitat upstream of any potential project impacts. Mussels may be relocated downstream if habitat upstream is determined unsuitable by VDGIF and FWS. If not determined prior to the relocation, the surveyor is responsible for determining the most suitable relocation area. All relocated mussels must be at least partially placed in the substrate, anterior end down. Project applicants may be required to monitor relocated mussels to determine relocation success/failure.

The location of all relocated federally-listed mussels must be accurately documented (preferably with geographic coordinates) and reported to FWS and VDGIF. All federally-listed mussel species also must be tagged and measured for potential future monitoring.

All mussel survey and relocation results must be submitted to FWS and VDGIF for review, prior to instream construction activities. Reviews will be expedited due to the potential short timeframe between surveys and/or relocations and the start of instream work. Reports must contain, at a minimum; number of species found, number of individuals per species and their sizes, number of individuals tagged, etc.

Project applicants may be required to adhere to time of year restrictions (Enclosure 5) for mussel relocations as recommended by FWS and VDGIF. Time of year restrictions will be specified in a letter or in FWS's biological opinion.

Enclosure 3: Surveyor List for Atlantic Slope Mussels in Virginia

ATLANTIC SLOPE FRESHWATER MUSSELS
SURVEY CONTACTS IN VIRGINIA

This list contains individuals who we have already determined are qualified to conduct surveys for the species listed above. This list does not include all individuals qualified or authorized to survey for this species. If you select someone not on this pre-approved surveyor list, please provide the proposed surveyor's qualifications to FWS and VDGIF 30 days prior to the start of the survey. Please send copies of all survey results to both agencies. If the survey determines that any rare species are present, please contact FWS and VDGIF to allow us the opportunity to work with you to ensure that a project avoids or minimizes adverse effects to rare species and their habitats. Inclusion of names on this list does not constitute endorsement by FWS, VDGIF, or any other Virginia or U.S. Government agency. Listed alphabetically. March 4, 2008

John Alderman
244 Red Gate Road
Pittsboro, NC 27312
(919) 542-5331
aldermanjm@mindspring.com

Braven Beaty
334 Whites Mill Road
Abingdon, VA 24210
(276) 676-2209
bbeaty@tnc.org

Richard Neves
Dept of Fish and Wildlife
Virginia Tech
Blacksburg, VA 24061-0321
(540) 231-5927
mussel@vt.edu

Brett Ostby
Dept of Fish and Wildlife
Virginia Tech
Blacksburg, VA 24061-0321
(540) 230-1042
bostby@vt.edu

Melissa Petty
110 Phlegar St.
Christiansburg, VA 24073
(540) 250-2182
mepetty@vt.edu

Steve Roble
Virginia Div of Natural Heritage
217 Governor St, 3rd Floor
Richmond, VA 23219
(804) 786-7951
steve.roble@dcr.virginia.gov

Tim Savidge
The Catena Group
410-B Millstone Dr
Hillsborough, NC 27278
(919) 732-1300
tsavidge@thecatenagroup.com

Philip Stevenson
Creek Laboratory, LLC
P.O. Box 953
Fredericksburg, VA 22404
(877) 433-8962
phil@creeklab.com

Enclosure 4: Surveyor List for Upper Tennessee River Basin Mussels in Virginia

TENNESSEE RIVER DRAINAGE FRESHWATER MUSSELS
SURVEY CONTACTS IN VIRGINIA

This list contains individuals who we have already determined are qualified to conduct surveys for the species listed above. This list does not include all individuals qualified or authorized to survey for this species. If you select someone not on this pre-approved surveyor list, please provide the proposed surveyor's qualifications to FWS and VDGIF 30 days prior to the start of the survey. Please send copies of all survey results to both agencies. If the survey determines that any rare species are present, please contact FWS and VDGIF to allow us the opportunity to work with you to ensure that a project avoids or minimizes adverse effects to rare species and their habitats. Inclusion of names on this list does not constitute endorsement by FWS, VDGIF, or any other Virginia or U.S. Government agency. Listed alphabetically. March 4, 2008

Steven A. Ahlstedt
P.O. Box 460
57 Deer Ridge Road
Norris, TN 37828
(865) 545-4140 x17
ahlstedt@usgs.gov

Braven Beaty
334 Whites Mill Rd.
Abingdon, VA 24210
(276) 676-2209
bbeaty@tnc.org

Arthur Bogan
Curator of Aquatic Invertebrates
N.C. State Museum of Natural Sciences
11 West Jones Street
Raleigh, NC 27603
(919) 733-7450 x753
arthur.bogan@ncmail.net

Gerald R. Dinkins
Dinkins Environmental Consulting, LLC
3716 West Beaver Creek Drive
Powell, TN 37849
(865) 938-7739
biodink@frontiernet.net

Mr. Mark Fagg
551 Ravenwood Drive
Morristown, Tennessee 37814
(423) 231-3314
musselhead@charter.net

Richard Neves
Dept of Fish and Wildlife
Virginia Tech
Blacksburg, VA 24061-0321
(540) 231-5927
mussel@vt.edu

Brett Ostby
Dept of Fish and Wildlife
Virginia Tech
Blacksburg, VA 24061-0321
(540) 230-1042
bostby@vt.edu

Melissa Petty
110 Phlegar St.
Christiansburg, VA 24073
(540) 250-2182
mepetty@vt.edu

Enclosure 5: Time of Year Restrictions

--*Alasmidonta heterodon* (Dwarf wedgemussel) - no instream work from March 15 through May 31 (release of glochidia), and August 15 through October 15 (spawning).

--*Villosa perpurpurea* (Purple bean) - no instream work from February 15 through June 15 (release of glochidia), August 15 through September 30 (spawning).

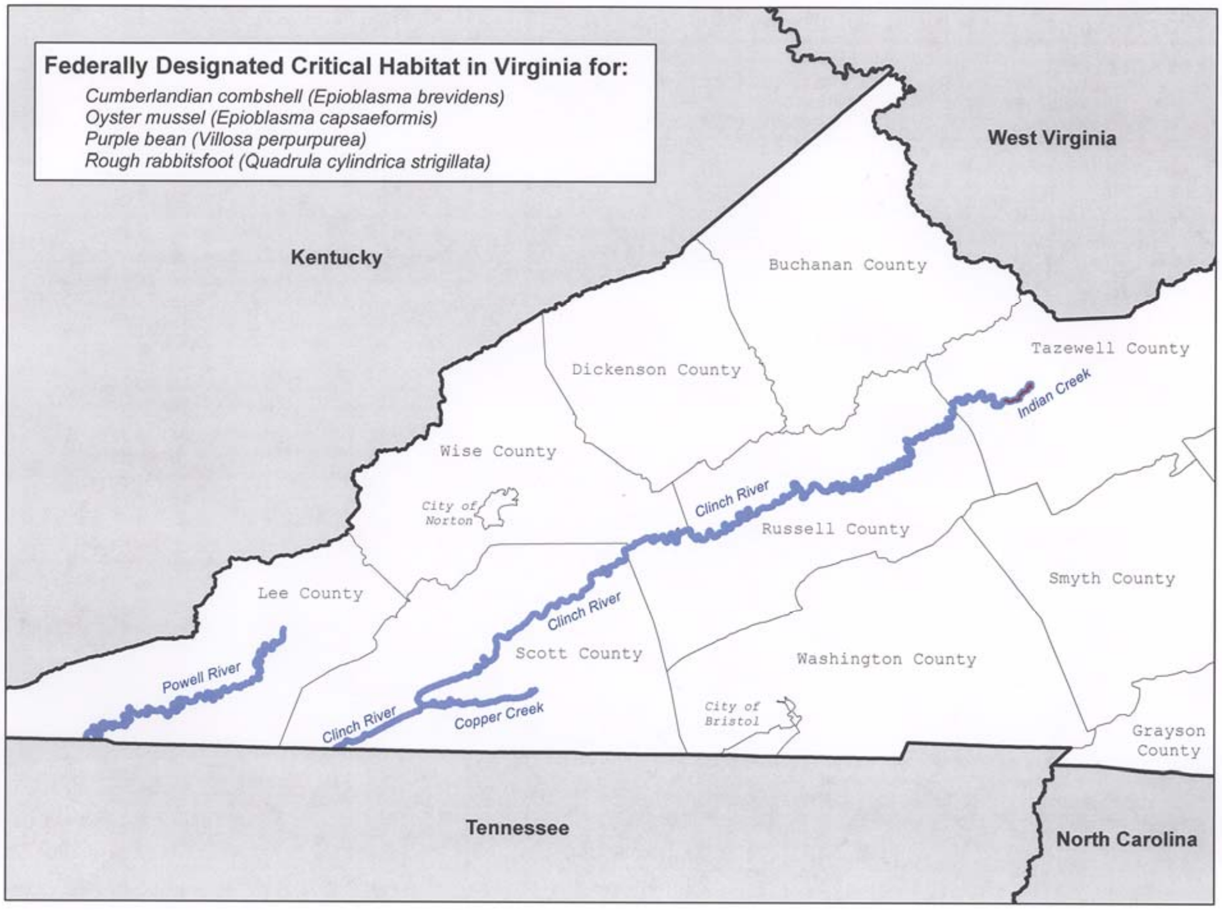
--Long-term brooders - no instream work April 15 through June 15 (release of glochidia), and 15 August through September 30 (spawning).

Alasmidonta varicosa	brook floater
Alasmidonta viridis	slippershell mussel
Cumberlandia monodonta	spectaclecase
Cyprogenia stegaria	fanshell
Dromus dromas	dromedary pearlymussel
Epioblasma brevidens	Cumberlandian combshell
Epioblasma capsaeformis	oyster mussel
Epioblasma florentina walkeri	tan riffleshell
Epioblasma torulosa gubernaculum	green blossom
Epioblasma triquetra	snuffbox
Lampsilis abrupta	pink mucket
Lasmigona holstonia	Tennessee heelsplitter
Lasmigona subviridis	green floater
Lemiox rimosus	birdwing pearlymussel
Leptodea fragilis	fragile papershell
Ligumia recta	black sandshell
Pegias fabula	littlewing pearlymussel
Ptychobranchus subtentum	fluted kidneyshell
Toxolasma lividus	purple lilliput
Truncilla truncata	deertoe
Villosa fabalis	rayed bean
Villosa trabalis	Cumberland bean

--Short-term brooders - No instream work May 15 through July 31.

Elliptio crassidens	elephantear
Fusconaia cor	shiny pigtoe
Fusconaia cuneolus	fine rayed pigtoe
Fusconaia masoni	Atlantic pigtoe
Hemistena lata	cracking pearlymussel
Lexingtonia dolabelloides	slabside pearlymussel
Plethobasus cyphyus	sheepnose
Pleurobema collina	James spinymussel
Pleurobema cordatum	Ohio pigtoe
Pleurobema plenum	rough pigtoe
Pleurobema rubrum	pyramid pigtoe
Quadrula cylindrica strigillata	rough rabbitsfoot
Quadrula intermedia	Cumberland monkeyface
Quadrula pustulosa pustulosa	pimpleback
Quadrula sparsa	Appalachian monkeyface
Tritogonia verrucosa	pistolgrip

Enclosure 6 - Map of Federally-Designated Critical Habitat for Mussels in Virginia



Federally Designated Critical Habitat in Virginia for:
Cumberlandian combshell (Epioblasma brevidens)
Oyster mussel (Epioblasma capsaeformis)
Purple bean (Villosa perpurpurea)
Rough rabbitsfoot (Quadrula cylindrica strigillata)
West Virginia
Kentucky
Buchanan County
Tazewell County
Dickenson County
Indian Creek
Wise County
Clinch River
City of Norton
Russell County
Lee County
Clinch River
Smyth County
Scott County
Washington County
Powell River
City of Bristol
Clinch River
Copper Creek
Grayson County
Tennessee
North Carolina

ENSR | AECOM

ENSR
1601 Prospect Parkway, Fort Collins, CO 80525-9769
T **970.493.8878**   F **970.493.0213**   www.ensr.aecom.com

Memorandum

Date: May 6, 2008

To: Gabrielle Borin, Jessica Rubado

From: Jim Burrell

Subject: Sediment transport distances at selected NiSource pipeline crossing locations; re: potential impacts to mussel species

Distribution:

The attached table indicates estimated sediment deposition distances at selected wet open-cut watercourse crossings for the NiSource pipeline project (ENSR Project 01776-034). The following summary is a brief discussion of the estimation approach, site selection, and the interpretation of results. Further documentation is planned.

A. Estimation Approach. Guidance from the management team indicated that a rapid approach to estimating sediment yield, transport, and deposition for wet open-cut pipeline crossings was required. Since the potential existed for applying the technique to many (e.g., 50 or more) project locations on an accelerated schedule, the approach needed to be relatively simple and formulated on available data and GIS applications. On that basis, a simplified procedure was developed to formulate and quantify the three processes of 1) suspended sediment supply to a stream from site disturbance, 2) instream transport and dispersion of the sediment by representative size fractions, and 3) sediment deposition on the streambed. From this, the likely zones of impacts to mussel species can be further approximated. A general chart of the sediment approximation approach is depicted in the attached figure.

1) Sediment Supply. Sediment supply from bank excavations was estimated by applying the Universal Soil Loss Equation (USLE) to soils information retrieved from the NRCS STATSGO database for the crossing locations. Soil associations on the floodplains were characterized by their grain-size distributions (cobble and gravel, sand, silt and clay) and erodibility factors. A typical working excavation site geometry of 75 feet long by 75 feet wide was used for each streambank. Appropriate Best Management Practices (BMPs) to control erosion and sediment yield were assumed to be successfully employed outside this working area. Sediment that could be eroded or tracked into the flow was generated in the USLE application by a rainfall factor representing a one-year storm event. This is smaller than a mean annual thunderstorm. Due to the proximity of the bank excavation to the streams, one hundred percent sediment yield was employed.

Average sediment supply from instream trenching was calculated by an empirical equation developed for the Interstate Natural Gas Association of America (INGAA) and the Gas Research Institute (GRI) (Golder Associates 1998). The equation was adapted to site conditions on the basis of testing and comparison to other published equations (Reid, et. al. 2004). Equations from both references were reasonably correlated to sets of field measurements at wet open-cut pipeline crossings. Streambed sediment characteristics for input were determined from available data at U.S. Geological Survey (USGS) locations for similar settings within the crossing regions.

2) Instream Transport. Transport of suspended sediment was determined by stream hydraulic factors determined from field measurements at USGS stream gages. Each of the selected watercourse crossing locations is reasonably close to a USGS gage where measurements of flow

ENSR
1601 Prospect Parkway, Fort Collins, CO 80525-9769
T **970.493.8878** F **970.493.0213** www.ensr.aecom.com

rates, velocities, and flow geometries have been recorded by the agency. Rectangular cross sections were assumed, and were reasonably supported by geometric data. Average cross-sectional velocities were used.

Dispersion of the fine suspended particles (silts and clays) in the flow was determined using longitudinal ("X") and lateral ("Y") dispersion coefficients and related equations presented in a number of USGS open-channel hydraulics publications. Distances for complete vertical ("Z") mixing of suspended silts and clays were determined to be well within the upstream portion of the dispersion fields.

3) Deposition. Sediment deposition downstream of the open-cut crossings was determined by calculating the fall velocities of representative grain diameters for the various sediment size classes (cobbles and gravels, sands, silts and clays) at each selected crossing location. Recent research (Wu and Wang, 2004) developed a well-correlated fall velocity approach for a range of sediment diameters. It is presented as being in line with, but more broadly applicable than, results from previous investigators. This was used for representative grain diameters greater than 0.2 millimeters. Fall velocities for silts and clays were based on suspended concentrations, using an averaging equation from the U.S. Bureau of Reclamation (US BurRec 2006). Turbulence effects on the settling of silts and clays were generally incorporated through the dispersion results.

The extent of deposition was based on the distance required for a vertically well-mixed suspended sediment load to fall through the water column as represented by the streamflow depth. The deposition distances were derived by a simple linear relationship based on the fall velocities of sediment size fractions and the downstream flow velocities. Although general in nature, this approach is supported in literature (Einstein 1967, Golder Associates 1998, and others). As footnoted, final deposition values indicated in the attached table represent the distances within which two criteria for mussel mortality were satisfied: 1) a burial depth of 0.6 centimeters or more, and 2) a suspended sediment concentration of 600 milligrams per liter or more. These criteria were obtained from related research into mussel mortality from siltation (Ellis 1936). Suspended silts and clays at lower concentrations would pass further downstream.

B. Site Selection and Flow Conditions. Three sites (the Duck River in Tennessee, the Elk River in West Virginia, and the James River in Virginia) were selected for application of the estimation procedure. Site selection was based on 1) a list of "may-affect" stream crossings for the NiSource project, 2) the availability of nearby USGS streamgaging data and related measurements, and 3) a likelihood of moderate flow conditions (discharge, depth and width, velocity) during the anticipated construction season (July through December). The anticipated crossing construction season is based on communications with the ENSR project staff regarding other wildlife considerations (e.g., bird nesting) that may affect the timing of construction near watercourses.

Moderate flow conditions may be the most significant in terms of potential effects of sediment deposition on mussel beds. Under the procedure, small discharges and slower flow velocities or shallow depths generally will not result in the calculation of sediment transport and deposition at distances as great as in the larger streams. Very large rivers, such as the Ohio and Tennessee rivers, absorb sediment inputs within their background conditions and disperse them fairly quickly within the cross-section to levels below the criteria.

Flow conditions used in the calculations represent the lower flow conditions late in the season. Average monthly flows were further averaged to obtain a seasonal average flow rate at each location, based on USGS gaging and watershed area. These flow conditions represent a narrower and shallower hydraulic geometry than "bankfull flows". The latter are much more likely to occur in springtime, outside the construction timing window.

ENSR
1601 Prospect Parkway, Fort Collins, CO 80525-9769
T **970.493.8878** F **970.493.0213** www.ensr.aecom.com

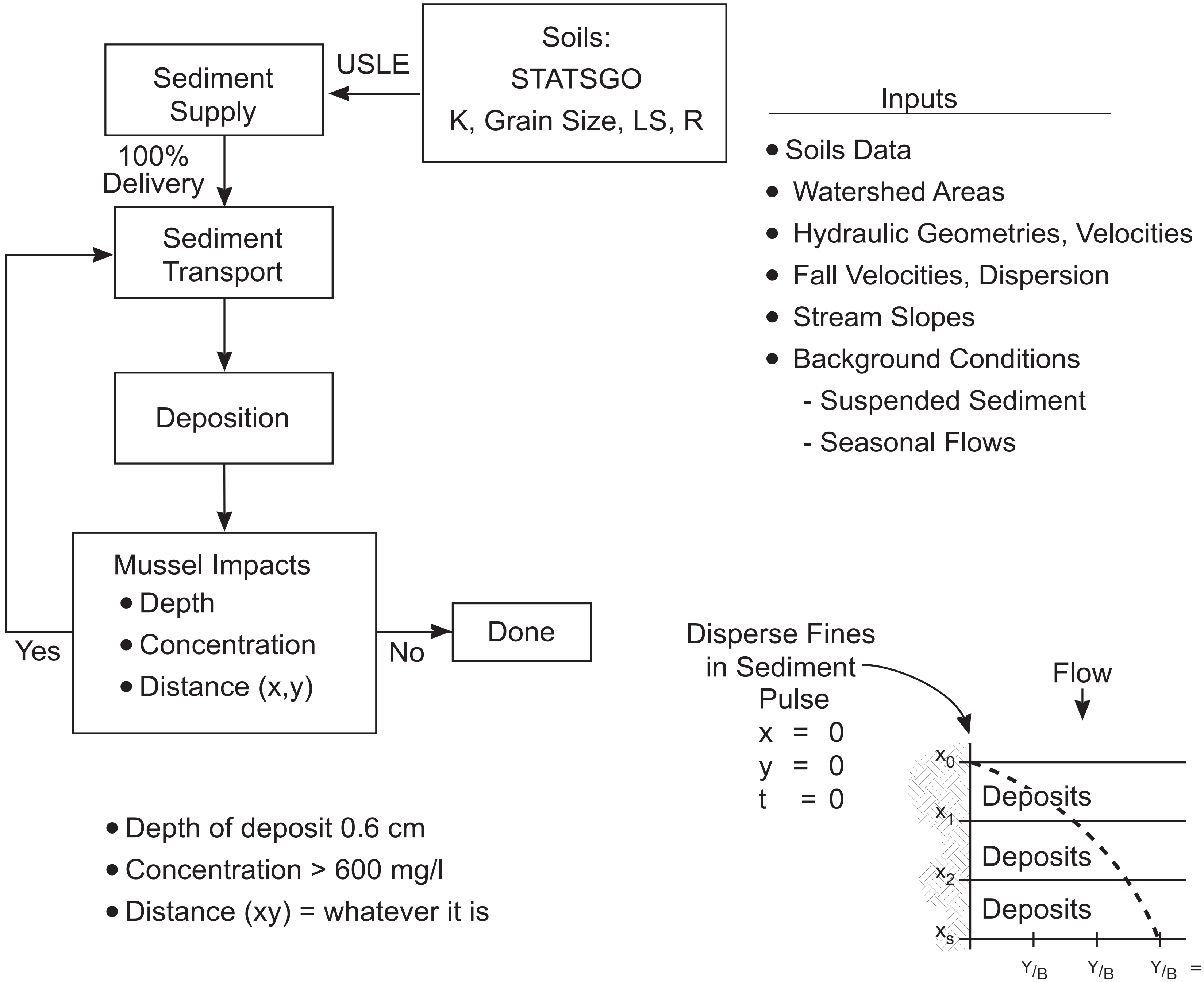
C. Interpretation of Results. The results of the estimation approach for the three streams are shown in the attached table. Of the sites selected, the Duck River is a relatively deep and fast-flowing stream with fine-grained materials in the banks and bed. Correspondingly, its transport and deposition distances are the longest. This is also due to the portion of silt and clay in suspension. In contrast, the Elk River is much deeper and slower. The overall grain size fractions along the Elk River are coarser, and its transport and deposition distances are the shortest. In addition to stream hydraulics, the silt and clay fraction never exceeds the concentration criterion or provides enough settleable mass in the water column to exceed the burial criterion. Therefore, the deposition distance for the criteria is governed by the faster settling rates of the sand fraction. The James River is in between the other two. While slower, it is also shallower, and has somewhat finer banks and bed than the Elk River. As a result, the silt and clay fraction exceeds the concentration criterion to the distance indicated in the table. However, for the same reason as on the Elk River (lack of settleable mass), the burial criterion is not exceeded by silt and clay on the James River. The deposition distance for the burial criterion is governed by the faster settling rates of the sand fraction.

It should be noted that the distances tabulated for the sediment supply from the bank (Part 1: USLE-derived sediment supply) relate to a portion of the flow field that extends out a distance of about 20 feet from the late summer and autumn seasonal shoreline on the James River, and out about 35 feet for the Duck River. A similar “wedge” of deposition occurs for fine sands on the Elk River. This is due to the transport mechanics, and the dispersion calculations, for a point source at each bank. This phenomenon is depicted on the attached figure, as well. For the trench calculations shown in Part 2, the distances pertain across the entire stream width.

The actual occurrence of mussel beds within these areas is subject to further analysis or data-gathering.

Sincerely yours,

Jim Burrell, EIT, MSCE
Senior Hydrologist
ENSR – Fort Collins, CO



Soils:
STATSGO
K, Grain Size, LS, R
USLE
Sediment Supply
100% Delivery
Sediment Transport
Deposition
Mussel Impacts
• Depth
• Concentration
• Distance (x,y)
Yes
No
Done
Inputs
• Soils Data
• Watershed Areas
• Hydraulic Geometries, Velocities
• Fall Velocities, Dispersion
• Stream Slopes
• Background Conditions
- Suspended Sediment
- Seasonal Flows
• Depth of deposit 0.6 cm
• Concentration > 600 mg/l
• Distance (xy) = whatever it is
Disperse Fines in Sediment Pulse
x = 0
y = 0
t = 0
Flow
Deposits
Deposits
Deposits

SEDIMENT TRANSPORT DISTANCES FROM WET OPEN-CUT CROSSINGS

1. From Bank Excavations, from USLE sediment supply approach

River	Location	TRANSECT ID	Flow Depth D, ft	Velocity, Vmean, ft/s	Representative Gravel Size, mm	Downstream Transport Distance, ft	Representative Sand Size, mm	Downstream Transport Distance, ft	Representative Silt & Clay Size, mm	% Silt & Clay	Downstream Transport Distance, ft *	Downstream Burial Distance, ft **
Duck	Maury County TN	1	5.32	2.45	12.44	7	0.30	94	< 0.05	80.2	3,700	1,060
Elk	Kanawha County WV	2	7.42	1.48	33.26	4	0.20	167	< 0.05	41.6	NA	167
James	Botetourt County VA	3	4.43	1.30	26.81	2	0.22	70	< 0.05	45.6	2,640	70

* Settling distance downstream for which suspended sediment concentration exceeds 600 mg/L
** Distance over which sediment settling forms deposits 0.6 cm thick or more
NA: neither criterion above was exceeded by the silt & clay fraction on the Elk River.

2. From Bed Trenching, per Gas Research Institute 1998 approach

River	Location	TRANSECT ID	Flow Depth D, ft	Vmean, ft/s	Representative Grain Size, d50, mm	Downstream Transport Distance, ft
Duck	Maury County TN	1	5.32	2.45	0.4231	65
Elk	Kanawha County WV	2	7.42	1.48	0.4420	53
James	Botetourt County VA	3	4.43	1.30	0.4420	28

Disinfection Techniques/ Options:
Preventing Spread of Pathogens, Bacteria and Invasives

Boats, Motors, Trailers, Equipment

U.S. Fish and Wildlife Service-Region 3

Develop and Implement a HACCP Plan! Comply with Federal and State Mandates

Methods below are only effective after proper preparation of infected equipment. After loading and securing boat and equipment on trailer at boat landing, boat, motor, trailer, and gear must have all aquatic vegetation, visible organisms/animals, soil, and water drained and removed BEFORE TRANSPORT. Upon leaving a worksite possibly infected with pathogens or invasive species, a proper disinfection must be completed before re-use of boat, motor, trailer, and any exposed gear in another waterway. Contact time is crucial for complete disinfection. Contact time reflects exposure of air, water, or disinfectant to a specific area, and not the total amount of time spent disinfecting. For example, if you are using 70^{o} C water to disinfect your boat, you must apply 70^{o} C water to each area for one minute or longer (see options and procedures below). Disinfection is MANDATORY for all exposed equipment and gear!

Methods	**Procedures**	**Positives**	**Negatives**
Heat + Air (Drying in hot sun/air)	**30C (86 F)** 24 hours minimum (time at temp contact period crucial) (exposure to hot sun/ air while dry)	Chemical free Effective, but only if properly done under ideal conditions	Time consuming Weather/Temperature criteria critical to reliable results
Heat + Water Spray &/or immerse	**50C (122 F)** contact time 10 minutes (time and temp contact crucial) (source of very hot water needed)	Chemical free Same as above	Must maintain high water temp/contact; hotter than normal tap or carwash. Use ppg.
Heat + Water Spray &/or immerse	**70C (158 F)** contact time 1 minute (time and temp contact crucial) (source of super hot water needed)	Chemical free Same as above	Must maintain very high water temp/contact; (i.e. steam pressure sprayer). Risk of burns; use personal protective gear (ppg)
Virkon Aquatic	**Follow product directions for proper mixture and minimum contact time.** (immerse in solution, apply directly, or spray-on with pressure washer & rinse)	Environmentally friendly Designed for aquatic use Quick inactivation time Sewer compatible	Follow MSDS directions for health risks and use personal protective gear ppg. when mixing Corrosive in concentrate form Chemical based
Quarternary Ammonium+Water ***(family of products)**	**Follow product directions for proper mixture and minimum contact time.** (immerse in solution, apply directly, or spray-on with pressure washer & rinse)	Effective, user friendly Low health risks Sewer compatible	Chemical based Follow MSDS directions for health risks and use ppg.
Chlorine + Water	**Min. 98 mg/liter** water for 2 minutes (immerse in solution, apply directly, or spray-on with pressure washer and rinse/neutralize thoroughly)	Widely available Effective	Follow MSDS directions for health risks and use personal protective gear ppg. Highly Corrosive

This is a partial list; research, choose, & use the most effective option available for you. Feel free to print, laminate, and post this page. USFWS Contacts are: Corey Puzach, La Crosse Fish Health Center-608 783-8445, or Dave Wedan R3 Watercraft Safety Coordinator-608 783-8435. HACCP Webpage http://www.haccp-nrm.org/

Appendix L-19

Riparian Buffer Mitigation Monitoring Protocol

Mitigation for all aquatic HCP species includes the establishment or protection of riparian buffers. A requirement of Chapter 7 is to monitor the riparian buffer mitigation sites both for effectiveness and Chapter 10 requires assessment if a mitigation site is impacted by a changed circumstance (e.g., flood, fire, disease). The following outlines the general provisions required to monitor these sites. Specific additional measures may be required as monitoring is implemented to ensure effectiveness of the monitoring protocols.

NiSource will record the lat long coordinates to accurately delimit the easement polygon boundary and will clearly and permanently mark the boundaries of the easement on the ground (typically metal fence posts) within six months of the easement being recorded or as otherwise specified.

Once the easement vegetation is established, NiSource will examine updated aerial photos every five years to determine the general condition of the easement (e.g., presence of significant erosion, evidence of fire or disease, and clearing, trails, dumping, or other human impacts) and to ensure that the structure and percent cover of the mitigation vegetation (trees or grasses) meet minimum requirements established in Chapter 6 and Appendix XX. At least once every 10 years NiSource will do an on-the-ground verification of the remotely sensed data (i.e., structure and percent cover of mitigation vegetation) and to more effectively assess the easement site for indications of invasive species, disease, significant erosion that threatens site integrity and other potential threats to the functioning of the easement as designed. Additional assessment using both remotely sensed data and on-the-ground verification may be required to determine the impacts of changed circumstances should a changed circumstance (e.g., flood, fire, invasive species) affect a mitigation site. NiSource will coordinate with the Service, which will determine whether remotely sensed data are sufficient or whether on-the-ground assessment is required in the case of changed circumstances.

Google Map Photos (or other aerial photo data) are acceptable data for remote assessment if they meet the following requirements: a) they provide data acquired in the appropriate year and season necessary to determine the structure and percent cover of the mitigation site (or to evaluate a changed circumstance); b) the aerial photos are of sufficient quality and resolution to determine the structure and percent cover of the mitigation site and provide information on possible threats to its integrity. The attached GoogleMap image of Big Darby Creek in Ohio (Photo 1) represents information that may be sufficient to determine structure (i.e., trees versus crop field) but not of sufficient resolution to make a determination on percent cover or other impacts. Photo 2, another Google image of the White River in Indiana may represent an image of sufficient quality to determine both structure and percent cover.

NiSource will employ accepted guidelines for evaluating remotely sensed data and for conducting on-the-ground assessment of percent cover and structure. To the extent feasible, the methods will be quantitative and will allow comparison of the assessments among years. NiSource will provide the specific protocols for both remote sensing and on-the-ground surveys in writing to the Service for approval before the first assessment using either method. With respect to threats to mitigation site integrity, at minimum, NiSource will assess the entire shoreline for erosion, and the entire mitigation site for other impacts (e.g., trails, erosion, clearing, dumping) that might affect the integrity of the mitigation.

Photo 1 – Fictional Riparian Buffer Boundary on Big Darby Creek in Ohio (Google 2010 Image)

Photo 2 – Fictional Riparian Buffer Boundary on the White River in Indiana (Google 2010 Image)

APPENDIX L-19

RIPARIAN RESTORATION STANDARD

Mitigation Option B) There is uncertainty associated with the survival of vegetation planted to restore riparian corridors.

The hypothesis relevant to riparian corridor restoration planting survival is:

A minimum of 75 percent of trees and grasses will survive after three years.

Adaptive management will be employed to ensure that the minimum survival rate of 75 percent is achieved. A person with qualifications and expertise in evaluating tree planting survival will survey all riparian restoration sites during the growing season of the third year after planting to determine the survival rate.

The threshold for adaptive management will be less than 75 percent survival of trees at three years after planting. In addition, if fewer than 50% of the planted trees and shrubs or less than 50% of the area of planted grasses is alive after the first year, the mitigation will be determined a failure and corrective will be required during the next growing season. If by the third growing season, greater than 75% of the trees and shrubs survive or 75% of the area of grasses survive, but it is determined as above that the greater than 50% will be permanently impaired (e.g., inordinately subject to disease, blow-down, etc.) then corrective action will be required

Alternatives to evaluate if the threshold is reached:

A) Replant the original tree species back to 100 percent of the original planting density.

B) Replant a different suite of native species back to the 100 percent of the original planting density.

C) Modify the site to facilitate better survival of planted trees and implement A or B above.

D) Plant a different type of native vegetation that provides the same suite of benefits to mussels.

E) Re-establish the original level of mitigation at a new site where the mitigation would provide compensatory mitigation for Nashville crayfish.

NATURAL RESOURCES CONSERVATION SERVICE Vermont NRCS

SPECIFICATION GUIDE SHEET

for Riparian Forest Buffer (391)

SCOPE:

This work will consist of establishing adapted and compatible native trees and shrubs adjacent to and up gradient from watercourses or waterbodies. The purpose for this practice may include creating shade to improve aquatic habitat, provide riparian habitat, provide for a source of detritus and large woody debris, reduce excess sediment and other pollutants in surface and shallow groundwater, reduce pesticide drift, restore riparian plant communities, and increase carbon storage.

GENERAL SPECIFICATIONS APPLICABLE TO ALL PURPOSES

To be able to plan for the restoration of a riparian area one must understand what its functions are and where it lies in the landscape. A good definition defines the **riparian area** as "the aquatic ecosystem and the portions of the adjacent terrestrial ecosystem that directly affect or are affected by the aquatic environment. This includes streams, rivers, lakes, and bays and their adjacent side channels, flood plain, and wetlands. In specific cases, the riparian area may also include a portion of the hillslope that directly serves as streamside habitats for wildlife."

The Three Zone System

A three-zone system has been developed to help plan riparian forest buffers. This three-zone concept is intended to be flexible in order to achieve both resource protection and landowner objectives.

All buffers, as a minimum, will consist of Management Zones 1and 2. The minimum width of these combined 2 zones, for all purposes, is 35 feet. Wider buffers are encouraged and may be required depending on the purpose. Wider buffers will provide more functions and values than narrow strips. Forested buffers that will connect two or more forested patches are considered corridors for wildlife. Minimum widths for travel corridors for wildlife are 50 feet where it is an identified objective of the practice.

Zone 1

This zone begins at top of bank and will contain trees and shrubs needed to provide aquatic shade, bank stability, detritus, large woody debris, and retain nutrients bound to soils. Large woody debris and tree roots in the water create habitat complexity and niches for invertebrates and aquatic organisms. Detritus such as leaves, twigs and fruit seeds entering the water and held by woody debris provide a base to the aquatic food chain.

Zone 1 is most subject to inundation. Species with the greatest tolerance to these conditions are listed in VT Forestry Technical Note 2 – VT Tree and Shrubs for Conservation. Silver maple, black willow, boxelder, alder, dogwood, and eastern cottonwood have evolved in and are best suited for these conditions in most locations throughout Vermont floodplains. Silver maple floodplain forests and alluvial shrub swamps are two natural community types that are commonly the target for restoration with this practice. The fast growth rate and brittle habit of these species withstand the periodic trauma of heavy floods. Instead of washing away and exposing unstabilized banks, these species shed branches, regrowing from the remaining trunk. Because of their fast growth rate, they are established relatively easily and rapidly reach canopy closure. These species facilitate the important goal of stream shading and promote establishment of the riparian forest buffer.

The minimum width for this zone for all purposes is 15 feet from top of bank.

Zone 2

This zone is landward of Zone 1 and will contain the trees and shrubs and other vegetation needed to filter runoff and provide uptake of nutrients and pollutants. Together, Zone 1 and 2 will provide a travel corridor and habitat for wildlife in addition to providing shade and a source of woody debris.

Zone 2 can include commercially viable canopy species such as red oak and sugar maple where site conditions permit; areas with high terraces and drier conditions. More flood and wet soil tolerant species, similar to Zone 1, will likely be necessary in Zone 2 depending on the natural community and soil moisture. Generally, for most buffers being planned and implemented in Vermont, Zone 2 is functionally an extension of Zone 1. Except in very wide buffers or near abrupt slope breaks, the species used for both zones will be essentially the same. An understory of shrubs will provide additional shade and structure to Zone 2. Where shading needs for the water body are met, the transition from Zone 2 to 3 can be planted with early successional species such as elderberry, dogwoods, and viburnums to limit the encroachment of invasive plants into Zone 2 and to provide a soft edge between the grass and forest habitats. **The minimum width for this zone for all purposes is 20 feet.**

Zone 3

This zone is landward of Zone 2 and consists of a strip of grass or herbaceous cover to spread, slow and filter runoff which may be transporting sediment, nutrients, and pesticides off cropland or other erosive areas. **The minimum width for this zone, where necessary, is 15 feet.**

Additional Specifications to Reduce Excess Amounts of Sediment, Organic Material, Nutrients and Pesticides in Surface Runoff and Reduce Excess Nutrients and Other Chemicals in Shallow Ground Water Flow

The riparian forest buffer will consist of Zones 1, 2 and, in some cases, Zone 3. Establishment of Zone 3 filter area will be required where there is sheet flow from cropland toward the forest buffer and stream. A hundred foot buffer has been shown to provide even greater water quality benefits and may be necessary depending on site conditions.

Fast growing species with high nutrient uptake potential should be favored for Zone 2. Zone 2 width will be expanded beyond the 20 foot minimum where necessary to capture excess nutrients, accommodate topography (slope) of the site and or accommodate stream adjustment processes (see Unstable River Channels section).

Where Zone 3 is required, the total combined buffer width shall be no less than 50 feet. Zone 3 will be established and managed according to the Filter Strip Specification Sheet 393.

Unstable River Channels

Planning buffers on unstable river channels requires a greater level of analysis. Many rivers in Vermont are undergoing adjustments due to past and current alterations and managements. Establishing a riparian forest buffer must account for the nature of these systems and for the extent of adjustment and change that could be expected. This will require using geomorphic assessment data and consultations with river scientists or other resource professionals. This consultation will help verify the form and extent of the instability.

Where an unstable channel exists on a project area and where Phase 1 assessments have been completed, use the defined river corridor from the internet based **River Management Stream Geomorphic Assessment Data Viewer** (Mapserve) as the potential foot print of the buffer area which may be refined with site visits. The corridor is intended to include the area that will allow for stream equilibrium condition to develop and stabilize over the long term.

Where there is no phase 1 data, a river corridor can be defined using the belt-width approach. See the DEC River Management 'Defining River Corridors Fact Sheet.' Adding an additional channel width on each side of the stream belt-width will approximate the river corridor for planning purposes.

Plantings should be set back from the top of bank and eroding channel commensurate with the rate of erosion. Bioengineering using stakes and wattles may help to slow the rate of erosion and aid in woody establishment on the buffer.

Additional Specifications to Maintain or Restore Water Temperatures and Provide Large Woody Debris

The riparian forest buffer will consist of Zones 1 and 2 and the total combined width will be a minimum of 35 feet. Zone 1 will be planted to fast growing, tall species that will quickly address the lack of shading and provide large woody debris. Canopy density should be kept at least at 80 percent coverage. Maximum shading ability is reached within a width of 80 feet, with 90 percent of the maximum reached within 55 feet.

Large woody debris (>4 inch diameter) usually originates within 60 feet of the stream. Ideally, streams supporting fish should have 75 to 200 pieces of large woody debris per stream mile.

Additional Specifications to Provide Fish and Wildlife Habitat

The riparian forest buffer will consist of Zones 1 and 2 and the total combined buffer width shall be no less than 50 feet. This will require that Zone two be expanded beyond the minimum to 35 feet. Zone 3 will be used in addition to Zones 1 and 2 where excess nutrients, sediments, etc. are also a concern. Buffers more 100 feet wide or more are recommended as they provide the most fish and wildlife habitat value. See Table 1 for more information about species or groups and buffer requirements. Design buffers to meet or exceed the minimum requirements of local species of concern.

Design buffers to connect upland habitats and wetlands if possible. Numerous species that use aquatic and riparian/wetland habitats will also use upland habitats at some point of their life cycle (e.g. wood turtle).

Planting Plan

The planting plan will be recorded on the approved VT NRCS 391 Job Sheet and will include the natural community type, species and sizes, numbers to be planted for the restoration, spacing, specifications for protection if applicable, and any associated bioengineering that will compliment the tree and shrub establishment. A pre-planting meeting will be held on site with the planters to ensure that the planting plan is properly followed based upon the site conditions.

Riparian forest buffers will be designed to meet the intended purpose of the practice and will also mimic natural plant communities native to the site. Locally developed, native Vermont plant materials or seeds should be considered for planting. See VT Forestry Technical Note 2 – VT Trees and Shrubs for Conservation for more information. Do not order or plant species developed outside of Vermont which are uncommon or rare in the State. This will maintain the genetic integrity of this species in Vermont. Plant a minimum of 5 species of trees and or shrubs for each site. For specifications on tree and shrub planting see Tree and Shrub Establishment (612) Specification Guide Sheet.

Determining Natural Plant Community

Various tools are available to assist in determining the natural community type and species typical of a specific site. The primary reference for determining natural community and species composition is Wetland Woodland Wildland – A Guide to the Natural Communities of Vermont. The companion reference is the Vermont NRCS Soil Series of Vermont and their associated Natural Communities found within section IIA of the electronic Field Office Technical Guide (eFOTG).

Steps: For a given site, the planner may determine the soil series from the County Soil Survey or onsite review. Next, refer to the Soil Series Natural Community guide and find the soil series; read across the table to find the natural community typical of that soil series. Refer to Wetlands Woodlands Wildland for more information about the natural communities including tree and shrub species.

It is also important for the planner to evaluate nearby plant communities on similar site conditions to determine what is appropriate or typical for the specific site. There may be inclusions of other non-forested communities such as emergent shallow marsh or sedge meadows which may provide good habitat diversity in concert with the forested areas. These naturally open communities should not be planted to trees without consideration.

Finally, the planting plan will also need to account for the availability of plant materials. Some species are difficult to grow locally and may be better established through natural regeneration on site.

Note: Be aware of local potential pathogens or pests known to be associated with plant materials that may be ordered from outside Vermont. For example, hemlock should generally not be imported due to wooly adelgid concerns.

Site Planning

Once the appropriate natural community and species are determined for the site, it is important to have a planting plan that specifies how and where different species will be planted based upon site conditions. It is not a good practice to indiscriminately plant species, regardless of habit, across the entire buffer area unless site conditions are uniform. There may be a good amount of variability in soil moisture, herbaceous vegetation height, and topography across this buffer area that should be planned for in the planting plan. For example, if there is a low floodplain or depressions within the buffer area, the planner will need to specify that species adapted to wet soils and inundation be planted in these locations and more upland species at the higher sites. Live stakes and wattles may be a good alternative to tree planting in very wet sites that are frequently flooded. This specific planting information should be made clear to the contracted planters at the pre-planting meeting on site. For information about tree species habits and characteristics and species suitable for bioengineering refer to VT Forestry Technical Note 2 – VT Trees and Shrubs for Conservation and the Tree and Shrub Establishment (612) Specification Guide Sheet.

Natural Regeneration

Natural regeneration can be a cost effective way to allow riparian forest buffer establishment and plant succession to occur on site. It is a slower process than planting but it is one that will select the most suitable species for the site and there is no concern about origins of the growing stock. However, it may not provide uniform stem density and closed canopy coverage for the site in as short a period of time as planting.

Determine if natural regeneration can successfully meet the purpose of the riparian forest buffer. If closed canopy conditions throughout the entire buffer area are required in a short period of time; then natural regeneration may not be the best choice. Recognize that natural regeneration has limitations and that certain buffer functions such as shading, nutrient uptake, habitat corridors, natural communities may need to be met with a planted buffer.

The first step in determining if natural regeneration will meet the purpose of the buffer is to determine how many stems per acre and what species are currently present. This can be done by using the Systematic Line Plot Cruise developed by the Maryland Department of Natural Resources Forest Service or other methods. For detailed description of this process see VT Forestry Technical 1 – Stems per Acre Line Plot.

Natural Regeneration Specifications

Where other buffer functions have been accounted for within the zones, then 150 existing woody stems per acre on site will be considered an established riparian forest buffer. Invasive plants will not be included in this count. This number of stems will approximate the number of stems that are expected to survive from a minimum planting of 200 stems per acre (see Plant Spacing and Density). Generally 75% survival is expected for a planted riparian forest buffer.

Once woody stems have been established it should lead to further regeneration through changes in the site condition (shading favoring trees and shrubs), seed dispersal by birds and mammals and root suckering. This additional regeneration will meet or exceed stems/acre on many planted buffers in Vermont.

Pay careful attention to Zone 1 of the Buffer when considering using natural regeneration instead of planting. This is a critical zone for development of favorable aquatic habitat and conditions. There should be very good evidence of natural regeneration in this Zone. Where there is not, plant accordingly even if the minimum numbers of stems per acre are present.

When considering potential establishment through natural regeneration, consider the site conditions and potential for establishment. Dense sod will likely need to be harrowed while idle crop fields or pastures may be well suited. Often pastures have some woody component that has been suppressed.

Consider the surrounding riparian areas or forest areas for seed sources. Natural regeneration is not a good option if the buffer area is surrounded by agricultural land with no favorable seed sources or potential for vegetative reproduction. Where there are perches for birds (e.g. fence posts, trees on site, etc.) there is a better likelihood of colonization for some woody species; in particular, shrubs whose fruits are fed upon by birds will be seeded into these areas.

When planning for natural regeneration to occur in the buffer, consider mode of dispersal, distance between seed source and target area, seed source strength (number and size of mature seed bearing specimens) and seed size. Generally, heavy seeded species will disperse short distances (one study found 150 feet or less) while wind and bird dispersed seeds may travel greater distances (same study found 450 feet or less). Obviously all seeds can travel greater distances but the probabilities are less. See Tree and Shrub Establishment Specification Sheet 612 (Table 1) for examples of seed sizes and dispersal mechanisms for various trees and shrubs.

Wind and bird dispersed seeds will be most likely to colonize a site with some stems present. Where there are no perching sites in a buffer, wind dispersed seeds will be the primary form of regeneration. Heavy seeded species such as oak and hickory will take longer to naturally establish; particularly over longer distances. Consider planting species such as oak and hickory in regenerating buffers to aid in establishment where they are a component of the targeted natural community.

Buffers that are not planted may persist in an early successional state for decades. This may provide good habitat for certain species of concern in the Northeast (e.g. shrubland birds) but it can also provide favorable conditions for invasive plants such as buckthorn and honeysuckle. Monitoring is important to prevent their initial establishment.

Plant Spacing and Density

In mature riparian floodplain forests, canopy tree stem density is roughly 150 stems per acre, indicating a tree spacing of 16 to 18 feet. Conversely, in an alluvial shrub swamp there may be thousands of stems per acre. Determine what plant spacing and density best meets the purpose of the buffer and best matches the natural community. It is likely that in many cases it is not feasible to plant to meet the natural condition stems per acre in some shrub natural communities so the goal should be to plant in a manner that will allow for succession to this natural community condition.

Initial plant to plant densities for trees and shrubs will depend on their potential height at 20 years of age. Riparian forest buffers are expected to reach crown closure at 10-20 years when stocked at the minimal level of 200 tall trees an acre (greater than 25 feet). Heights may be estimated based on:

- Performance of the individual species (or comparable species) in nearby areas on similar sites.
- Predetermined and documented heights from VT Forestry Technical Note 1 – VT Trees and Shrubs for Conservation.

When establishing a new planted buffer, a minimum of two staggered rows of trees and or shrubs will be established along the water body. Generally this will be within Zone 1. Favor species that will provide shading in a short amount of time. See VT Forestry Technical Note 2 – VT Trees and Shrubs for Conservation.

Planting density should be higher than the final stem density desired, to allow for losses due to competition, stress, and animal damage. Generally, 75% is the expected survival rate for planted buffers. For a floodplain forest, a minimum of 200 plants are needed to be planted per acre to ensure 150 stems per acre. Natural regeneration is also expected to contribute trees and shrubs. In a study in Maryland of 130 buffer sites, 36% of total stocking of woody species was from natural regeneration.

Plant Types/ Community	Plants per Acre	Plant-to-Plant Spacing (Feet)
Shrub Community – shrub dominated, mostly shrubs	450 to 300	10 to 12
Forest Community – tree dominated, mix of trees and shrubs	300 to 200	12 to 15

Plant a mix of trees and shrubs to add habitat value; even when planting the minimum 200 stems per acre. When planting the minimum number of trees and shrubs together in a forest community, do not exceed 25% shrubs in the planting plan. Except in narrow buffers (35-50 feet), it is unlikely necessary to have tall trees for shading on the entire buffer. Adding shrubs to the planting will provide a successional component and important habitat value for wildlife. Adding vertical strata (shrub layer) to the vegetative community will increase the available niches to be used by more species of wildlife. For buffers greater than 50 feet, up to 25% of the buffer area may be left open and intermixed with planting areas. This approach would work well with planting clumps of shrubs. Individual open areas should not exceed 1/10 acre in size. Species of concern such as wood turtles will use open areas for foraging or basking; particularly in or near alluvial shrub swamps.

Establishment Period

The riparian forest buffer will be considered established when 75% of the planted trees and shrubs are alive after 2 growing seasons. If, after 2 growing seasons, there are less than 75% live planted trees on site and natural regeneration has not made up the loss of stems, then re-planting will be necessary.

For Natural Regeneration, assuming other buffer purposes have been accounted for, then 150 existing woody stems per acre on site will be considered an established riparian forest buffer. No additional planting will be necessary unless specified by the planner.

Planting trees and shrubs is not required in all cases where existing stem density is less than 150 per acre. Sites that have evidence of regeneration, where there is a high likelihood of attaining the minimum 150 stems per acre in two growing seasons do not require planting. For instance, a crop field that has initial establishment of silver maple seedlings (not required density) adjacent to mature silver maples will likely exceed the minimum 150 stems per acre through natural regeneration in two growing seasons simply by stopping tillage and herbicide application. Also, a heavily grazed pasture with a 100 native woody stems per acre may easily reach 150 stems per acre in two growing seasons simply by removing livestock. If, after 2 growing seasons, there are less than 150 live native woody stems per acre on site then planting will be necessary.

Direct Seeding Guidelines

Refer to Tree and Shrub Establishment (612) Specification Guide Sheet for information regarding direct seeding. Plant enough seeds to reach the desired stems per acre. Be aware that mortality is generally much higher when direct seeding.

Site Preparation/Weed Control for Buffer Establishment

Refer to Tree and Shrub Establishment (612) Specification Guide Sheet for information regarding site preparation and weed control.

Planting Dates

Refer to Tree and Shrub Establishment (612) Specification Guide Sheet for information regarding planting dates for seeds, seedlings, cuttings and larger planting stock.

Planting Requirements/Techniques

Refer to Tree and Shrub Establishment (612) Specification Guide Sheet for information regarding planting requirements and techniques.

Plant Protection

Refer to Tree and Shrub Establishment (612) Specification Guide Sheet for information regarding protection for planting stock.

REFERENCES:

Buffers for Habitat - Riparian Buffers for the Connecticut River Watershed Fact Sheet Number 4 1998. Connecticut River Joint Commission (CRJC). http://www.crjc.org/riparianbuffers.htm

Buffer Maintenance and Monitoring. 2004. Alliance for the Chesapeake Bay. http://www.acb-online.org/pubs.cfm

Chesapeake Bay riparian handbook: a guide for establishing and maintaining riparian forest buffers. 1997. Palone, R.S. and A.H. Todd (editors.) USDA Forest Service. NA-TP-02-97. Radnor, PA.

Riparian Buffers and Corridors – Technical Papers. 2005. VT Agency of Natural Resources.

Riparian Forest Buffers - Function and Design for Protection and Enhancement of Water Resources, NA-PR-07-91. 1991 David J. Welsch. USDA Forest Service, Northeastern Area State and Private Forestry, St. Paul,MN. http://www.na.fs.fed.us/spfo/pubs/n_resource/buffer/cover.htm

Riparian Forest Buffer Success and Survival in Maryland. 2001. Maryland DNR Forest Service. Research Report DNR/FS-01-01.

Tree dispersal among forest fragments: II – Dispersal abilities and biogeographical controls. 2002. Nina Hewitt and Martin Kellman. Journal of Biogeography, 29:351-363.

Table 1. Riparian Forest Buffer Widths for Fish and Wildlife

SPECIES	DESIRED WIDTH (in feet)
Wildlife dependent on wetlands or watercourses	**30-600'**
Bald eagle, nesting heron, cavity nesting ducks	600
Pileated woodpecker	450
Beaver, dabbling ducks, mink	300
Bobcat, red fox, fisher, otter, muskrat	330
Amphibians and reptiles	100-330
Belted kingfisher	100-200
Songbirds	**40-660**
Scarlet tanager, American redstart, rufous-sided towhee	660
Brown thrasher, hairy woodpecker, red-eyed vireo	130
Blue jay, black capped chickadee, downy woodpecker	50
Cardinal	40
Cold water fisheries	**100-300**

Source - Connecticut River Joint Commission (CRJC) Buffers for Habitat - in the series *Riparian Buffers for the Connecticut River Watershed*

Table 2. Natural Community types associated with rivers and lakes.

Open Upland Shores	Open Wet Shores	Marshes and Sedge Meadows	Shrub Swamps	Floodplain Forests and Swamps
Riverside Outcrop	Outwash Plain Pondshore	Shallow Emergent Marsh	Alluvial Shrub Swamp	Lakeside Floodplain Forest
Erosional River Bluff	River Mud Shore	Sedge Meadow	Sweet Gale Shoreline Swamp	Red or Silver Maple-Green Ash Swamp
Lake Shale or Cobble Beach	River Sand or Gravel Shore	Cattail Marsh		Red Maple-Northern White Cedar Swamp
Lake Sand Beach	River Cobble Shore	Deep Broadleaf Marsh		Silver Maple-Ostrich Fern Riverine Floodplain Forest
Sand Dune	Calcareous Riverside Seep	Wild Rice Marsh		Silver Maple-Sensitive Fern Riverine Floodplain Forest
	Rivershore Grassland	Deep Bulrush Marsh		Sugar Maple-Ostrich Fern Riverine Floodplain Forest
	Lakeshore Grassland			

Source – Riparian Buffers and Corridors – VTANR

Table 3.

Number of Trees per Acre by Various Methods of Spacing

Spacing (feet)	Trees (number)	Spacing (feet)	Trees (number)	Spacing (feet)	Trees (number)
2x2	10,890	7x9	691	12x15	242
3x3	4,840	7x10	622	12x18	202
4x4	2,722	7x12	519	12x20	182
4x5	2,178	7x15	415	12x25	145
4x6	1,815	8x8	681	13x13	258
4x7	1,556	8x9	605	13x15	223
4x8	1,361	8x10	544	13x20	168
4x9	1,210	8x12	454	13x25	134
4x10	1,089	8x15	363	14x14	222
5x5	1,742	8x25	218	14x15	207
5x6	1,452	9x9	538	14x20	156
5x7	1,245	9x10	484	14x25	124
5x8	1,089	9x12	403	15x15	194
5x9	968	9x15	323	15x20	145
5x10	871	10x10	436	15x25	116
6x6	1,210	10x12	363	16x16	170
6x7	1,037	10x15	290	16x20	136
6x8	908	10x18	242	16x25	109
6x9	807	11x11	360	18x18	134
6x10	726	11x12	330	18x20	121
6x12	605	11x15	264	18x25	97
6x15	484	11x20	198	20x20	109
7x7	889	11x25	158	20x25	87
7x8	778	12x12	302	25x25	70

Source - Chesapeake Bay riparian handbook

Appendix L-21

NASHVILLE CRAYFISH SURVEY PROTOCOLS

These protocols are currently being prepared and will be included in this MSHCP when available from the Service. These protocols will be based, in part, on the specifications provided in Nowicki et al. 2008, *Monitoring crayfish using a mark-recapture method: potentials, recommendations, and limitations* (attached).

Biodivers Conserv (2008) 17:3513–3530
DOI 10.1007/s10531-008-9425-1

ORIGINAL PAPER

Monitoring crayfish using a mark-recapture method: potentials, recommendations, and limitations

Piotr Nowicki · Tina Tirelli · Rocco Mussat Sartor · Francesca Bona · Daniela Pessani

Received: 28 September 2007 / Accepted: 26 June 2008 / Published online: 16 July 2008

Abstract Crayfish are regarded as useful indicators of environmental quality and freshwater biodiversity. However, reliable methods for monitoring their populations are needed so that this potential can be fully utilised. We report and discuss methodological aspects of the white-clawed crayfish (*Austropotamobius pallipes* complex) survey conducted in Piedmont, Italy, with the use of mark-recapture. The results suggest that the method can serve as a convenient tool for estimating the size of crayfish populations and inferring their temporal trends. The two populations investigated appeared closed except for wintertime and July. Consequently, the Robust Design, which is regarded as the most reliable mark-recapture approach, can be easily applied. The minimum effective sampling plan for monitoring purposes should comprise one primary period per year, conducted in the summer–autumn season, and consisting of three capture sessions. If gaining insight into the ecology of the investigated species is the prime objective and sufficient resources are available, the optimal plan should include two primary periods (in spring and the summer–autumn season) of five capture sessions each. Capture sessions need to be separated by roughly 2-week intervals in order to avoid the strong, but short-term, negative effect of capturing crayfish on their recapture chances. As the model without heterogeneity in capture probabilities ensures better estimate precision we recommend that data collected for both sexes are analysed separately. Taking into consideration higher male catchabilities and sex ratio being invariably 1:1, it also seems beneficial to estimate only male numbers and double them to achieve total population sizes.

Keywords *Austropotamobius pallipes* complex · Jolly-Seber model · Model selection · Population size estimation · Relative abundance methods · Robust design · Sampling intensity · Survival patterns

P. Nowicki (✉)
Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, 30-387 Krakow, Poland
e-mail: piotr.nowicki@uj.edu.pl

T. Tirelli · R. Mussat Sartor · F. Bona · D. Pessani
Dipartimento di Biologia Animale e dell'Uomo, University of Turin, Via Accademia Albertina, 13-10123 Torino, Italy

Introduction

Crayfish are a highly diverse and important invertebrate group, with many species playing a prominent role in freshwater ecosystems. They are keystone consumers (Nyström et al. 1996) feeding on algae, macrophytes, invertebrates, and detritus (e.g. Lodge et al. 1994; Whitledge and Rabeni 1997). In turn, they are preyed upon by various fish, birds, and mammals (Holdich and Lowery 1988; Reynolds 1998; Holdich 2003). In addition, their burrowing behaviour considerably modifies river banks (Dorn and Mittelbach 1999), creating microhabitats that constitute a refuge from drought and extreme winter conditions for many small organisms (Usio and Townsend 2004; Zhang et al. 2004; Pintor and Soluk 2006). For the above reasons crayfish have recently been regarded as potential useful indicators of freshwater biodiversity (Reynolds and Souty-Grosset 2003; Gherardi and Souty-Grosset 2006). Moreover, some crayfish, especially long-lived species of cool waters, are sensitive to pollution and thus may serve as useful bioindicators of water quality (Jay and Holdich 1981; Holdich and Reeve 1991; Reynolds et al. 2001).

Several freshwater crayfish species are currently endangered in various parts of the world and listed in the IUCN Red List (Baillie and Groombridge 1996; Souty-Grosset et al. 2006). Apart from their sensitivity to pollution, this is mainly caused by the competition with exotic crayfish introduced by man as well as disease and parasite transmission or even predation by them (Gherardi and Holdich 1999; Taugbøl and Skurdal 1999; Lodge et al. 2000; Gherardi 2006). The negative impact of these invasive exotics is not restricted to native crayfish species; in fact they have been reported to seriously reduce biomass and species richness of many other groups of fauna and flora too (Wilson et al. 2004; Rodríguez et al. 2005; Crawford et al. 2006; Rogowski and Stockwell 2006; Rosenthal et al. 2006; Willis and Magnuson 2006).

Consequently, monitoring both native and invasive crayfish species is not only essential for assessing the status of the former (in many cases being a legal obligation), but also important in much broader conservation programmes targeting whole communities or even the entire biodiversity of freshwater areas. The problem, however, lies in the lack of a well-established methodology for monitoring crayfish populations. The methods traditionally used for assessing crayfish abundance, such as manual searching, trapping, and night viewing, are not fully reliable (Rabeni et al. 1997; Peay 2003; Dorn et al. 2005; also see the Discussion section for further explanation).

One of the potential remedies could be the application of mark-recapture methods, which are particularly useful for studying population trends in small animals, and have been successfully harnessed in monitoring programmes for rodents, birds, reptiles, amphibians, and butterflies (Baillie 1995; Marunouchi et al. 2002; Flowerdew et al. 2004; Julliard et al. 2004; Moore et al. 2007; Nowicki et al. 2008). In crayfish, these methods have been used mainly for investigating dispersal (e.g. Robinson et al. 2000; Gherardi et al. 2000; Byron and Wilson 2001), whereas studies aimed at population parameters were short-termed and focused on population structure (Parkyn et al. 2002; Maguire et al. 2004; Jones and Coulson 2006) or spatial abundance patterns (Guan and Wiles 1996; Hicks 2003; Hockley et al. 2005) rather than on temporal trends.

The aim of the present study was to test the applicability of mark-recapture for monitoring crayfish populations. Our motivation was stimulated by the fact that crayfish may be expected to be rather easy to sample with mark-recapture, based on characteristics of their biology. The ease of sampling crayfish derives from their relatively high local densities (e.g. Guan and Wiles 1996; Hicks 2003; Jones et al. 2005), high site-fidelity (Bubb et al. 2002, 2006; Webb and Richardson 2004), and considerable longevity (Parkyn et al. 2002;

Holdich 2003), which together make it possible to achieve adequate recapture rates. We were also interested in assessing which mark-recapture models would fit the data best, thus ensuring unbiased and relatively precise population size estimates. Finally, our intention was to propose a simplified protocol for data collection and analysis in monitoring of freshwater crayfish populations.

Materials and methods

Study species and sites

As a model for the analyses we chose the white-clawed crayfish (*Austropotamobius pallipes* complex). The white-clawed crayfish are native to western Europe with a wide historical distribution ranging from the Balkans and Italy in the south-east to Ireland in the north-west (Reynolds 1998; Holdich 2003; Souty-Grosset et al. 2006). However, over the last 150 years they experienced a dramatic decline, and are currently mostly confined to small and isolated relict populations (Holdich and Lowery 1988; Reynolds 1998). Consequently, the white-clawed crayfish are protected by national laws in the countries where they occur as well as listed in the Annexes II and V of the Habitats Directive (van Helddingen et al. 1996; Holdich 2003). The distinction between the two recently separated white-clawed crayfish species, *A. pallipes* and *A. italicus*, is possible only on basis of genetic data (Santucci et al. 1997; Grandjean et al. 2002; Fratini et al. 2005). As species identity, however, is of little relevance due to their similar life-history traits, we did not classify the species sampled as either of the two, but refer to it as the *A. pallipes* complex.

We investigated two local populations of the *A. pallipes* complex inhabiting the Rio dell'Osio and the Rio Pilatu streams in the hydrographical basin of the Malone river, located north of Turin in the foothills of the Italian Alps (Fig. 1). Rio dell'Osio (N 45°18′52″, E 7°33′31″, 530 m a.s.l.) has an average width of ca. 4 m and consists of rapid flow stretches with a depth of 20–30 cm, interspersed every 10–40 m with slow flow pools (max. dimensions: 9 × 7 m; avg. depth: 2 m). Rio Pilatu (N 45°17′26″, E 7°29′19″, 570 m a.s.l.) shows the same general characteristics, but it is only ca. 2.5 m wide and with smaller pools (max. dimensions: 3 × 2 m; avg. depth: 1.2 m). In both streams we sampled approximately 450 m long sections, where the habitat is apparently optimal for the white-clawed crayfish. Diverse banks with numerous tree roots, trunks and holes serve as potential refuges, while the surrounding lush vegetation provides shade and a large supply of organic material. More importantly for our purposes, crayfish populations within the sampled sections were effectively spatially isolated. Upstream, the sections are blocked by man-made cascades, while downstream the habitat becomes unsuitable for crayfish due to strong anthropogenic impact.

Field sampling

The mark-recapture surveys of the white-clawed crayfish populations in Rio dell'Osio and Rio Pilatu have been carried out since 2005 within the framework of the Action Plan for the species in the Piedmont region (Tirelli et al. in press). Our study is based on the data gathered so far, comprising years 2005–2006 and the first half of 2007. In 2005 the sampling was conducted from April to November with 13 two-day capture sessions held roughly every 2 weeks. In the following year, the capture sessions were made more

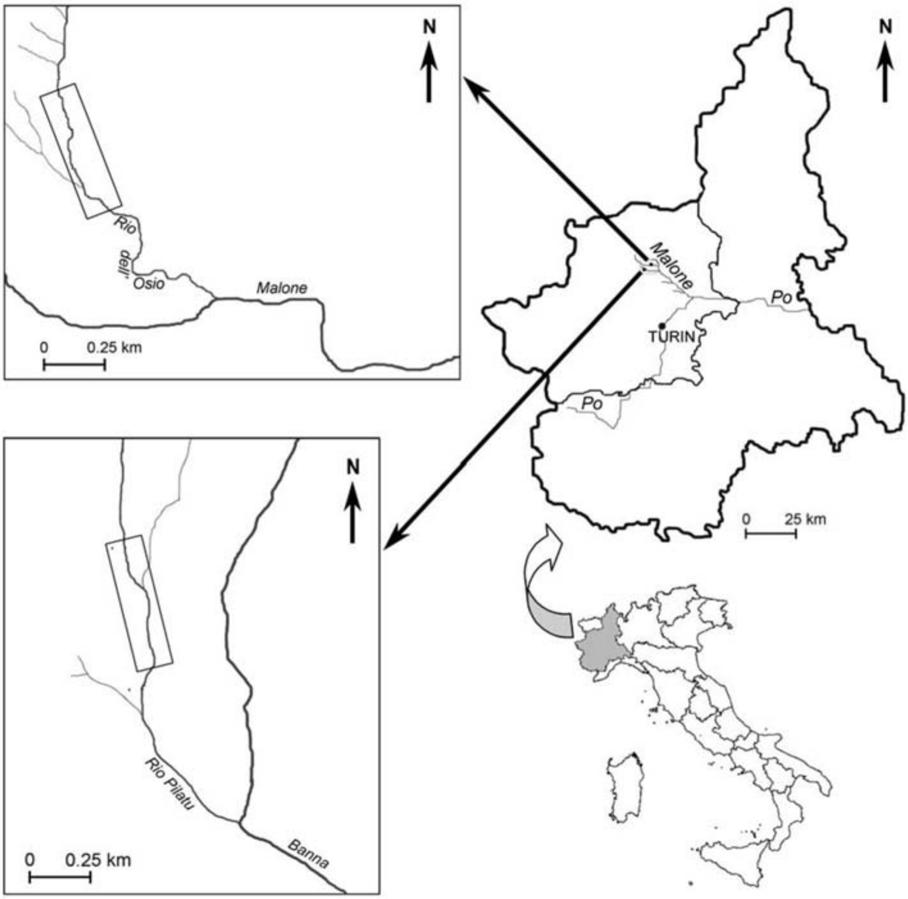


Fig. 1 Schematic map showing location of the two study sites in the Piedmont region, Northern Italy

intensive as the investigated sections of both streams (hereafter called sites) were subdivided into 5 units of approximately equal length (80–100 m). Each unit was sampled on a different day, and thus a single capture session lasted 5 days. There were six capture sessions conducted roughly once a month from May to October. Finally, in 2007 the sampling plan resembled that of 2006, but the intervals between capture sessions were shortened to two weeks, which made it possible to have three sessions in April and May.

Two people were involved in sampling that typically lasted for ca. 2–3 h per day on each site, though its intensity had to be lower on many occasions in spring 2006 due to rainy weather, and in spring 2007 due to high water conditions. Crayfish were either actively searched for and hand-netted in shallow water during daytime or caught using eight traps set in deeper places in evenings and examined the following mornings. The traps were 50 × 25 × 25 cm, and with pig or chicken liver used as bait. All individuals captured were sexed and measured. Subsequently those with a total length greater than 40 mm were considered adults and were individually marked. In this way, during the three years of the study we recorded altogether 1,709 captures of 747 males and 439 females in Rio dell'Osio, and 1,278 captures of 521 males and 434 females in Rio Pilatu.

Marking was done with the method described by Guan (1997), which uses a code system based on holes punched in different positions of telson and uropods. The trouble with Guan's method is that the duration of marks depends on their position. However, as we applied marks on the most durable positions (for details see Guan 1997) they should last for at least 2–3 moulting events, which corresponds to over 1 year in full-grown white-clawed crayfish (Lowery 1988), or even more as our findings suggest (see the Results section). Juveniles were released without marking for both ethical and practical reasons. The former are related to the fact that marking is known to reduce crayfish growth rate (Guan 1997). A strong practical argument against marking juveniles is little feasibility of their use in mark-recapture studies due to very low catchability (Rabeni et al. 1997; Gherardi et al. 2000; Dorn et al. 2005) as well as frequent moultings (up to six per year) leading to increased loss of marks.

Data analysis

The sampling plan, at least for the first 2 years of the study, was designed under the conservative assumption that the investigated crayfish populations would be open for most of the time. Nevertheless, in the first step of our analysis we evaluated the validity of this assumption. This was done through assessing the survival (ϕ) and recruitment (B) of individuals between capture sessions with the open population Jolly-Seber type models (Schwarz and Arnason 1996; Schwarz and Seber 1999). Strictly-speaking ϕ should be called residence as it is affected not only by mortality but also by emigration, yet we retain the term survival for the sake of consistency with the standard mark-recapture nomenclature. Recruitment in turn includes both births (in fact in our case it is maturation as we only investigated the adult fraction of crayfish populations) and immigration. Either survival significantly lower than 1 or recruitment significantly different from 0 would indicate population openness. The models were fitted using the program POPAN (Arnason and Schwarz 1999). The program provides the estimates of capture probabilities ($\hat{p}_i$) for consecutive capture sessions as well as estimates of survival ($\hat{\phi}_i$) and the 'probability of entrance' into a population ($\hat{b}_i$, which is a relative measure of recruitment $\hat{b} = \hat{B}_i / \sum \hat{B}_i$) for intervals between sessions. Both temporal variation (t) and inter-sexual differences (s) in parameters were of prime interest. Thus we only considered the estimates of the unconstrained model $p(s*t)\phi(s*t)b(s*t)$, which anyway performed very well as indicated by the Akaike's Information Criterion (Akaike 1973; Hurvich and Tsai 1989).

Since the analysis of survival and recruitment patterns revealed that the investigated populations remained effectively closed for long periods (see the Results section), we decided to apply the Robust Design model (Pollock 1982; Pollock et al. 1990). The Robust Design is a mixed model using two types of capture periods: each primary period consists of several secondary periods. Population is assumed to be closed within primary periods, but open between them. Data on captures and recaptures during each secondary period are used to estimate population sizes within primary periods, while the data pooled within each primary period are used to estimate survival and recruitment between the periods. We adopted five primary periods comprising spring seasons 2005, 2006, and 2007 with respectively 6, 3, and 3 capture sessions constituting secondary periods, as well as summer–autumn seasons 2005 and 2006 with respectively 7 and 3 secondary periods.

For comparative purposes the data were analysed separately for males and females as well as jointly for the entire adult population. The analysis was conducted with the software MARK 4.3 (White and Burnham 1999), including the program CAPTURE (Otis et al.

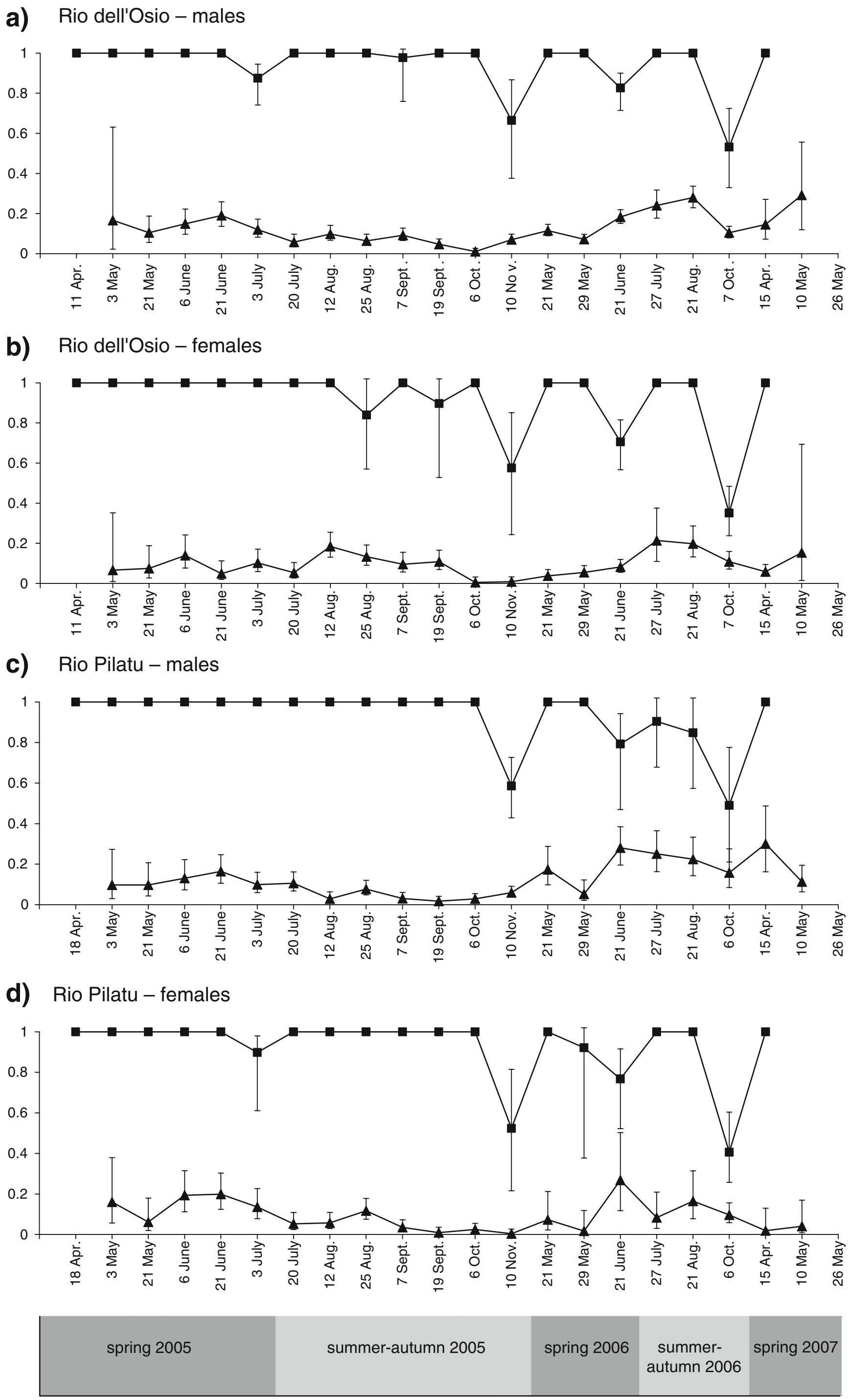
Fig. 2 Parameter estimates (squares = survival, triangles = capture probability; both with 95% confidence intervals) of the open population Jolly-Seber model applied to the crayfish populations investigated in 2005–2007. It should be noted that survival estimates presented for any given capture session actually refer to the interval between this session and the following one (e.g. the estimates given for 21 June 2006 represent the fractions of individuals surviving between 21 June and 27 July 2006). Estimates of probability of entrance into population are not included, but they were never significantly different from zero and generally of low precision. The bottom bar shows the resulting division of capture sessions into Robust Design primary periods

1978; Rexstad and Burnham 1991) incorporated as an independent module into MARK. The program CAPTURE was applied for selecting the most appropriate closed population models and the subsequent derivation of population size estimates for primary periods. The candidate models involved the null model assuming equal and constant capture probability for each individual (M_0) and models accounting for different types of violations to this assumption, such as time variation (M_t), heterogeneity (M_h), behaviour response (M_b), or their combinations (M_{bh}, M_{tb}, M_{th}, M_{tbh}) (Otis et al. 1978). As it was reasonable to expect that the nature of possible violations to equal catchability assumption was similar for both investigated populations and across seasons we opted to use the same closed model for all primary periods within a particular system (i.e. male/female fractions or entire adult population) as recommended by Williams et al. (2002). The selection of the most appropriate models followed the routine of the program CAPTURE in its first step. Subsequently, based on its outcome for the two primary periods of 2005, we calculated the weighted mean fit of different models with weights being numbers of individuals captured. The primary periods of 2006–2007 were not used in this analysis, because with only three secondary periods available the selection routine of the program CAPTURE would have had too little power (Otis et al. 1978; Menkens and Anderson 1988).

In addition, we investigated how representative the five sampling units were for the entire study sites in 2006–2007. For this purpose, population size estimates were also derived, using the Robust Design, from the data collected within sampling units. The division of sampling sessions into primary periods and the closed population models applied within them were identical as for the entire data sets. Obviously, with individuals moving between the sampling units spatial closure was not maintained and thus the population size estimates produced for the units should be expected to be positively biased (Kendall 1999). However, the biases were likely to be only slight, thanks to the aforementioned high site-fidelity of crayfish.

Results

Capture probability estimates yielded by the Jolly-Seber model were generally higher for males, although for both sexes their temporal variation was much more pronounced (Fig. 2). As expected capture probabilities grew substantially between 2005 and the two following years, corresponding to the increased sampling intensity, but even within the same season they were extremely variable. The recruitment between consecutive capture sessions according to the Jolly-Seber model was never significantly different from zero. In contrast, the analysis of survival revealed a fairly distinct and consistent pattern with significant losses of individuals occurring in July (though less clearly so in 2005) and over winter, but not in any other period (Fig. 2). Consequently, while applying the Robust Design, we divided capture sessions into the following five primary periods: April–early July 2005 (spring 2005); late July–November 2005 (summer–autumn 2005); May–June


a) Rio dell'Osio – males
1
0.8
0.6
0.4
0.2
0
11 Apr.
3 May
21 May
6 June
21 June
3 July
20 July
12 Aug.
25 Aug.
7 Sept.
19 Sept.
6 Oct.
10 Nov.
21 May
29 May
21 June
27 July
21 Aug.
7 Oct.
15 Apr.
10 May
26 May
b) Rio dell'Osio – females
c) Rio Pilatu – males
18 Apr.
6 Oct.
d) Rio Pilatu – females
spring 2005
summer-autumn 2005
spring 2006
summer-autumn 2006
spring 2007

2006 (spring 2006); late July–October 2006 (summer–autumn 2006); April–May 2007 (spring 2007).

The closed model selection procedure within primary periods indicated that the model with temporal variation in capture probabilities (M_t) was the most appropriate for both sexes (Table 1), which concurs with the aforementioned results of the Jolly-Seber model. In the case of the data pooled together for both sexes, heterogeneity in capture probabilities, apparently reflecting inter-sexual differences, could also be detected, and the M_{th} model performed the best (Table 1). At the same time intra-sexual heterogeneity was rather unlikely since the M_h model performed poorly within any sex. Similarly, there was very little indication of behavioural response effect on capture probabilities. However, the above refers only to the effect between capture sessions, i.e. over periods of 2 weeks or more, and does not preclude a strong negative behavioural response within the few days following capture. We hardly ever (altogether less than 10 cases) managed to recapture an individual within the same session.

The results of the M_t and M_{th} models applied to the investigated crayfish populations within the five seasons of the survey are given in Table 2. Capture probabilities were highest summer–autumn 2006 when the sampling was intensive and conducted in optimal conditions, but considerably lower in the springs of 2006 and 2007 with occasional unfavourable conditions, and even lower in 2005 when the capture sessions were less intensive, despite their twice higher number. With the slight exception of Rio Pilatu in spring 2005, males had catchabilities approximately twice as high as females ($\hat{p} = 0.24 - 0.53$ vs. $0.11 - 0.39$). Consequently, the precision index of their seasonal number estimates was about twice as good (Table 2). Also noticeable was a generally better precision of population size estimates obtained through summing male and female numbers as compared with those derived from the pooled data, which reflected the advantage of using the M_t rather than the M_{th} model. Nevertheless, estimates yielded by both methods were highly concordant (Kendall's $\tau = 0.733$, $n = 10$, $P = 0.0032$). The Rio dell'Osio population appeared relatively stable, while the Rio Pilatu population less so ($CV = 0.19$ and 0.43 respectively; based on the summed male and female numbers). However, this pattern can be explained at least partly by worse precision of the estimates for the latter site. Through the course of the study adult crayfish numbers grew gradually from ca. 900 to 1,100 individuals in Rio dell'Osio and ca. 600–700 individuals in Rio Pilatu in 2005 to roughly 1,400 individuals per site in summer–autumn 2006, but in the following spring they dropped back to initial (or even a bit lower) levels (Table 2, Fig. 3). Estimated sex ratio was invariably very close to 1:1.

Approximately 90% of individuals survived from spring to summer–autumn season each year, whereas the survival over winter was only about 50% in Rio dell'Osio and 30–40% in Rio Pilatu (Fig. 3). The average adult survival rate over the entire survey period was 0.952 per month (SE = 0.012) in Rio dell'Osio and 0.939 per month (SE = 0.009) in Rio Pilatu, with absolutely no inter-sexual differences. These correspond to the average residence time of 20 months (95% CI: 12–33) and 16 months (95% CI: 12–22) respectively for both populations. Because of the possible problem of mark loss, these figures should actually be regarded as the lower limits of mark duration. In this respect it is also worth mentioning that in spring 2007 we recaptured a considerable number (15 in Rio dell'Osio and 9 in Rio Pilatu) of individuals that had been marked 2 years before.

With few exceptions season-to-season trends (defined as $\hat{r}_i = \hat{N}_{i+1}/\hat{N}_i$) recorded within the sampling units were highly consistent for each site and period, regardless of whether local abundance estimates were produced separately for both sexes and then summed (intra-class correlation coefficient $r_I = 0.801$, $P = 0.0010$) or derived from the pooled data

Table 1 Performance of different closed population models (see the Data analysis section for their description) within the first two primary periods of the Robust Design

System	Site	Season	Model							
			M_0	M_h	M_b	M_t	M_{bh}	M_{th}	M_{tb}	M_{tbh}
Males	Rio dell'Osio	Spring 2005	0.75	0.83	0.05	1	0	0.31	0.41	0.15
	Rio dell'Osio	Summer–autumn 2005	0.12	0	0.03	1	0.07	0.70	0.26	0.17
	Rio Pilatu	Spring 2005	0.80	0	0.17	0.90	0.13	0.28	0.44	1
	Rio Pilatu	Summer–autumn 2005	0.14	0	0.04	1	0.06	0.68	0.33	0.21
	Weighted mean		0.41	0.24	0.06	0.99	0.06	0.52	0.35	0.29
Females	Rio dell'Osio	Spring 2005	0.46	0.24	0	1	0.24	0.97	0.31	0.56
	Rio dell'Osio	Summer–autumn 2005	0.13	0	0.34	0.58	0.18	0.38	1	0.40
	Rio Pilatu	Spring 2005	0.11	0.90	0.15	1	0	0.28	0.43	0.79
	Rio Pilatu	Summer–autumn 2005	0.13	0	0.36	1	0.18	0.58	0.39	0.39
	Weighted mean		0.19	0.24	0.24	0.83	0.15	0.51	0.62	0.51
All adults	Rio dell'Osio	Spring 2005	0.86	1	0.30	0	0.37	0.74	0.53	0.99
	Rio dell'Osio	Summer–autumn 2005	0.18	0	0.56	0.54	0.14	1	0.83	0.58
	Rio Pilatu	Spring 2005	0.76	0.85	0.05	0.04	0	1	0.40	0.29
	Rio Pilatu	Summer–autumn 2005	0.14	0	0.35	0.63	0.17	0.72	1	0.40
	Weighted mean		0.45	0.40	0.36	0.33	0.18	0.88	0.71	0.59

Values in the table represent relative fit of the models according to the model selection routine of the program CAPTURE, with 1s indicating the best-fitting models, and 0s being the worst ones. Weights used in the calculations of weighted mean fit of the models for each system (i.e. males, females, or entire adult fraction) were the numbers of captured crayfish as presented in Table 2

Table 2 Crayfish population characteristics within primary periods of the Robust Design

Site	Season	Males (M_t)				Females (M_t)				Sex ratio	Males (M_t) + females (M_t)				All adults (M_{th})			
		n	$\hat{N}$	$\hat{p}$	PI	n	$\hat{N}$	$\hat{p}$	PI		n	$\hat{N}$	$\hat{p}$	PI	n	$\hat{N}$	$\hat{p}$	PI
Rio dell'Osio	Spring 2005	110	432 (283–712)	0.25	0.24	46	429 (152–1,430)	0.11	0.65	50:50	156	861 (473–1,724)	0.18	0.35	156	1,085 (614–2,045)	0.14	0.32
	Summer–autumn 2005	132	419 (291–649)	0.32	0.21	99	491 (279–954)	0.20	0.33	46:54	231	910 (633–1,378)	0.25	0.20	231	1,007 (706–1,500)	0.23	0.20
	Spring 2006	184	571 (418–824)	0.32	0.18	50	400 (147–1,320)	0.13	0.64	59:41	234	971 (596–1,735)	0.24	0.28	234	1,134 (592–2,497)	0.21	0.40
	Summer–autumn 2006	385	725 (629–859)	0.53	0.08	239	616 (484–821)	0.39	0.14	54:46	624	1,341 (1166–1,572)	0.47	0.08	624	1,448 (1,179–1,846)	0.43	0.12
	Spring 2007	164	508 (350–803)	0.32	0.22	66	510 (212–1,417)	0.13	0.54	50:50	230	1,018 (617–1,836)	0.23	0.29	230	952 (479–2,330)	0.24	0.45
Rio Pilatu	Spring 2005	55	309 (144–779)	0.18	0.47	56	322 (150–813)	0.17	0.47	49:51	111	631 (353–1,230)	0.18	0.34	111	706 (378–1,440)	0.16	0.36
	Summer–autumn 2005	76	311 (181–607)	0.24	0.33	50	339 (145–931)	0.15	0.53	48:52	126	650 (375–1,227)	0.19	0.32	126	728 (417–1,374)	0.17	0.32
	Spring 2006	131	534 (323–977)	0.25	0.30	74	380 (174–1,015)	0.19	0.50	58:42	205	914 (569–1,585)	0.22	0.27	205	867 (501–1,690)	0.24	0.33
	Summer–autumn 2006	291	663 (541–843)	0.44	0.11	258	770 (589–1,051)	0.34	0.15	46:54	549	1,433 (1,200–1,749)	0.38	0.10	549	1,733 (1,318–2,374)	0.32	0.15
	Spring 2007	107	293 (199–485)	0.37	0.24	39	250 (97–814)	0.16	0.63	54:46	146	543 (322–1,040)	0.27	0.32	146	450 (246–1,078)	0.32	0.42

Parameters presented comprise the number of captured individuals (n), estimated population size ($\hat{N}$, with 95% confidence intervals in parentheses), capture probability $(\hat{p} = n/\hat{N})$, precision index defined as standard error of population size scaled to its estimate $(PI = SE(\hat{N})/\hat{N})$, and sex ratio based on estimated population sizes (males:females). Estimates for males and females were obtained with the M_t model (Chao 1989), while those for the entire adult fraction with the M_{th} model (Chao et al. 1992)

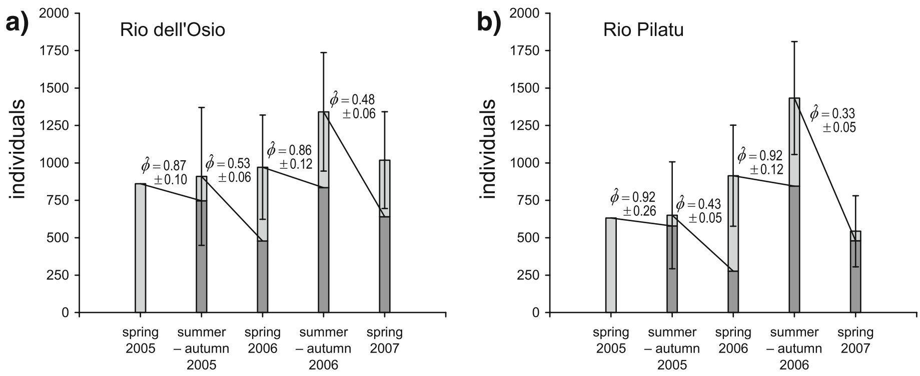


Fig. 3 Population dynamics of the investigated crayfish populations as revealed by the Robust Design. Except for the first season, when all the individuals were new to the survey, population size estimates were partitioned into recruitments of new individuals (light bars; shown with 95% confidence intervals), and fractions surviving from previous seasons (dark bars). Survival estimates (ϕ, presented with SE) refer to the entire adult fraction, while population sizes were estimated separately for males and females and then added up, since there was no difference in survival, but clear difference in capture probabilities between both sexes

($r_I = 0.760$, $P = 0.0026$). They were significantly affected by period, but not by site nor by interaction between site and period (MANOVA: period effect $F_{1,16} = 26.81$, $P < 0.0001$; site effect $F_{1,16} = 0.01$, $P = 0.9298$; interaction $F_{1,16} = 0.23$, $P = 0.6368$; the trends analysed were based on the summed male and female numbers, but the pattern was the same in the case of pooled data). This implies synchrony of population trends between the two sites. Most importantly, trends recorded within the sampling units reflected well those estimated for the entire study sites (Fig. 4). The sums of crayfish numbers estimated for the units exceeded the population size estimates for the entire sites only marginally, if at all, which suggests that there is no major effect of violations to the assumption of population closure within seasons resulting from possible crayfish movements between the units. The above prediction is confirmed by the low mobility that we recorded. During the whole survey only 20.3% of individuals recaptured in Rio dell'Osio and 21.7% in Rio Pilatu moved between the units, and among them less than half did so within a single season.

Discussion

Applicability of mark-recapture in comparison with other crayfish monitoring methods

A detailed review of methods for monitoring freshwater crayfish abundance has been provided by Peay (2003). Electrofishing, despite its efficiency (Rabeni et al. 1997), is highly destructive not only to crayfish investigated, but also to the whole community of co-occurring water organisms. Consequently, it should not be considered for application in monitoring programmes, especially those motivated by conservation purposes. Searching over fixed areas, typically quadrats, has a clear advantage of yielding absolute density estimates. On the other hand, this method is very labour-intensive and thus sampling plots have to be small. This alone does not preclude its use in fairly large-scale studies (e.g. DiStefano et al. 2003). However, it is doubtful whether the estimates obtained for sampling

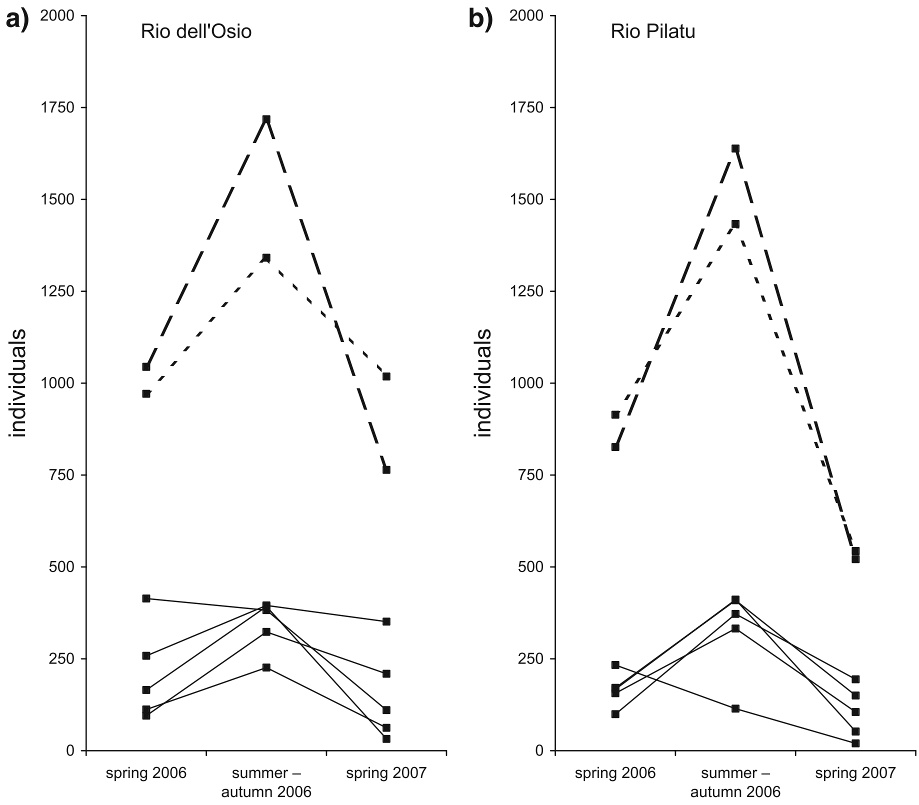


Fig. 4 Changes in crayfish abundance in 2006–2007 according to (i) individual numbers estimated for the five units of each study site (solid lines), (ii) the sums of numbers estimated within units (broken lines), and (iii) population sizes estimated for the entire study sites (dotted lines). In all cases the results presented are the sums of estimates obtained separately for males and females with the M_h model; the estimates obtained for the entire adult fraction with the M_{th} model indicated almost identical patterns

plots can be extrapolated to larger areas, especially that applying the method requires particular hydrological conditions.

Other methods reviewed by Peay (2003), i.e. searching for individuals in their refuges, night viewing, and trapping with baited or unbaited traps, are relatively labour-effective and non-destructive to crayfish populations. Nevertheless, one should remember that they are all relative abundance methods, which do not provide information about actual population size, and their results can serve at best as relative indices of abundance (e.g. Westman et al. 2002). Such indices may be useful for monitoring population trends only as long as the proportion of population sampled on each occasion remains constant. This last assumption is unlikely to be met, because the efficiencies of all the aforementioned methods are highly influenced by various environmental factors (Abrahamsson 1983; Skurdal et al. 1990; Acosta and Perry 2000; Maguire et al. 2002; Peay 2003). In the present study the proportion of individuals captured within each session was highly variable not only between season, which is not surprising because of differences in sampling intensity, but also within seasons, when this intensity was uniform. Hence, the proportion of population sampled may be expected to vary greatly even in the case of a standardised sampling protocol, such as the one proposed by Peay (2003).

We postulate that the mark-recapture approach offers a useful alternative. In its case variation in the proportion of population sampled is no longer a hindrance, because differences in catchability are estimated from the data and accounted for in the derivation of population size estimates. The applicability of mark-recapture depends on ensuring that the assumptions of theoretical models are met. For crayfish this seems relatively easy as compared to many other animals. The above statement is, of course, only true for their adult fraction, but surveys restricted to adults, which define effective population size, are sufficient for monitoring purposes, especially in invertebrates.

The populations of the white-clawed crayfish that we investigated were closed except for wintertime and the month of July. Openness in the former period apparently reflects high mortality during winter due to starvation, predation, and mating stress. The lack of closure in July is more difficult to explain, but it is probably associated with increased predation risk at moulting and (in females) release of juveniles as well as considerable mobility in this period (Gledhill et al. 1993; Reynolds 1998; Maguire et al. 2002). Obviously, the timing of periods of population openness (in particular the summer one) is likely to differ between regions (Reynolds 1998; Holdich 2003), and possibly even more so between species. Nevertheless, we strongly believe that a similar temporal pattern with long periods of population closure occurs in other freshwater crayfish of the temperate zone as well. Consequently, the Robust Design, which is regarded as the most reliable among mark-recapture models as it allows for unequal capture probability (Lancia et al. 1994; Williams et al. 2002), appears quite suitable for investigating freshwater crayfish populations. This model is in fact much more suitable for crayfish than for several other species groups, such as e.g. butterflies or fish, where it has already been tried successfully (Nowicki et al. 2005; Pollock et al. 2007).

Recommendations for optimal sampling plan

Under the Robust Design requirements the optimal survey plan for European freshwater crayfish should comprise two primary sampling periods per year: in spring and in summer–autumn season. The precise timing of these periods should be adjusted to the specific situation, but the months that turned out to be the most appropriate for the white-clawed crayfish populations in the present study, i.e. April–June and August–October respectively, may be used as preliminary settings if no a priori knowledge of the investigated system is available. In the case of logistic and/or financial constraints, a useful option may be to restrict the sampling to one primary period per year, especially if a survey is conducted for monitoring purposes rather than in order to gain an insight into the ecology of the investigated species. Our findings indicate that it would be more reasonable to omit spring sampling in such a situation, because of higher capture probabilities and slightly better chances for favourable weather conditions in the summer-autumn season.

Each primary period should optimally consist of five capture sessions (i.e. secondary periods) separated by two-week intervals. Shorter breaks are not recommended due to the fact that crayfish are very likely to strongly avoid being recaptured within a few days after the initial capture (Robinson et al. 2000; this study). Conducting five capture sessions separated by 2-week intervals within a 3-month period implies a rather densely-packed schedule and may be logistically difficult if several sites are going to be surveyed. Furthermore, bad weather or water conditions may occasionally make it impossible to perform some of the planned sessions. Fortunately, our results from the summer–autumn season of 2006 prove that even only three sessions may be sufficient for achieving precise estimates of crayfish population size provided that capture probabilities are high enough. The main disadvantage of having less than five capture sessions within a primary period is that the most appropriate closed population model cannot be reliably selected (Otis et al. 1978), but this problem is not insurmountable. Firstly, as highlighted by Skalski and Robson (1992) although inappropriate models lead to biased population size estimates, the biases should be consistent as long as the same model is used all the time. This results in unbiased estimates of population trends, which are typically the prime aim of monitoring programmes. Secondly, our findings concerning the closed model selection were fairly straightforward and repeatable, providing clear guidelines for dealing with crayfish populations in this respect. Therefore, unless there is significant evidence that some other model fits the data considerably better we recommend that (i) the model with time variation in capture probabilities (M_t) be applied for male and female fractions analysed separately, whereas (ii) the model with time variation and heterogeneity in capture probabilities (M_{th}) is used in the case of entire adult population analysed jointly. The former option is preferable because of higher precision of the M_t model.

Moreover, as both our survey and many other studies into crayfish population structure conducted for a wide array of species indicated a perfectly balanced sex ratio (Guan and Wiles 1996; Gherardi et al. 2000; Parkyn et al. 2002; Maguire et al. 2002, 2004; Scalici and Gibertini 2005) estimating only male numbers and extrapolating them to total population sizes by multiplying by two is worth considering. Such an approach should not only increase estimate precision, but also reduce the amount of work through refraining from marking females. Sexual dimorphism in crayfish is evident enough (Holdich and Lowery 1988) so that females can be identified and released immediately after capture, which in addition should save them and their offsprings some stress.

Practical constraints in the use of mark-recapture

Practical issues are very important to consider since it is the high labour-intensity of mark-recapture methods that is the usual argument against their use in the monitoring of many species groups. The peculiarity of crayfish monitoring is that virtually all other methods of abundance assessment involve capturing individuals as well (Peay 2003). Hence the effort required for applying mark-recapture, even though substantial, is only slightly higher than for relative abundance methods. According to our experience, two people should be easily able to conduct a simple capture session on a ca. 500-m long stream section within 2 days, and three people could potentially do it in a day. This translates into the annual field effort of 9–12 person-days in the case of the minimal sampling plan (a single primary period with three capture sessions), and 30–40 person-days for the optimal one (two primary periods of five sessions each). Optimistically, it seems that this substantial effort does not have to be reproduced over a large number of sites. Between- and within-site synchrony of crayfish population dynamics suggests that trends recorded at single sites, or even fragments of sites, are likely to be representative for broader areas. Nevertheless, testing the above hypothesis in a large-scale research is highly desirable.

Apart from labour-intensity a major obstacle to a wider use of mark-recapture is the difficulty in marking crayfish (cf. Westman and Savolainen 2002). With traditional marking techniques marks disappear after several moulting events (Guan 1997), although the outcome of the present study proves that the problem is less acute than expected. We discovered that marks on adults last for about a year and a half on average. With such a mark duration their loss should not affect population size estimates obtained with the Robust Design model, but only the parameters describing the turnover of individuals between seasons, leading to the underestimation of survival and the overestimation of recruitment (Arnason and Mills 1981). In other words, even though mark loss makes the investigation of the underlying demographic processes difficult, at least it does not obstruct the assessment of population trends, which meets the needs of most monitoring programmes. In addition, recent developments in marking techniques achieved in marine crayfish (e.g. Frisch and Hobbs 2006) give hope for cheap and long-lasting marks available to be applied in the near future.

Acknowledgements This survey was funded by the Piedmont regional government through the project "Action plan for the crayfish *Austropotamobius pallipes* complex (Crustacea Decapoda Astacidae) in Piedmont", while the data analysis was supported by the European Commission within its STREP project EuMon (contract no. 006463). We would like to thank Giulia Bemporad, Luca Buonerba, Livio Favaro, Andrea Forchino, and Valentina Jackson for their help in the fieldwork, and James Brookes for improving the English of the manuscript.

References

Abrahamsson S (1983) Trappability, locomotion, and diel pattern of activity of the crayfish *Astacus astacus* and *Pacifastacus leniusculus* Dana. Freshw Crayfish 5:239–253

Acosta CA, Perry SA (2000) Effective sampling area: a quantitative method for sampling crayfish population in freshwater marshes. Crustaceana 73:425–431. doi:10.1163/156854000504516

Akaike H (1973) Information theory and an extension of the maximum likelihood principle. In: Petrov BN, Csaki F (eds) Second international symposium on information theory. Akademiai Kiado, Budapest, pp 267–281

Arnason AN, Mills KH (1981) Bias and loss of precision due to tag loss in Jolly-Seber estimates for mark-recapture experiments. Can J Fish Aquat Sci 38:1077–1095

Arnason AN, Schwarz CJ (1999) Using POPAN-5 to analyse banding data. Bird Study 46(Suppl):157–168

Baillie SR (1995) Uses of ringing data for the conservation and management of bird populations: a ringing scheme perspective. J Appl Stat 22:967–987. doi:10.1080/02664769524748

Baillie J, Groombridge B (eds) (1996) 1996 IUCN red list of threatened animals. IUCN, Gland

Bubb DH, Lucas MC, Timothy J, Thom TJ (2002) Winter movements and activity of signal crayfish *Pacifastacus leniusculus* in an upland river, determined by radio telemetry. Hydrobiologia 483:111–119. doi:10.1023/A:1021363109155

Bubb DH, Thom TJ, Lucas MC (2006) Movement patterns of the invasive signal crayfish determined by PIT telemetry. Can J Zool 84:1202–1209. doi:10.1139/Z06-100

Byron CJ, Wilson A (2001) Rusty crayfish (*Orconectes rusticus*) movement within and between habitats in Trout Lake, Vilas County, Wisconsin. J North Am Benthol Soc 20:606–614. doi:10.2307/1468091

Chao A (1989) Estimating population size for sparse data in capture-recapture experiments. Biometrics 45:427–438. doi:10.2307/2531487

Chao A, Lee SM, Jeng SL (1992) Estimation of population size for capture-recapture data when capture probabilities vary by time and individual animal. Biometrics 48:201–216. doi:10.2307/2532750

Crawford L, Yeomans WE, Adams CE (2006) The impact of introduced signal crayfish *Pacifastacus leniusculus* on stream invertebrate communities. Aquat Conserv 16:611–621. doi:10.1002/aqc.761

DiStefano RJ, Gale CM, Wagner BA, Zweifel RD (2003) A sampling method to assess lotic crayfish communities. J Crust Biol 23:678–690. doi:10.1651/C-2364

Dorn NJ, Mittelbach GG (1999) More than predator and prey: a review of interactions between fish and crayfish. Vie Milieu 49:229–237

Dorn NJ, Urgelles R, Trexler JC (2005) Evaluating active and passive sampling methods to quantify crayfish density in a freshwater wetland. J North Am Benthol Soc 24:346–356. doi:10.1899/04-037.1

Flowerdew JR, Shore RF, Poulton SMC, Sparks TH (2004) Live trapping to monitor small mammals in Britain. Mammal Rev 34:31–50. doi:10.1046/j.0305-1838.2003.00025.x

Fratini S, Zaccara S, Barbaresi S, Grandjean F, Souty-Grosset C, Crosa G et al (2005) Phylogeography of the threatened crayfish (genus *Austropotamobius*) in Italy: implications for its taxonomy and conservation. Heredity 94:108–118. doi:10.1038/sj.hdy.6800891

Frisch AJ, Hobbs JPA (2006) Long term retention of internal elastomer tags in a wild population of painted crayfish (*Panulirus versicolor* [Latreille]) on the Great Barrier Reef. J Exp Mar Biol Ecol 339:104–110. doi:10.1016/j.jembe.2006.07.016

Gherardi F (2006) Crayfish invading Europe: the case study of *Procambarus clarkii*. Mar Freshw Behav Physiol 39:175–191. doi:10.1080/10236240600869702

Gherardi F, Holdich D (eds) (1999) Crayfish in Europe as alien species: how to make the best of a bad situation? Crustacean Issues, vol 11. Balkema, Rotterdam

Gherardi F, Souty-Grosset C (eds) (2006) European crayfish as heritage species-linking research and management strategies to conservation and socio-economic development, CRAYNET, vol 4. Bull Fr Pêche Piscic 380–381:1–566

Gherardi F, Barbaresi S, Salvi G (2000) Spatial and temporal patterns in the movement of *Procambarus clarkii*, an invasive crayfish. Aquat Sci 62:179–193

Gledhill T, Sutcliffe DW, Williams WD (1993) British freshwater Crustacea Malacostraca, 2nd edn. Freshwater Biological Association Scientific Publications 52. Freshwater Biological Association, Ambleside

Grandjean F, Frelon-Raimond M, Souty-Grosset C (2002) Compilation of molecular data for the phylogeny of the genus *Austropotamobius*: one species or several? Bull Fr Peche Piscic 367:671–680

Guan RZ (1997) An improved method for marking crayfish. Crustaceana 70:641–652. doi: 10.1163/156854097X00104

Guan RZ, Wiles PR (1996) Growth, density and biomass of crayfish, *Pacifastacus leniusculus*, in a British lowland river. Aquat Living Resour 9:265–272. doi:10.1051/alr:1996030

Hicks BJ (2003) Distribution and abundance of fish and crayfish in a Waikato stream in relation to basin area. NZ J Zool 30:149–160

Hockley NJ, Jones JPG, Andriahajaina FB, Manica A, Ranambitsoa EH, Randriamboahary JA (2005) When should communities and conservationists monitor exploited resources? Biodivers Conserv 14:2795–2806. doi:10.1007/s10531-005-8416-8

Holdich DM (2003) Ecology of the white-clawed crayfish *Austropotamobius pallipes*. Conserving natura 2000 rivers, ecology series no. 1. English Nature, Peterborough

Holdich DM, Lowery RS (eds) (1988) Freshwater crayfish—biology, management and exploitation. Croom Helm, London

Holdich DM, Reeve ID (1991) The distribution of freshwater crayfish in the British Isles with particular reference to crayfish plague, alien introductions and water quality. Aquat Conserv 1:139–158. doi: 10.1002/aqc.3270010204

Hurvich CM, Tsai C (1989) Regression and time series model selection in small samples. Biometrika 76:297–307. doi:10.1093/biomet/76.2.297

Jay D, Holdich DM (1981) The distribution of the crayfish, *Austropotamobius pallipes*, in British waters. Freshw Biol 11:121–129. doi:10.1111/j.1365-2427.1981.tb01248.x

Jones JPG, Andriahajaina FB, Hockley NJ, Balmford A, Ravoahangimalala OR (2005) A multidisciplinary approach to assessing the sustainability of freshwater crayfish harvesting in Madagascar. Conserv Biol 19:1863–1871. doi:10.1111/j.1523-1739.2005.00267.x

Jones JPG, Coulson T (2006) Population regulation and demography in a harvested freshwater crayfish from Madagascar. Oikos 112:602–611. doi:10.1111/j.0030-1299.2006.14301.x

Julliard R, Jiguet F, Couvet D (2004) Evidence for the impact of global warming on the long-term population dynamics of common birds. Proc Biol Sci 271:490–492. doi:10.1098/rsbl.2004.0229

Kendall WL (1999) Robustness of closed capture-recapture methods to violations of the closure assumption. Ecology 80:2517–2525

Lancia RA, Nichols JD, Pollock KH (1994) Estimating the number of animals in wildlife populations. In: Bookhout TA (ed) Research and management techniques for wildlife and habitats, 5th edn. The Wildlife Society, Bethesda, pp 215–253

Lodge DM, Kershner MW, Aloi JE, Covich AP (1994) Effects of an omnivorous crayfish (*Orconectes rusticus*) on a freshwater littoral food web. Ecology 75:1265–1281. doi:10.2307/1937452

Lodge DM, Taylor CA, Holdich DM, Skurdal J (2000) Nonindigenous crayfishes threaten North American freshwater biodiversity: lessons from Europe. Fisheries 25:7–20 doi:10.1577/1548-8446(2000)025 <0007:NCTNAF>2.0.CO;2

Lowery RS (1988) Growth, moulting and reproduction. In: Holdich DM, Lowery RS (eds) Freshwater crayfish: biology, management and exploitation. Croom Helm, London, pp 83–113

Maguire I, Erben R, Klobucar GIV, Lajtner J (2002) Year cycle of *Austropotamobius torrentium* (Schrank) in streams on Medvednica Mountain (Croatia). Bull Fr Peche Piscic 367:943–957

Maguire I, Hudina S, Erben R (2004) Estimation of noble crayfish (*Astacus astacus* L.) population size in the Velika Paklenica Stream (Croatia). Bull Fr Peche Piscic 372:353–366. doi:10.1051/kmae:2004009

Marunouchi J, Kusano T, Ueda H (2002) Fluctuation in abundance and age structure of a breeding population of the Japanese brown frog, *Rana japonica* Gunther (Amphibia, Anura). Zool Sci 19:343–350. doi:10.2108/zsj.19.343

Menkens GE Jr, Anderson SH (1988) Estimation of small-mammal population size. Ecology 69:1952–1959. doi:10.2307/1941172

Moore JA, Hoare JM, Daugherty CH, Nelson NJ (2007) Waiting reveals waning weight: monitoring over 54 years shows a decline in body condition of a long-lived reptile (tuatara, *Sphenodon punctatus*). Biol Conserv 135:181–188. doi:10.1016/j.biocon.2006.10.029

Nowicki P, Witek M, Skórka P, Settele J, Woyciechowski M (2005) Population ecology of endangered butterflies *Maculinea teleius* and *M. nausithous* and its conservation implications. Popul Ecol 47:193–202. doi:10.1007/s10144-005-0222-3

Nowicki P, Settele J, Henry P-Y, Woyciechowski M (2008) Butterfly monitoring methods: the ideal and the real world. Isr J Ecol Evol 54:69–88

Nyström P, Brönmark C, Graneli W (1996) Patterns in benthic food webs: a role for omnivorous crayfish? Freshw Biol 36:631–646. doi:10.1046/j.1365-2427.1996.d01-528.x

Otis DL, Burnham KP, White DC, Anderson DR (1978) Statistical inference from capture data on closed animal populations. Wildl Monogr 62:1–135

Parkyn SM, Collier KJ, Hicks BJ (2002) Growth and population dynamics of crayfish *Paranephrops planifrons* in streams within native forest and pastoral land uses. NZ J Mar Freshw 36:847–861

Peay S (2003) Monitoring the White-clawed crayfish *Austropotamobius p. pallipes*. Conserving natura 2000 rivers, monitoring series no. 1. English Nature, Peterborough

Pintor LM, Soluk DA (2006) Evaluating the non-consumptive, positive effects of a predator in the persistence of an endangered species. Biol Conserv 130:584–591. doi:10.1016/j.biocon.2006.01.021

Pollock KH (1982) A capture-recapture design robust to unequal probabilities of capture. J Wildl Manage 46:757–760. doi:10.2307/3808569

Pollock KH, Nichols JD, Brownie C, Hines JE (1990) Statistical inference for capture recapture experiments. Wildl Monogr 107:1–97

Pollock KH, Yoshizaki J, Fabrizio MC, Schram ST (2007) Factors affecting survival rates of a recovering lake trout population estimated by mark-recapture in Lake Superior, 1969–1996. Trans Am Fish Soc 136:185–194. doi:10.1577/T05-317.1

Rabeni CF, Collier KJ, Parkyn SM, Hicks BJ (1997) Evaluating methods of sampling stream crayfish. NZ J Mar Freshw 31:693–700

Rexstad EA, Burnham KP (1991) User's guide for interactive program CAPTURE. Abundance estimation of closed animal populations. Colorado State University, Fort Collins

Reynolds JD (1998) Conservation management of the white-clawed crayfish, *Austropotamobius pallipes*. Part 1. Irish Wildlife Manuals 1, Dublin

Reynolds J, Souty-Grosset C (eds) (2003) The endangered native crayfish Austropotamobius pallipes, bioindicator and heritage species, CRAYNET, vol 1. Bull Fr Pêche Piscic 370–371:1–230

Reynolds JD, Gouin N, Pain S, Grandjean F, Demers A, Souty-Grosset C (2001) Irish crayfish populations: ecological survey and preliminary genetic findings. Freshw Crayfish 13:584–594

Robinson CA, Thom TJ, Lucas MC (2000) Ranging behaviour of a large freshwater invertebrate, the white-clawed crayfish *Austropotamobius pallipes*. Freshw Biol 44:509–521. doi:10.1046/j.1365-2427.2000.00603.x

Rodríguez CF, Bécares E, Fernández-Aláez M, Fernández-Aláez C (2005) Loss of diversity and degradation of wetlands as a result of introducing exotic crayfish. Biol Invasions 7:75–85. doi:10.1007/s10530-004-9636-7

Rogowski DL, Stockwell CA (2006) Assessment of potential impacts of exotic species on populations of a threatened species, White Sands pupfish, *Cyprinodon tularosa*. Biol Invasions 8:79–87. doi:10.1007/s10530-005-0238-9

Rosenthal SK, Stevens SS, Lodge DM (2006) Whole-lake effects of invasive crayfish (*Orconectes* spp.) and the potential for restoration. Can J Fish Aquat Sci 63:1276–1285. doi:10.1139/F06-037

Santucci F, Iaconelli M, Andreani P, Cianchi R, Nascetti G, Bullini L (1997) Allozyme diversity of European freshwater crayfish of the genus *Austropotamobius*. Bull Fr Pêche Piscic 347:663–676

Scalici M, Gibertini G (2005) Can *Austropotamobius italicus meridionalis* be used as a monitoring instrument in Central Italy? Preliminary observations. Bull Fr Peche Piscic 376–377:613–625

Schwarz CJ, Arnason AN (1996) A general methodology for the analysis of capture-recapture experiments in open populations. Biometrics 52:860–873. doi:10.2307/2533048

Schwarz CJ, Seber GAF (1999) Estimating animal abundance. Stat Sci 14:427–456. Review III. doi:10.1214/ss/1009212521

Skalski JR, Robson DS (1992) Techniques for wildlife investigations. Academic Press, San Diego

Skurdal J, Qvenild T, Taugbøl T, Fjeld E (1990) A 6-year study of *Thelohania contejeani* parasitism of the noble crayfish, *Astacus astacus* L., in lake Steinsfjorden, SE Norway. J Fish Dis 13:411–415. doi:10.1111/j.1365-2761.1990.tb00800.x

Souty-Grosset C, Holdich DM, Noel PY, Reynolds JD, Haffner P (eds) (2006) Atlas of crayfish in Europe. Muséum National d'Histoire Naturelle, Paris

Taugbøl T, Skurdal J (1999) The future of native crayfish in Europe—how to make the best of a bad situation? Crustac Issues 11:271–279

Tirelli T, Mussat Sartor R, Bona F, De Biaggi E, Zocco D, Badino G et al Census of *Austropotamobius* genus in four Districts of Piedmont (Western Italy). Bol Mus Reg Sci Nat Torino (in press)

Usio N, Townsend CR (2004) Roles of crayfish: consequences of predation and bioturbation for stream invertebrates. Ecology 85:807–822. doi:10.1890/02-0618

van Helddingen PJ, Willemse I, Speight MCD (eds) (1996) Background information on the invertebrates of the habitats directive and the bern convention. Part 1-Crustacea, Coleoptera and Lepidoptera. Nature and environment no. 79. Council of Europe Publishing, Strasbourg

Webb M, Richardson A (2004) A radio telemetry study of movement in the giant Tasmanian freshwater crayfish, *Astacopsis gouldi*. Freshw Crayfish 14:197–204

Westman K, Savolainen R (2002) Growth of the signal crayfish, *Pacifastacus leniusculus*, in a small lake in Finland. Boreal Environ Res 7:53–61

Westman K, Savolainen R, Julkunen M (2002) Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a small, enclosed Finnish lake: a 30-year study. Ecography 25:53–73. doi:10.1034/j.1600-0587.2002.250107.x

White GC, Burnham KP (1999) Program MARK: survival estimation from populations of marked animals. Bird Study 46:120–138

Whitledge GW, Rabeni CF (1997) Energy sources and ecological role of crayfishes in an Ozark stream: insights from stable isotopes and gut analysis. Can J Fish Aquat Sci 54:2555–2563. doi:10.1139/cjfas-54-11-2555

Williams BK, Nichols JD, Conroy MJ (2002) Analysis and management of animal populations. Academic Press, San Diego

Willis TV, Magnuson JJ (2006) Response of fish communities in five north temperate lakes to exotic species and climate. Limnol Oceanogr 51:2808–2820

Wilson KA, Magnuson JJ, Lodge DM, Hill AM, Kratz TK, Perry WL et al (2004) A long-term rusty crayfish (*Orconectes rusticus*) invasion: dispersal patterns and community change in a north temperate lake. Can J Fish Aquat Sci 61:2255–2266. doi:10.1139/f04-170

Zhang YX, Richardson JS, Negishi JN (2004) Detritus processing, ecosystem engineering and benthic diversity: a test of predator-omnivore interference. J Anim Ecol 73:756–766. doi:10.1111/j.0021-8790.2004.00849.x

United States Department of the Interior
FISH AND WILDLIFE SERVICE
Division of Ecological Services
9014 East 21st Street
Tulsa, Oklahoma 74129-1428
918/581-7458 / (FAX) 918/581-7467

American Burying Beetle *Nicrophorus Americanus*
Survey Guidance for Oklahoma
Updated May 20, 2009

Introduction

The goal of this document is to provide guidance in designing and conducting presence/absence surveys for the ABB as a means of complying with section 7 and 9 of the Endangered Species Act (ESA). Section 9 of the Endangered Species Act (ESA) prohibits all persons from the taking of federally listed species. Take includes harming, harassing, or killing. Section 7 of the ESA requires federal agencies to consult with the Service if a project they authorize, fund, or carry out may adversely affect a federally listed species. Baited pitfall traps are the most effective method known for surveying for ABBs (Creighton *et al.,* 1993; Service, 1991; Bedick *et al,.* 2004). The below baited pitfall survey methodology has proven to be successful in safely capturing the ABB. Following this guidance should help to ensure the validity of survey results. Implementing other survey methods, not recommend by the Service, may not result in confidence of survey results. Due to the ABBs life history, false negative survey results can occur. Further, data gathered using this guidance will allow for comparison of results between surveys. Surveys for ABBs that are conducted for research purposes are beyond the scope of this guidance and should be coordinated with the Service.

Time for Surveys

As a means of determining the presence or absence of ABBs, the Service recommends surveys be conducted during the ABBs primary active season, which is between May 20 and September 20 in Oklahoma. Surveys should begin no earlier than May 20 and end no later than September 17 in Oklahoma (dates may vary among states and will need to be verified for each state). Additional nights of survey are needed for each night during a survey effort where the night-time ambient temperature falls below 60 degrees Fahrenheit [ºF] and when rain events deposit ½ inch or more during 7 pm to 7 am.

The ABB's nocturnal activity and trapping success decreases or is absent when temperatures drop below 60 (Bedick *et al*. 1999, Kozol 1991). Further, ABB nocturnal activity is shown to be delayed when temperatures are greater than 75ºF (Bedick *et al*. 1999).

The determination of the ABB's activity period in Oklahoma is based on the past eleven years of weather data in eastern Oklahoma (Oklahoma Mesonet) where nighttime ambient temperatures are consistently above 60ºF. Mesonet data from the Tahlequah and Broken Bow stations were analyzed. May 20 is typically the day commencing the onset of a five day period were nighttime ambient temperatures remain 60ºF or higher, and September 20 is the first day of a five day period with temperatures below 60ºF. There is the potential that the temperature will drop below 60ºF for a night or two, but typically not for five consecutive nights or more.

Timing

Although the capture rates of ABBs are known to be higher during certain dates during their prime active period, May 20 and September 20, the Service is not recommending restricting survey implementation to specific dates during this prime active period.

Both Bedick *et al.* (2004) and the U.S. Fish and Wildlife Service (Service) (1991) reported an increase in capture rates during certain times during the ABBs active season and during the ABBs nightly activity. Capture rates for ABBs are highest from mid-June to mid-July and again in mid-August. Bedick *et al.* (2004) reported that a peak in capture success also occurred in mid-August. In Nebraska, Bedick et al (1999) reported two peak activity periods. One in late June and early July when ABB are most actively searching for carrion, and in late August and early September when teneral ABBs emerge.

Surveying during the above two peak timeframes would result in the greatest potential for trapping success and the Service would prefer that surveys be conducted during these periods. However, we understand that limiting surveys to these time periods is too restrictive and therefore conducting surveys during these time periods is not a requirement. Further, this survey protocol is only a method to determine presence/absence and not an abundance or population survey.

Timeframe a Survey is Valid

Survey results are only valid for one calendar year from the last date of the survey effort. This determination is based on the fluctuating nature of ABB populations. Data indicates that ABBs likely have moved and numbers have fluctuated within a given area from year to year.

Much of the long-term information concerning the life history of the ABB pertinent to Oklahoma has come from work done at Fort Chaffee, Arkansas; McAlester Army Ammunition Plant (McAlester), Oklahoma; and Camp Gruber, Oklahoma. While the land use at Fort Chaffee, McAlester, and Camp Gruber is different among each entity, each entity maintains a relatively consistent land use pattern of its own through time. However, Hiott and Schnell (2003) reported the number of ABBs captured and the location of high density ABB concentrations typically varies annually or biennially at each site. This trend indicates ABBs are cyclic, where there are high numbers and abundance for one or two years, followed by a decline in numbers the following year or two, and repetition of the cycle over time. In addition, each year they reported that the high concentration areas of ABBs appeared to shift annually throughout the sites. Further, the ABB is an annual species (living for only one year) and the following year's numbers are dependent upon the reproductive success of the previous year.

False negatives are possible outcomes of with ABB surveys. The Service (1991) reported that during late July ABBs were easy to attract to carrion bait but were difficult to capture in pitfall traps. Standard transects on Camp Gruber that resulted in ABB captures in one year showed no capture of ABBs in another year. Other surveys conducted in a given area of Oklahoma have resulted in ABB captures during one survey but surveys conducted in the same given area and the same active season have resulted in negative ABB captures.

Survey effort radius

A survey is valid for a 5 mile radius. Considering ABBs mobility, small size, recorded movement distances, and distance from which they can detect carrion, a presence/absence survey was effective only over 5 miles and that this erred on the side of the species.

An ABB may move as little as 0.15 mile per night to a maximum of 6.2 miles in 6 nights (1.03 miles per night), with a mean movement distance of 0.52 mile per night (Schnell and Hiott 1995, 2003). Bedick *et al.* (2004) reported average nightly movements of 0.62 mile with the large proportion (85%) of recaptures moving distances of 0.31 mile per night. Creighton *et al.* (1992) reported that individual ABBs moved over 4 miles in only a few days. Creighton and Schnell (1998) reported an average nightly movement of 0.76 mile, and maximum distances of four miles in five nights (0.8 mile per night) and 6.2 miles in 6 nights (1.03 miles per night), a minimum distance of 0.15 mile in one night, and a mean nightly movement of 1.67 miles.

While this data could be interpreted to imply that an ABB could move 95 miles [0.62 (mean nightly movement) X 154 days (May 20 – September 20)] during the active season, the Service does not believe this is an accurate interpretation. Mark and recapture data at Camp Gruber and Fort Chaffee did not find any ABBs that moved between these installations, a distance of about 54 miles (Schnell and Hiott, 1997-2003). Even if ABBs moved such long distances, the Service assumes it is unlikely ABBs move in such a consistently linear direction.

The group of ABBs at Camp Gruber has persisted for at least 11 years. While the numbers and high density areas of ABBs have changed annually or biennially, indicating ABBs are typically a cyclic species (Schnell and Hiott, 2003), they appear to be self-sustaining. The Service assumed it was not likely that all, the majority, or even a single ABB on these installations moved from 54 to 95 miles away from Camp Gruber. Further, for a species to survive it cannot exert more energy than it consumes and would only expend the minimal amount of energy needed to secure necessary resources.

Minimum Survey Effort

To effectively determine presence or absence of ABBs in a given area, surveys need to be conducted for a minimum of 3 consecutive nights [or 24 trapnights, 8 traps (or 1 transect) x 3 nights = 24 trapnights] not hampered by rain or temperature as described below. The effective radius of a transect is 0.5 miles. So to effectively survey a particular area, transects should be spaced at 0.5 mile increments across the project area until the entire project area is within the effective trapping area of at least one transect. Each transect deployed should be open for 3 consecutive nights. Keep in mind that a transect does not have to be linear.

For projects with less than a 0.5 mile radius from the center to any point along the perimeter, a minimum of 1 complete transect (8 traps spaced 66 feet apart) is required. One transect with 8 traps for 3 nights results in 24 trapnights (the number of traps times the number of survey nights). Again, the placement of the transect does not need to be linear

Based on ABB movement data described above, the effective trapping area of a transect is a 0.5 mile radius. Creighton *et al.* (1993) and the Service (1991) recommend surveying for a minimum of 3 consecutive nights to adequately determine the presence or absence of ABBs in an area. Bedick *et al.* (2004) recommends a range of 3 to 5 nights, preferably 5 nights. However, after 3 consecutive nights of trapping, surveyor observations report trapping success can decline. If ABBs have not been captured within the 3 consecutive nights of surveying then ABBs are unlikely, although not impossible, to be captured (Personal communication with Ana Hiott, 2003) with additional trapnights.

Minimum project acreage

According to our 2005 analysis of ABB density estimates, projects which disturb less than 1.2 acres would have, on average, no more than a one percent chance of impacting an individual ABB. In addition, the Service evaluates numerous other factors including the project: type, construction/installation duration, permanency, location, land use, implementation methods, habitat, and time/season.

Using recently collected survey data, the Service's Oklahoma Ecological Services Field Office derived densities of ABBs in their known range within Oklahoma. We used all known survey records to determine the total number of ABBs captured and total transects deployed per survey. In using this information, we assumed all captures were obtained using the methodology described by Creighton, *et al*. (1993) unless stated otherwise. Data that were not collected using the Creighton *et al*. (1993) methodology were excluded from the analysis.

The Service then estimated the area each transect would effectively trap. Creighton, *et al*. (1993) determined, based on known movements of ABBs, that transects do not need to be spaced any closer than 0.5 miles. Past and ongoing research demonstrates this trapping recommendation is still appropriate. Schnell and Hiott (1997-2003) annually determined the average nightly movements of the ABB, using marked individuals over a nine-year period at Camp Gruber to be 0.62 miles. The smallest average nightly movement for any given active season over that same period was 0.52 miles. Consequently, we believe each transect effectively traps beetles from an area of 0.5 mile. As such, the effective trapping area (ETA) for one transect is about 153.5 acres. Using the ETA and number of ABBs collected, we estimated average ABB densities to be 0.0084 ABBs/acre for their known range in Oklahoma. The Service then used a standard z test to determine the probability of encountering an individual ABB in a given area. We determined that disturbance of less than 1.2 acres would have, on average, no more than a one percent chance of impacting an individual ABB.

Weather Requirements

An additional night of surveying is required when the temperature falls below 60ºF between 7:00 PM and 7:00 AM and/or when rainfall greater than ½ inch occurs between 7:00 PM and 7:00 AM. All weather data for surveys should be collected using the Oklahoma Mesonet website, www.mesonet.org. Directions for using the Oklahoma Mesonet website are provided below. All additional nights of surveys conducted due to weather need to be specified in the "*ABB Survey Data Collection Form*" and the "*ABB Survey Summary Report*".

Bedick et al. (1999) reported the highest number of ABB captures 3 to 4 hours after sunset. However, captures occurred from 1 to 11 hours after sunset during this study. In Oklahoma, sunset times range from 7:18 PM to 8:44 PM between May 20 and September 20. Based on Bedick *et al*.'s (1999) findings, the peak timeframe for ABB captures in Oklahoma would be from 10:18 PM to 12:18 PM and the nightly timeframe of ABB activity would be from 10:18 PM to 7:24 AM.

This is just one study and the study site was in Nebraska, so to err on the side of the species, the Service has determined that the nighttime active period for ABBs to be between 7:00 PM to 7:00 AM in Oklahoma. The Service also considered the limiting factors of this type of survey effort, specifically we considered the fact that this type of survey provides only the presence or absence data of the ABB in a given area and that the minimum duration of the survey effort is 3 nights. To err on the side of the species, all feasible variables that could result in a false negative survey need to be eliminated if possible. Temperature and rainfall are variables that can be easily monitored and adjusted for.

Oklahoma Mesonet Instructions:

1. At www.mesonet.org, click on "Past Data and Files" under "Mesonet Data" (Left side of the screen)
2. Click on the Mesonet station closest to the survey site. Then under the "Station Monthly Summary" heading select the month and year of the survey. Then click "Get summary."

   Temperature:
   - In "Summary Report" of this Mesonet page, find the date of the survey. Daily Mesonet data is measured from midnight to midnight, so if traps are set on June 3rd, temperature data from both the 3rd and the 4th will be needed to address the entire trapping night, which is between 7:00 PM to 7:00 AM, and because the nightly low temperature most often occur past midnight.

   Rainfall:
   - In "Summary Report" of this Mesonet page, find the date of the survey. Once again, if the traps are set on June 3rd, Mesonet data from the both the 3rd and 4th need to be reviewed to address the entire trapping night, which is between 7:00 PM to 7:00 AM. Rainfall over ½ inch during a trap night requires further analysis and reporting. The time of the rainfall needs to be determined. This Mesonet page only reports the entire rainfall that occurred in a 24 hour period (midnight to midnight). To determine when during a 24 hour period rainfall events occurred and how much rain fell, proceed to the paragraph below for instructions to navigate through Mesonet to the proper page.

   - Click on "Past Data and Files". Then under the heading "Data Files" on the bottom right of screen click on "Mesonet MTS Files". Then click on the relevant date. Then select the Mesonet station nearest to the survey area. Rain totals are given in 5 minute increments on this Mesonet page. Remember that daily Mesonet data is provided for a 24 hour period, beginning at 12:00 AM and ending at 12:00 AM. So the date the traps were set and the following date need to be reviewed to determine the rainfall for the entire trapnight.

Transect spacing

Transects should be spaced a minimum of 0.5 mile apart on small to medium sized projects and a maximum of 1 mile apart on large projects to achieve reliable survey results. The Service defines large projects as those that are over 20 square miles or over 20 linear miles.

Bedick *et al.* (2004) recommended that 0.31 mile be used as a minimum transect spacing for traps with large bait. Creighton *et al.* (1992) concluded that transects less than 0.5 mile apart were not required. In large sample areas, Creighton *et al.* (1992) stipulated that transects can be located as much as 1 mile apart. The Service's rationale considered these recommendations and the ABB movement data discussed previously in determining an effective transect spacing.

Transect Placement

Transects should be placed in a configuration that best represents the different habitat types present in the survey area and on the highest spots in the survey area. Transects are not limited to linear arrangements, they can be aligned to suit the shape of the project area for which you are surveying. For example, if a project is kidney bean shaped, transects can be curved to fit a kidney bean shape. However, transect spacing of 0.5 mile needs to be followed.

American burying beetles are feeding habitat generalists (Creighton *et al*, 1993). Consequently, it is recommended that transects be placed in all the different habitat types present in a survey area. Bedick *et al.* (2004) reported ABBs were significantly more attracted to traps placed on ridges than those placed in valleys. They believed that increased ABB captures on ridge tops may be a result of increased odor movement, thus increasing the possibility of detection by ABBs.

Ants

Traps should not be placed within 23 feet (7 meters) of ant hills. If ants are discovered in a trap it should be relocated at least 23 feet away. Ants can swarm and kill an ABB that is trapped in a pitfall trap (Creighton *et al,*. 1993 and Bedick *et al,*. 2004).

Transect

A transect is defined as 8 pitfall traps, spaced 66 feet (20 meters) apart (Figure 1), for a total length of 460 feet (140 meters). This definition is based on the transect protocol described in Creighton *et al.* (1993) and Bedick *et al.* (2004) which all utilize or recommend the same type of transect. Transects are not limited to linear arrangements, they can be aligned to suit the shape of the project area for which you are surveying. For example, if a project is kidney bean shaped, transects can be curved to fit a kidney bean shape. However, the above described quantity of traps per transect and the spatial arrangement (traps 66 feet apart) of traps must be followed.

Traps

The Service recommends using baited pitfall traps for ABB presence/absence surveys. Each pitfall trap consists of a trap cup, bait cup, wire, and cover. The traps are designed to utilize carrion to attract ABBs, keep captured ABBs alive, minimize bait and ABB contact, and prevent ABBs from escaping until traps are checked. The ABB flies toward the bait odor, lands near the trap, and crawls under the cover and into the trap cup. Once in the trap, the slick sides of the cup prevent the ABB from crawling out, and the cover and suspended bait cup keep the ABB from flying out of the trap cup. Figures 2 and 3 display the baited pitfall trap setup. If the utilization of other trap design and equipment is proposed this must be coordinated with and approved by the Service.

Traps in the form of 24 oz (0.7 L) plastic cups (similar to Solo cups) have proven effective. The Service recommends that the trap cup consist of two 24-oz Solo cups. Cups must have smooth sides, free of any texture or ridges to prevent ABBs from climbing out. American burying beetles can climb a surface with textured/ridged sides but cannot climb a smooth, slick surface. The 2 cups are stacked together and placed in an appropriate sized hole in the ground. Stacking the cups one inside the other facilitates removal of trapped insects. The top cup can easily be pulled out and replaced while the second cup remains in the ground to maintain the integrity of the hole. The lip of the trap cups should be 0.5 to 0.75 inch above ground level. This prevents water runoff from filling the cup. The cup should not exceed 0.75 inches from the ground because this could prohibit ABBs from being able to crawl over the lip and into the trap. A 1 to 2 inch squared piece of wetted sponge soil should be placed in the bottom of the trap cups. This has been shown to decrease mortality by desiccation of ABBs and providing a floatation device should the cup be inundated with water.

The bait cup consists of a plastic cup about 5 – 6 ounces (20 milliliters) in size. Examples of bait cup types that have proven effective are cups similar to those used in restaurants for carry out of salad dressing or styro-foam coffee cups with the top portion cut off leaving only the bottom 1 inch of the cup. The type of bait cup used is not as important as the trap cup; however the size of the bait cup is important. The bait cup should be large enough to hold the proper amount of bait (described below) but small enough to be suspended over the trap cups and still allow ABBs to crawl into the trap cup. Bait suspended over the pitfall trap via skewer or similar device is not recommended. The bait dries out and the odor emitted is greatly weakened.

Wire is used to secure and suspend the bait cup over the trap cup. To accomplish this, the wire needs to be hand malleable but sturdy enough to support a full bait cup. The wire is inserted into one side of the bait cup near the top and pushed all the way through the cup and out the other side of the cup; about 3 inches of wire should extend on either side of the exterior of the bait cup. Bend wire down and push into the ground to suspend and secure the bait cup over the trap cup. The reason for separate bait and trap cups is to protect the ABB from coming in contact with the bait. As the bait liquefies or becomes gummy ABBs could be harmed if they come into contact with the bait.

The cover should be hard, not transparent or opaque, weighted or secured to the ground, at least 6 inches in depth, at least 12 inches in diameter at its widest point, and raised off the ground about 1 to 2 inches. A hard, plastic dome structure has proven effective at protecting the trap (e.g. inverting nursery plant containers with holes on the side, or small gray paint buckets). A hard structure, secured to the ground is needed to prevent scavenger and rainfall from accessing the trap cup, and to provide shade to captured insects to avoid desiccation. Scavengers absconding with bait is common and potentially results in harm to ABBs. In addition, bait loss during trapping reduces the effectiveness of the trapping effort and therefore ABB capture success. The cover structure should be raised off the ground about 1 inch or holes should be cut along the lip of the container flush to the ground to allow ABBs to crawl into the trap. At least 40% of the lip should be open to allow ABBs access to trap. In lieu of a plastic dome structure, other similar structures can be used. For example, a piece of wood raised on legs can be used. However, all covers must be secured to the ground. Shingles or other such flimsy materials are not to be used.

In areas where scavengers are a significant problem wire mesh can be installed between the pitfall trap and the cover (Appe). The piece of wire mesh should be a minimum of 6 inches squared and mesh size should be at least 1 inch to allow ABBs access to trap but prevent larger animals from stealing the bait. The wire mesh should be secured to the ground with stakes, and a hard cover will still need to be used.

Exposure to full sunlight and temperatures over 77ºF for even a few hours can result in ABB mortality (Service, 1991). According to Bedick *et al.* (2004) wetted soil in the bottom of the trap helped maintain high humidity. This improved the longevity of ABBs and therefore decreased the mortality of ABBs from desiccation. They found this was feasible to use with stacked cup traps. However, saturated soil in the bottom of a trap cup can also pose a threat to ABBs. The Service (2008) found that saturated soil can result in mortality of ABBs through clogging of spiracles or drowning. Other permittees have used a 1 to 2 inch squared piece of wetted sponge in the bottom of traps. The Service recommends utilization of wetted sponges. Soil can still be placed in the bottom of the trap cup; however, this soil should not be wetted.

Bedick *et al.* (2004) found the primary cause of ABB mortality was drowning due to traps flooding with water. However, on multiple occasions, floating and apparently dead beetles were removed from traps, and they subsequently recovered after 10 to 20 minutes. Consequently, ABBs that appear dead or are lethargic should be held for at least 20 minutes to determine actual condition. Monitored ABBs should be placed in a roomy, open container, with air circulation, out of direct sunlight.

Bedick *et al.* (2004) evaluated six types of pitfall traps for capturing ABBs ranging from stacked cups, to PVC pipe, to buckets. They determined buckets with a dome cover had the greatest trapping success because it allowed for the largest piece of bait. However, they realized that this was not the most practical or economically feasible method to implement. They rated stacked cups as the second best trapping method. Creighton *et al.* (1993) also determined that stacked cups as baited pitfall traps proved effective at capturing ABBs and recommended this method.

Creighton *et al.* (1993) recommended using traps that are smooth, free from any bump or ridge to prevent ABBs from climbing out of the trap. Metal cans should be avoided because as soon as any rust appears, ABBs may be able climb out of the trap (Service, 1991). Creighton *et al.* (2004) also recommended the lip of the cup be flush with the ground. However, Bedick et al. (2004) and Hiott (2002 personal communication) recommend the lip of the pitfall cup trap be a 0.5 to 1 inch above the ground surface to prevent water runoff from filling up the trap. Bedick *et al.* (2004) took additional measures by building a berm around the raised portion of the cup.

Creighton *et al*. (1993) and Bedick *et al.* (2004) recommend suspending bait cups over trap cups to lure ABBs, and prevent contact between ABBs and the bait. While carrion beetles are seemingly well-adapted to moving around in carrion, Bedick *et al.* (2004) found that under some conditions spiracle blocking could occur. Bait placed in a small container with a water-tight bottom can decay into a near liquid state. Bait in this liquid form could adhere to ABBs, harden, and then clog spiracles. They did not think this was a common occurrence but could potentially occur.

Bedick *et al.* (2004) recommends using weighted, hard plastic rain-shields larger than the opening of the trap cup to protect the trap from rain. The cover over the pitfall trap should be secured to the ground to effectively protect the trap from rainfall and predators (Creighton *et al.*, 1993). Bedick *et al*. (2004) also recommended using wire mesh secured to the ground between the trap and the cover (Figure 4) to prevent scavenge of bait. Mesh was secured to the ground with three to four bent wire stakes, 4 inches (10 cm) long, driven into the ground. Covers were raised between 1-2 inches above the ground to allow ABBs access to trap.

Bait

Any type of carrion is suitable for use as bait for ABB pitfall traps. However, un-skinned chicken is preferred by multiple surveyors because it is inexpensive and remains moist longer than other baits due to the fat content of the skin. Others have successfully used liver, gizzard, or road kill. The only requirements are that the bait must be the appropriate size in correlation with trap size and must produce a pungent odor that ABBs are able to detect. The appropriate size of bait for stacked cup traps is between 0.5 to 0.7 ounces (15-20 grams). The bait must be rotten and emit a pungent odor to be effective. Adding a small amount of water to the bait cup is effective at prolonging the moisture content and odor. During trapping efforts any bait that has dried out or no longer emits a pungent odor must be replaced with new bait.

Fresh bait is not an attractant to ABBs (Creighton *et al.*, 1993). To prepare bait for use, cut into cubes about 0.5-0.7 ounces (15-20 grams) in size. Place the cubes into a sealed container or bag. Do not fill the container or bag completely full, as the bait rots gas pressure is increased and the extra room is need for this expansion. Place the container or bag in the sun for a minimum of one day (Creighton *et al.*, 1993). If the day is relatively cool (less than 85ºF [29ºC]), the bait should stay in the sun longer.

Once bait is prepared, the packaged (container or bag) bait should then be further enclosed in a larger sealed container, such as an ice chest or bucket with lid. The bait should be used within the next few days. Use of the larger sealed container prevents odor from escaping into unwanted areas and is ideal for transporting bait. The Service recommends that the bait or any containers holding bait not be placed inside a vehicle. You will never get the smell out of your vehicle! Instead, place the containers in the bed of a pick-up or secure to outside of vehicle. In addition, discarded or old bait should not be left at or near the current trapping area. This could lure ABBs away from the baited pitfall traps.

Bedick *et al.* (2004) reported no significant difference between capture rates of ABBs using various types of bait (Appendix 1). However, they did find a significant difference between the sizes of bait used and the number of ABBs captured. A large piece of bait positively correlated with an increase in the number of ABBs captured. Bedick *et al.* (2004) recommended that bait of 7 ounces (200 grams) be used. This amount of bait is feasible if a bucket trap is used but not if a cup is used. Creighton *et al.* (1993) and Service (1991) recommend that 0.5-0.7 ounces (15-20 grams) of bait be used.

Setting and Checking Traps

Each trap must be checked by 10:00 A.M. each morning. Checking traps entails: collecting all trapped ABBs, recording and releasing other *Nicrophorus* species; replacing any missing or dry bait, re-moistening sponge in trap cup if needed, replacing floatation device if needed, and replacing/resituating any disturbed parts of the trap.

Any injured or lethargic ABBs should be released immediately. ABBs that appear to be dead should be collected and monitored for at least 20 minutes, as described below under *Processing ABBs*, to accurately determine their condition. Any dead ABBs should be handled as described below under *Accidental Death of ABBs*.

The Service recommends using a 5-gallon bucket or similar container to carry the needed equipment used during trap checking. The items you will need to carry include: a container to hold ABBs, prepared bait, water, a trowel for digging, tongs and the data sheet. Use a hard, plastic container with a lid and air holes to hold ABBs collected along a transect. Carry a small container of prepared bait (and tongs to handle bait) to replace missing or dried bait. Carry a 20 oz plastic bottle of water to re-moisten sponge in the bottom of the cup trap, and the bait. Replace or re-situate any disturbed traps (this is where you may need the trowel to re-dig the hole for the trap). Record the species and number of any other *Nicrophorus* captured and then immediately release. The other *Nicrophorus* species should be released several feet away from a transect to avoid being crushed by foot traffic. Once all the traps along a transect have been checked, proceed in processing all the captured ABBs either at the end of the transect or at the vehicle. If processing occurs at the vehicle, the vehicle must be within 500 feet of the transect. This is to minimize handling time and for ABBs to be released in the area they were captured.

All traps must be in place and baited by 5:00 P.M. each night. Traps should be cleared of ABBs by 10:00 A.M. every day. Exposure to full sunlight and temperatures over 77ºF (25ºC) for even a few hours can result in ABB mortality (Service 1991). Traps can be baited at the same time they are checked each morning provided the bait does not dry out.

Since ABBs are nocturnal, there is not a risk of baited traps capturing ABBs during the day.

Disturbed bait or traps

Additional nights of trapping will need to be implemented if 4 or more traps and/or bait are disturbed, and no ABBs have been captured during a 3 night survey period. Predators or scavengers can cause bait loss and/or trap disturbance during ABB surveys. This can affect the results of the trapping effort. Disturbed traps or traps missing bait, reduces or eliminates the effectiveness of attracting and/or containing ABBs and therefore ABB capture success. Any negative results from these traps are not reliable. The Service calculated that a trap disturbance percentage of 20 was acceptable and still allowed for surveys that provided valuable data results.

The specific number of additional nights of trapping that will be needed is dependent on the number of traps and/or bait disturbed. The table below outlines the amount of additional trapping needed. Transects in which ABB have been captured, regardless of whether or not any traps have been disturbed or are missing bait, do not require additional nights of trapping since ABB have been confirmed detecting and finding baited pitfall traps along a transect.

# of traps &/or bait disturbed	# of additional nights to survey
0-4	0 additional trapping needed
4-8	1 transect for 1 night
9-16	2 transect for 1 night
17-24	3 transect for 1 night

All trap disturbances must be recorded on the "*ABB Survey Data Form*". There are specific sections on this form pertaining to bait disturbance and any additional nights of survey required. In addition, the amount of disturbed bait and/or traps needs to be summarized in the "*ABB Summary Report*".

Processing ABBs

Processing includes sexing, aging, taking measurements, marking (if authorized) and data recording.

Captured ABBs should only be held for a maximum of 30 minutes, preferably much less than this. ABBs held for longer than 10 minutes should be placed in a hard, plastic container with a damp sponge and then the container placed in an iced cooler. ABBs are sensitive to prolonged heat exposure.

An efficient method to process ABBs is to collect all the ABBs from all 8 traps along a transect and then process all the ABBs at one time. Processing of ABBs can then be completed at the end of the transect or at the vehicle. Multiple permittees find processing ABBs at their vehicle facilitates the task. This eliminates the need to carry all of the equipment during trap checking, quicker processing of ABBs, availability of a cooler if needed, and other advantages.

Clipping of the elytra is only applicable for mark and recapture surveys and is not appropriate without specific authorization from the Service. The survey protocol described here is to determine the presence or absence of ABBs only. Clipping of elytra causes hemoglobin to be secreted by ABBs. Although, there is currently no conclusive evidence that this is a direct or indirect adverse impact to the ABB, it is not recommended. It is the Service's responsibility to err on the side of the species. Further based on past survey reports, recaptures are highly unlikely during such a short survey effort. If a research project is being conducted and identification of individuals is needed then this research project must be approved by the Service.

Measuring the pronotum is voluntary; however the Service would appreciate the collection of this data. Measuring of the pronotum should be done with digital calipers.

Sexing

The sex of ABBs can be distinguished based on the orange-red marking located between the frons and mandibles on the head. These markings are rectangular on males and triangular on females (Figure 5).

Aging

Adults that have pupated during the current active season are known as newly eclosed. Newly eclosed ABBs (young) can be distinguished from ABBs produced the previous year (old) by their softer bodies and more shiny appearance (Creighton *et al.,* 1994). Also, the orange-red pronotum appears to be lighter and more orange in color in newly eclosed adults. Older adults often are missing body parts, especially legs or antennae. In addition, the mandibles of older adults appear to be a bit more worn at the tip.

ABB Release

ABBs should be released along transects where they were captured or within about 500 feet of the transect. Further, ABBs should be released a minimum of about 100 feet away from vehicle or foot traffic and outside of the pathway of vehicle and foot traffic to avoid trampling.

Identification and Other Nicrophorus Species

There are 6 other *Nicrophorus* species in Oklahoma that resemble the ABB. The ABB can usually be distinguished from other *Nicrophorus* species by the large orange-red spot on its pronotum (body segment between the head and abdomen) and on the frons (Figure 6). No other mature *Nicrophorus* species has an orange-red marking covering the pronotum and frons. However, newly eclosed (within 2 weeks after emerging from the ground) *N. orbicollis* can have a burnt orange marking on the pronotum. However, red-orange frons present on the ABB should allow for distinction from new *N. orbicollis*. The *N. orbicollis* has black frons. A description of the other *Nicrophorus* species in Oklahoma and an identification key is located in Appendices 2 and 3, respectively. In addition, photos of other *Nicrophorus* species in Oklahoma are available on our website for comparison.

Accidental Death of ABBs

The handling of all endangered species is strictly regulated by the Service. All accidental mortalities of ABBs must be accounted for and an "*ABB Accidental Death Form"* must be completed for each individual specimen and submitted within 14 calendar days to the Oklahoma Ecological Services Field Office. An "*ABB Accidental Death Summary Report"* must be completed in electronic and hardcopy formats and submitted to the Oklahoma Ecological Services Field Office by October 15 of each year and to the Regional Permit Coordinator along with your annual report.

Dead ABBs should be placed in cotton within a sealable, rigid container to prevent jostling of the ABB causing limb and antennae damage. Each specimen must have a unique alphanumeric name assigned. This alphanumeric name should be the first letter of the first 2 words of the permittee company or individual (e.g. Acme Company, first dead ABB = AC001). A label with the date found dead, permittee, legal description (down to quarter section at least), and specimen alphanumeric name should be placed inside each container to ensure future identification. Only place one ABB specimen per container to avoid mixing up specimens. Place the container on ice until the ABB can be prepared. Dead ABBs are to be submitted to the Service or a Service approved facility with their corresponding "*ABB Accidental Death Form"*.

Reporting

The Service has prepared a standard "*ABB Survey Data Collection Form*" (Appendix 4). Use of this form ensures that all of the needed data is recorded by all permittees. This form is to be completed for each transect, each night during a survey effort. Copies of all forms are to be submitted to the Oklahoma Ecological Services field office (see address in heading).

In addition, an "*ABB Survey Summary Report*" (Appendix 5 and 6) is to be completed for each survey effort. This is to be submitted electronically in excel file format to ABBcontact@fws.gov. Additionally, a hardcopy form is to be mailed to the Oklahoma Ecological Services Field Office along with the corresponding "*ABB Survey Data Collection Forms*". A description of the required fields to complete in the "*ABB Survey Summary Report*" is provided in Appendix 7.

All latitude and longitude data should be reported in decimal degrees and the coordinate system/projection should be in NAD 83. If a survey is conducted in compliance to the Endangered Species Act or the National Environmental Protection Act, project names and numbers need to correctly correspond. Only complete and accurate reporting forms will be accepted. Incomplete and/or inaccurate forms will be returned and the surveys will be considered invalid until the forms are corrected and/or properly completed, and submitted. When sending corrected forms, indicate that it is a correction, what specifically has been corrected, and the project name.

<u>*Protocols and Forms*</u>
All forms can be downloaded from the Oklahoma Ecological Services Field Office's website <http://www.fws.gov/southwest/es/oklahoma/beetle1.htm>.

References

Bedick, Jon, Brett Ratcliffe, and Leon Higley. 2004. A new sampling protocol for the endangered American burying beetle, *Nicrophorus americanus* Olivier (Coleoptera: Silphidae). The Coleopterists Bulletin 58(1):57-70.

Creighton, Curtis, Mark Lomolino, and Gary Schnell. 1993. Survey methods for the American burying beetle (*Nicrophorus americanus*) in Oklahoma and Arkansas. Unpublished.

Creighton, Curtis, and Gary Schnell. 1998. Short-term movement patterns of the endangered American burying beetle *Nicrophorus americanus*. Biological Conservation 86:281-287.

Schnell, Gary, Ana Hiott. 1995. 1995 Annual report of trapping and relocation activities concerning the endangered American burying beetle (*Nicrophorus americanus*). Sam Noble Oklahoma Museum of Natural History, University of Oklahoma. Unpublished.

Schnell, Gary, Ana Hiott. 2003. 2003 Annual report of trapping and relocation activities concerning the endangered American burying beetle (*Nicrophorus americanus*). Sam Noble Oklahoma Museum of Natural History, University of Oklahoma. Unpublished.

U.S. Fish and Wildlife Service. 1991. American burying beetle (*Nicrophorus americanus*) recovery plan. Newton Corner, Massachusetts. 80 pp.

This guidance was developed from the above references, U.S. Fish and Wildlife Service's July 14, 2005, "ABB Survey Guidance" and U.S. Fish and Wildlife Service Working Group on May 6, 2004, and other meetings between Service personnel and permittees in March and April 2009. The Oklahoma Ecological Services Field Office, in coordination with other Field Offices, update this survey protocol as necessary due to new findings. This guidance strives to streamline and update American burying beetle survey recommendations among the Arkansas, Oklahoma, Kansas, Nebraska, South Dakota, and Arlington, Texas Field Offices. However, due to the current habitat, land-use, development, other environmental considerations, and etc. there is variation among the different states. However, each state protocol may be different in some manners due to the land use and actions that occur in the different states. Each state Service office should be contacted for their most current protocols.

Appendix 1. Carrion Types Tested and Found Effective at Attracting ABBs

Appendix 1. Carrion types used in this study and the number of American burying beetles (ABB) attracted.

Carrion type	Trap-nights	ABB	ABB per trap-night*	Recaptures	Total ABB trap-night†
Badger	7	4	0.571	0	0.571
Cat (domestic)	19	13	0.684	3	0.842
Coyote	7	0	0	0	0
Jackrabbit	36	18	0.500	6	0.667
Opossum	5	0	0	0	0
Pig (domestic)	9	2	0.222	0	0.222
Rat (white lab rat)	178	149	0.837	28	0.994
Squirrel (gray)	27	56	2.074	34	3.333
Badger & squirrel	6	2	0.333	1	0.500
Cat & rat	3	3	1.000	0	1.000
Total: Mammals	**297**	**247**	**0.832**	**72**	**1.074**
Snake (rattlesnake)	6	10	1.667	5	2.500
Snake (bull snake)	12	13	1.083	5	1.500
Toad (*Bufo* species)	13	16	1.231	1	1.308
Turtle (ornate box turtle)	25	33	1.320	12	1.800
Toad & snake	14	11	0.786	7	1.286
Total: Herptiles	**70**	**83**	**1.186**	**30**	**1.614**
Dove (mourning dove)	30	31	1.033	9	1.333
Turkey (giblets)	3	1	0.333	0	0.333
Total: Birds	**33**	**32**	**0.970**	**9**	**1.242**
Fish (scraps)	6	6	1.000	3	1.500
Cat & green racer	2	1	0.500	0	0.500
Rat & ornate box turtle	4	2	0.500	0	0.500
Rat & bull snake	47	36	0.766	6	0.894
Rat & rattlesnake	36	39	1.083	15	1.500
Squirrel & rattlesnake	4	9	2.250	2	2.750
Squirrel, snake, box turtle	2	3	1.500	0	1.500
Mixed carrion (other)	55	80	1.455	26	1.927
Total: mixed carrion	150	170	1.133	49	1.460
Total: all carrion types	**562**	**538**	**0.957**	**163**	**1.247**

* ABB per trap night includes only first captures.
† Total ABB per trap-night includes recaptures with first captures.

attractiveness, the overall capture rate for *N. americanus* using the lab rats was comparable to other types of carrion (Table 1).

Trap Effectiveness and Topography. Sampling from paired bucket traps on ridge-top and valley locations produced a total of 83 *N. americanus* over a six day sampling period. The captures include recaptures, and the mean totals for six days are shown in Figure 5. Thirty *N. americanus* were captured in the valley and 53 on the ridges. Analysis of variance over time using SAS showed no significant difference for the effect of time ($F_{1,5}$ = 1.76, P = 0.15). The traps placed on the ridges attracted significantly more beetles than the traps placed in the valley ($F_{1,3}$ = 4.37, P = 0.0451).

Greater trap captures on ridge tops may have resulted from increased movement of odors associated with bait decay than occurred in valleys, thus improving the possibility of detection by beetles. In addition, beetle activity could be favored by warmer temperature on the ridge tops. During one session of nocturnal trapping, several

Appendix 2. Description of Nicrophorus Species in Oklahoma

TABLE 1. Description of burying beetles found in eastern Oklahoma and western Arkansas.

Nicrophorus americanus: Four elytral spots. Orange-red pronotum and frons distingiush this species from all other North American burying beetles.

Nicrophorus orbicollis: Black pronotum with some texturing to it. Four orange spots on elytra (two/elytron) that do not extend to edges of elytra. Typically, it is the most common species in wooded habitats.

Nicrophorus marginatus: Similar to *N. orbicollis* except that each pair of elytral spots on is connected along lateral edge of elytron. Species found almost exclusively in grassland areas.

Nicrophorus sayi: Very similar to *N. orbicollis* except femur of each back leg is distinctly curved instead of straight. Also, proximal pair of elytral spots extend to lateral edge of the elytra. Active in early spring and quite rare after late June.

Nicrophorus tomentosus: Pronotum covered with fine, golden hairs. Easily distinguished from all other species by this characteristic. Found in variety of habitats.

Nicrophorus pustulatus: Relatively dark appearance with faint or absent elytral spots. Four small, orange spots may be visible at distal end of elytra (two spots/elytron).

Nicrophorus carolinus: Similar to *N. orbicollis* except that pronotum very smooth and domelike. Usually found near large rivers.

Appendix 3. Identification Key to Nicrophorus Species in Oklahoma

Table 2. Key to burying beetles of eastern Oklahoma and western Arkansas.

1A. Pronotum covered with fine, golden hairs.......... *tomentosus*
1B. Pronotum not covered with hairs.......... 2

2A. Pronotum and frons red-orange.......... *americanus*
2B. Pronotum and frons black.......... 3

3A. Elytral spots faint or absent.......... *pustulatus*
3B. Elytral spots present.......... 4

4A. Pronotum round, smooth and domelike.......... *carolinus*
4B. Pronotum not round, smooth or domelike.......... 5

5A. Femur of back leg distinctly curved.......... *sayi*
5B. Femur of back leg straight.......... 6

6A. Spots on each elytron connected on lateral edge of elytron.......... *marginatus*
6B. Elytral spots distinct.......... *orbicollis*

AMERICAN BURYING BEETLE SURVEY DATA COLLECTION FORM

Project Name:________________Time[1]:_____ Date[1]:_______ Transect #:______ Survey Night: 1 2 3 4 5 6
M/D/Y

Survey Company: __________________ Surveyor:__________________ Project Proponent:________________
(Spell out)

State:_____ County:___________ Legal Description[2]:______________ General Location:_____________________
(Sec Township Range) (nearest town, city, landmark)

Decimal Degrees[2]:________________/__________________ Coordinate System[2]:__NAD83_

Vegetation Type:__________________________ Primary Soil Type:_____________________________
(Prairie, woodland, forest, pasture) (Refer to County Soil Survey)

Temp[3]: Min______ Max______ °F Wind[4]:_______mph Cloud Cover[4]:_____________%

Rain > 0.5 in.?[5] **yes** *or* **no** Additional survey night(s) required because of rain?[6] **yes** *or* **no**

Trap No.	*americanus*	*orbicollis*	*tomentosus*	*pustulatus*	*marginatus*	*carolinus*	*sayi*	*Other carrion beetles*
1. U D[7]								
2. U D								
3. U D								
4. U D								
5. U D								
6. U D								
7. U D								
8. U D								
Totals								

Number of disturbed traps and/or bait (D):______

Additional survey night(s) required because of disturbance?[8] : **yes** *or* **no**

List each individual American burying beetle captured below and complete the appropriate columns.

ABB	**Male**	**Female**	**Old**[9]	**New**[9]	**Age Unknown**[9]	**Recapture**[10]	**Newly Marked**[11]	**Tag #**	**Death**	**Pronotum Width**
1										
2										
3										
4										
5										
6										
7										
8										
9										

Comments:____

1. Date and time refer to when trap checked;
2. Check that legal description fits decimal degrees location. Lat/long MUST be in decimal degrees, NAD 83
3. Max/Min temp for previous 24-hour period prior to checking traps, must use data from www.mesonet.org
4. Wind and cloud cover data refer to current conditions.
5. Rain for previous 24 hour period starting at midnight of the night trapped, must use data from www.mesonet.org
6. Additional trapping required if > 0.5 in. of rainfall occurs between 7pm and 7 am on the night of survey, must use data from www.mesonet.org
7. U= trap undisturbed, bait present; D= trap disturbed AND/OR bait gone;
8. Determine total number of disturbed traps over all 3 survey nights. If between 4 and 8 traps are disturbed over the 3 survey nights, 1 additional night of surveys are required. If between 9 and 16 traps are disturbed, 2 additional nights required. If between 17 and 24 traps are disturbed, 3 additional nights required.
9. OLD=breeding adult; NEW=newly enclosed adult; UNK=age cannot be determined.
10. Recaptures refer to color and number of bee tag on beetles that have been previously marked.
11. Newly marked males and females refers to color, number of bee tag, and age of beetle (e.g. R54[old]).

American Burying Beetle Survey Summary Report (Electronic)

An electronic copy (in excel format, *NOT* PDF) of this is to be submitted to the Oklahoma Ecological S survey effort. In addition, a hard copy (previous worksheet) along with the corresponding ABB Surve Oklahoma Office within 30 days.

The data for each transect should be summarized and entered in the report below.

Project Information			Date			Location							
Transect	Proj_name	Proj_desc	month	day	year	county	state	T	R	S	lat (dec. degrees)	long (dec. degrees)	coordsyst (NAD 83)

* An ABB Trap and Relocate Reporting Form needs to be submitted to the Service, in addition to this data sh

Services Field Office within 30 days of each
ey Data Forms are to be submitted to the

	Involved Parties			Survey Information							
location	company	surveyor	proj_proponent	survey_method	survey_type	bait_type	area	Tabb	pos_neg	recap	male_total

heet.

ABB Capture Data											
male_young	male_adult	Munkage	female_total	female_young	female_adult	Funkage	unknsex	dead	Adult	YOY	unknage

Trap Effort					Habitat Data			Other Nicrophorus captured						
tot_trap_ night	tot_bait_ distub	TTN_tbd	abbptn	abbptn_tbd	soil	GenSoil	veg	orb	tom	pust	marg	caro	sayi	abb_released

Relocation Effort*								
gen_rel_ site	rlsmonth	rlsday	rlsyear	abb_died	lat	long	coorsys	Notes

American Burying Beetle Survey Summary Report

A hardcopy of this is to be submitted to the Oklahoma Ecological Services Field Office within the electronic copy (next worksheet) along with the corresponding ABB Survey Data Forms a within 30 days.

The data for each transect should be summarized and entered in the report below. In addition totaled (where stated).

	Project Information		Date		
Transect	Proj_name	Proj_desc	month	day	year
1					
2					
3					

	Location					
Transect	county	state	T	R	S	lat (dec)
1						
2						
3						

	Involved Parties			Survey Information		
Transect	company	surveyor	proj_proponent	survey_mentod	survey_type	bait_type
1						
2						
3						

	ABB Capture Data					
Transect	Tabb	pos_neg	recap	male_total	male_young	male_adult
1						
2						
3						
TOTAL						
Transect	female_adult	Funkage	unknsex	dead	Adult	YOY
1						
2						
3						
TOTAL						

	Trap Effort					
Transect	tot_trap_night	tot_bait_disturb	TTN_tbd	abbptn	abbptn_tbd	soil
1						
2						
3						
TOTAL						

	Other Nicrophorus captured					
Transect	orb	tom	pust	marg	caro	sayi
1						
2						
3						
TOTAL						

	Relocation Effort*				
Transect	abb_released	gen_rel_site	rlsmonth	rlsday	rlsyear
1					
2					
3					

Transect	lat	long	coorsys	abb_died
1			NAD 83	
2			NAD 83	
3			NAD 83	

Transect	Notes
1	
2	
3	

* An ABB Trap and Relocate Reporting Form needs to be submitted to the Service, in addition to this

30 days of each survey effort. In addition,
re to be submitted to the Oklahoma Office

ı, the data of all the transects should be

long (deg)	coordsyst	location
-	NAD 83	
-	NAD 83	
-	NAD 83	

area

Munkage	female_total	female_young

unknage

Habitat Data

GenSoil	veg

s data sheet.

Title Descriptions

Code	Meaning	Definition
abb_died	# ABBs died during transport	The total number of translocated ABBs that died during transport to new release location.
abbptn	ABBs per Trap night	Total number of ABBs trapped divided by the total number of trap nights = ABBs per trap night
abbptn_tbd	ABBs per undisturbed trap night	Total number of ABBs trapped divided by the total number of undisturbed trap nights = ABBs per undisturbed trap night
abb_released	# ABBs Captured to Relocate	The total number of ABBs captured to be translocated offsite.
adult	Adults	Total # of ABBs that were born the previous year.
Area	Approximate Survey Area (Acres)	Total number of acres of project area and/or survey area. For surveys conducted specifically to determine the presence/absence of ABBs in an area where a project is proposed then provide the total acreage of the project size. If surveys are being conducted for monitoring or research purposes, provide the total area of your research or monitoring area.
bait_type	Bait Type	Type of bait used in traps during surveys.
caro	Nicrophorus carolinus	provides the total number of this species captured
company	Company	Company/Institution that conducted the survey
coordsyst	Coordinate System	Type of coordinate system your lat/long is provided in.
county	County	County(s) where survey was conducted
day	Day	The last day of the survey.
dead	Dead/Killed	Total number of dead or injured ABBs encountered during survey. This includes ABBs killed while in the trap or during handling.
female_adult	female adult	total number of adult females captured
female_total	female total	total number of females captured
female_young	female young	total number of young females captured
Funk age	female unknown age	total number of females of unknown age captured
GenSoil	general soil	
gen_rel_site	general relocation site	A reference point to locate the survey site.
lat	Decimal Degree Latitude	Decimal Degree Latitude for relocation sites

location	Location	A reference point.
long	Decimal Degree Longitude	Decimal Degree Longitude for relocation sites
male_adult	male adults	total number of adult males captured
male_total	male total	total number of males captured
male_young	male young	total number of young males captured
marg	Nicrophorus marginatus	provides the total number of this species captured
month	Month	Month survey started.
Munk age	male unknown age	total number of males of unknown age captured
notes	notes	this is a blank cell available for comments not appropriate for the provided columns
orb	Nicrophorus orbicollis	provides the total number of this species captured
pos_neg	positive or negative	Results of survey. If any ABBs were captured, write "positive". If no ABBs were captured, write, "negative."
Proj_desc	Project description	description of the type of project to be implemented
Proj_name	Project name	name of the project
proj_proponent	Project Proponent	Company that funded or contracted out survey.
pust	Nicrophorus pustulatus	provides the total number of this species captured
R	Range	Range of legal description.
recap	Recaptures	Number of ABBs that were recaptured during this survey effort. Recaptures may be from your marking efforts or from a prior marking effort by someone else. Choices: Not a recapture, a recapture with a tag, or a recapture with a clipped elytra. If the recapture has a tag, note the tag number here.
rlsday	release day	
rlsmonth	release month	
rlsyear	release year	
S	Section	Section of legal description.
sayi	Nicrophorus sayi	provides the total number of this species captured
soil	Soil	Soil type as defined by Soil Survey Book.
state	State	State where project was conducted.
surveyor	Surveyor/Researcher Name	Name of individual(s) that conducted the survey.

survey_method	Survey Method	Type of approved survey protocol used. The Service has used 3 approved ABB survey protocols. One is from the recovery plan and one is from Creighton et al. The Service has recently provided a April 2005 survey guidance that we recommend be used. However, if you are doing research on a different type of survey method, list that here.
survey_type	Survey Type	Type of results trying to achieve.
T	Township	Township of legal description.
Tabb	Total # ABBs	Total number of abbs captured during the survey effort.
tom	Nicrophorus tomentosus	provides the total number of this species captured
tot_bait_disturb	Trap or Bait Disturbance	Total number of trapnights that were disturbed or where bait was missing during entire trapping effort. Any traps with bait missing need to be included in this category. Plus any traps where the cover, bait cup or pitfall trap were disturbed need to be included in this category.
tot_trap_night	Total Trap nights	Total number traps x total number of nights surveyed=total trap nights
TTN-tbd	Trap or bait disturbance minus the total bait disturbed.	The accurate amount of trapnights is dependent on the bait being present in the trap the entire night. When bait is removed or disturbed this eliminates the effectiveness of the trap. Consequently, the capture rate of ABBs is less and this needs to be considered.
Transect	Transect Number	Number designated when there are multiple transects for one project
unkn age	Age unknown	Total number of ABBs from the survey effort where age could not be determined. Including recaptures.
unkn sex	Sex unknown	Total number of ABBs from the survey effort where sex could not be determined. Including recaptures.
veg	Vegetation	Type of vegetation present at site. The Habitat Inventory Protocol established by Creighton et al should be used to determine this, unless new research is being conducted. If you are not collecting habitat data then provide a general description of the vegetation at the survey site.
year	Year	Year survey started.
YOY	Young of year	# of ABBs that were born the current year.

Example

55/72=0.76

Should always be NAD 83

If the survey was conducted from August 9 to August 13, 2004, then 13 should be entered into this field.

1

(i.e. sandy loam, etc.)

(i.e. nearest city, mountain, state wildlife management area, state park, refuge, etc)

(i.e. nearest city, mountain, state wildlife management area, state park, refuge, etc)

i.e. New oil well

i.e. Martin oil well

i.e. Oklahoma Department of Transportation

ABBs can be marked with a bee tag or by making a small cut on one of their elytra.

i.e. hectorville complex, etc.

One of 4 choices is available. 1-Recovery Plan, 2-Creighton et al, 3-Service April 2005, or new research method.

i.e. presence/absence, density, abundance

55

One trapnight equals 1 open trap for night. So if 3 traps were disturbed for 3 nights, the total traps or baits disturbed equals 9 trapnights

24 traps (3 transects with 8 traps) x 3 nights = 72

48 trap nights - 12 traps disturbed = 36

i.e. Project name: Tahlequah pipeline, Transect: 4

5

3

General Description: Open oak-hickory woods with herbaceous understory; thick pine woods with shrubby understory; native prairie; non-native pasture; cropland; bare soil; Bermuda grass; mostly native grasses with scattered trees; or etc.

Figure 1. Diagram of survey transect.

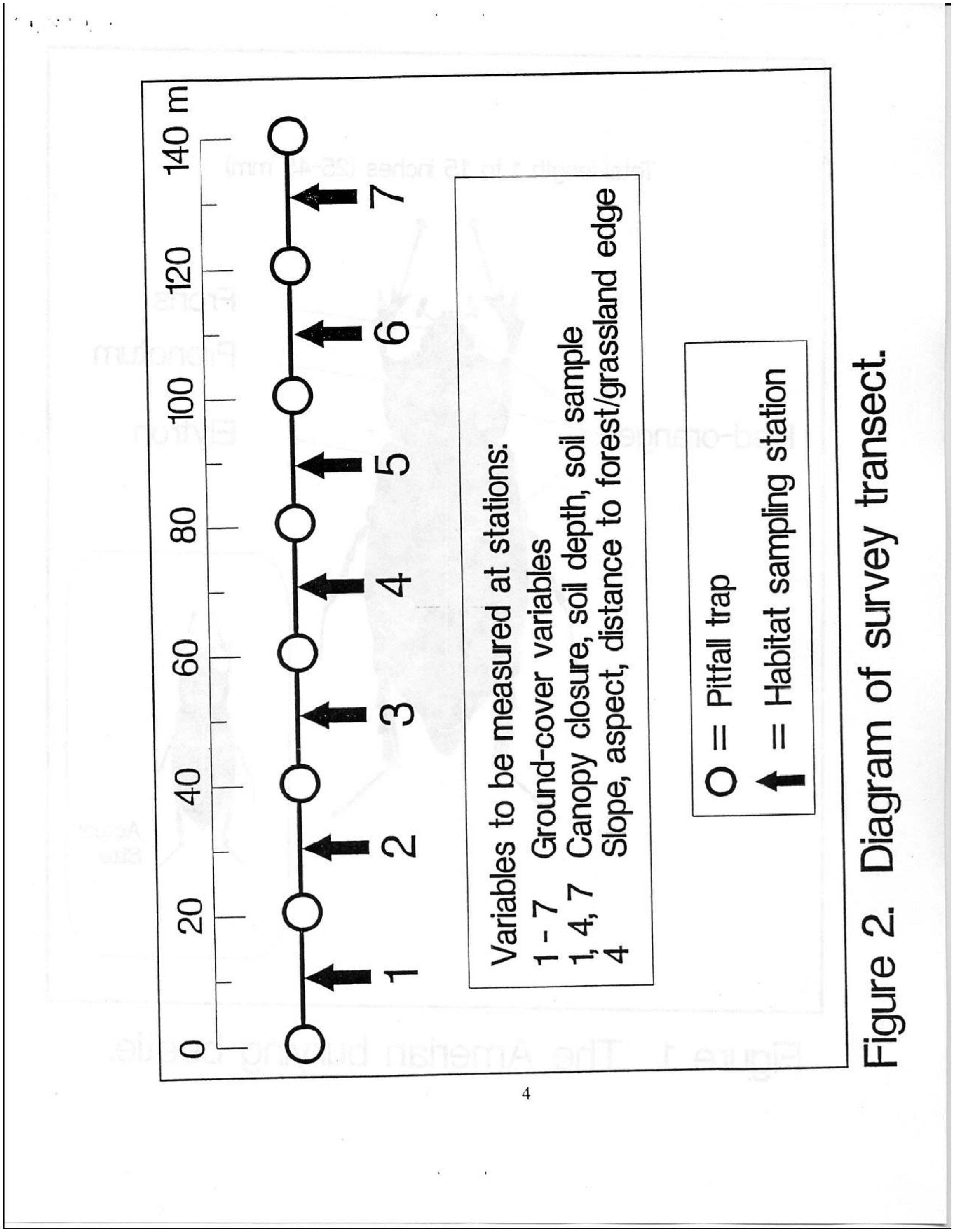


Figure 2. Bucket Pitfall Trap with Wood Cover

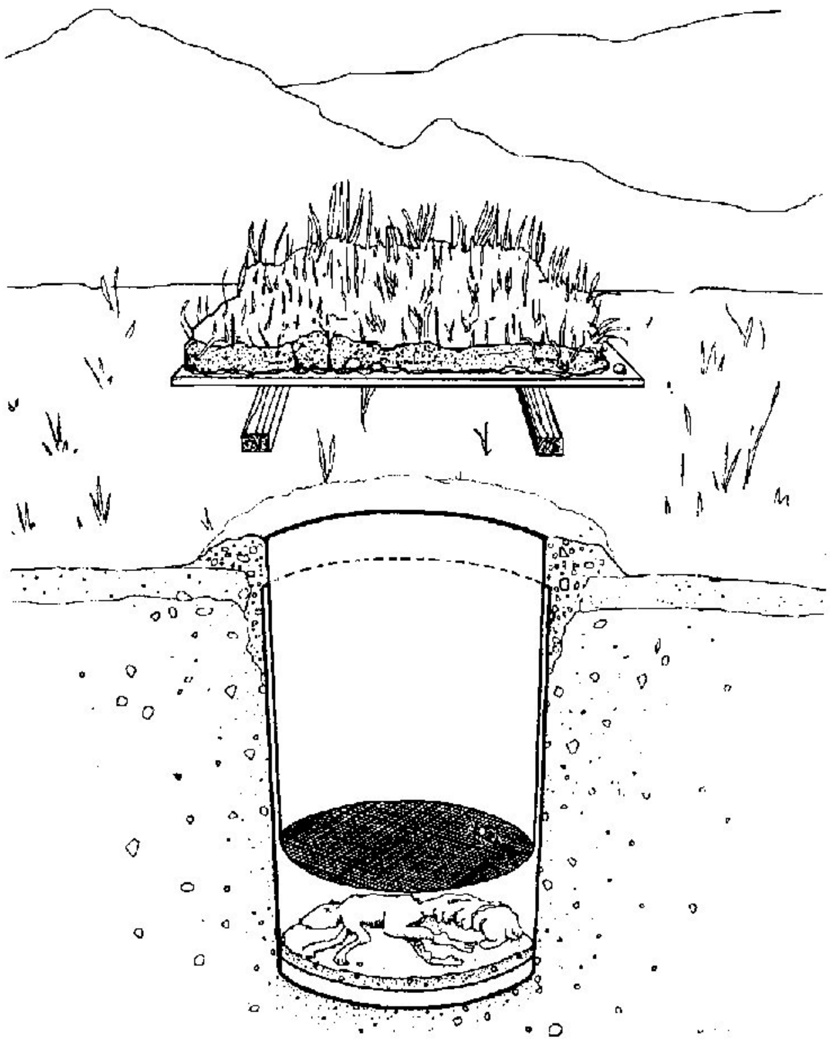


Fig. 2. Double bucket trap. The bottom of one bucket was removed and replaced by window screen; the screen bucket was placed within the second bucket containing whole carrion.

valley in Lincoln County) and along the ridge tops surrounding it. Eight pitfall buckets were used, and each pair was baited with the same carrion mixture. The pitfalls were paired: one trap in the valley and one trap on an adjoining ridge (Fig. 4). Four replicates were placed in a north-south direction. The pairs were alternated to the east side of the valley/west ridge and then the west side of the valley/east ridge. Each pair of traps was placed 200–400 m apart. Each set of traps was placed a minimum of 1 km apart. Trapping continued for six consecutive days (14–19 August 1996). The traps were checked each morning, and the beetles were quantified as previously described.

Figure 3. Diagram of pitfall trap.

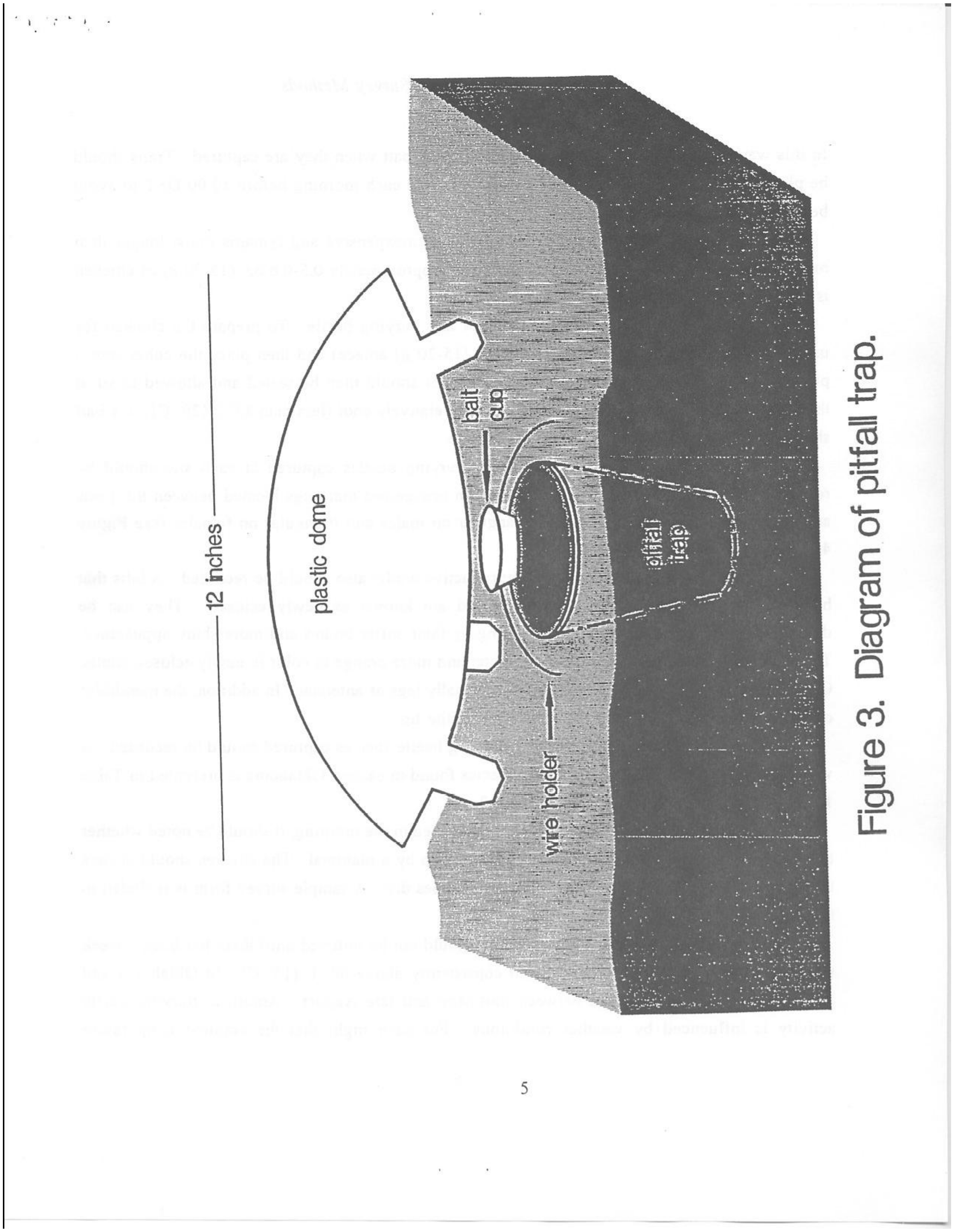


Figure 4. Bucket Trap with Wire Mesh Vertebrate Exclusion Cover

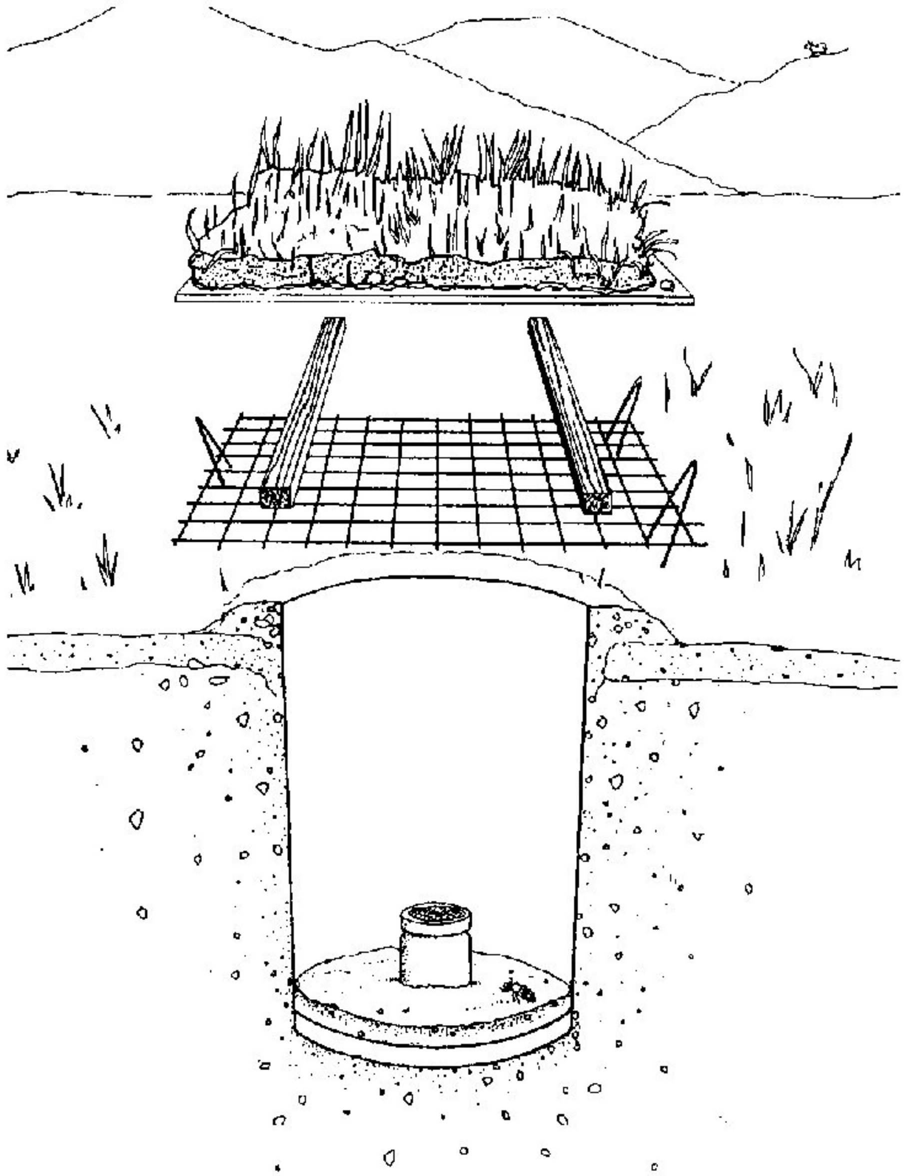


Fig. 4. The 18.9 liter bucket used for the majority of this study. The bucket was buried in the ground and a berm of soil (6–8 cm) was formed around the bucket lip. Bait is placed in the screen container. A plastic rain cover is placed over the bucket and supported by wood or stones. Wire screen is used over the bucket in areas with vertebrate scavenger activity.

Results and Discussion

Trapping Techniques. For most insect sampling, the key issue is sampling efficiency and numbers collected per unit of effort. However, in sampling an endangered species, minimizing mortality during sampling is a key requirement. In evaluating trapping methods for *N. americanus*, we needed to weigh both efficiency and survivorship with emphasis on survivorship.

With these requirements, trapping results indicated that a five-gallon bucket was the best, although perhaps not the most practical, pitfall trap. Small diameter cups and pipe

Figure 6. Characteristics distinguishing male from female American burying beetles.

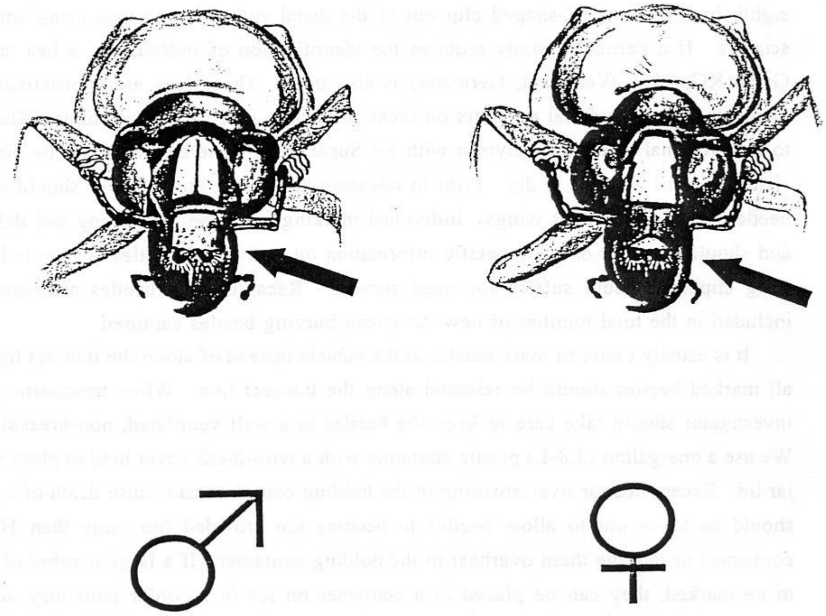


Figure 4. Characteristics distinguishing male from female American burying beetles.

Figure 5. Diagram of the American burying beetle.

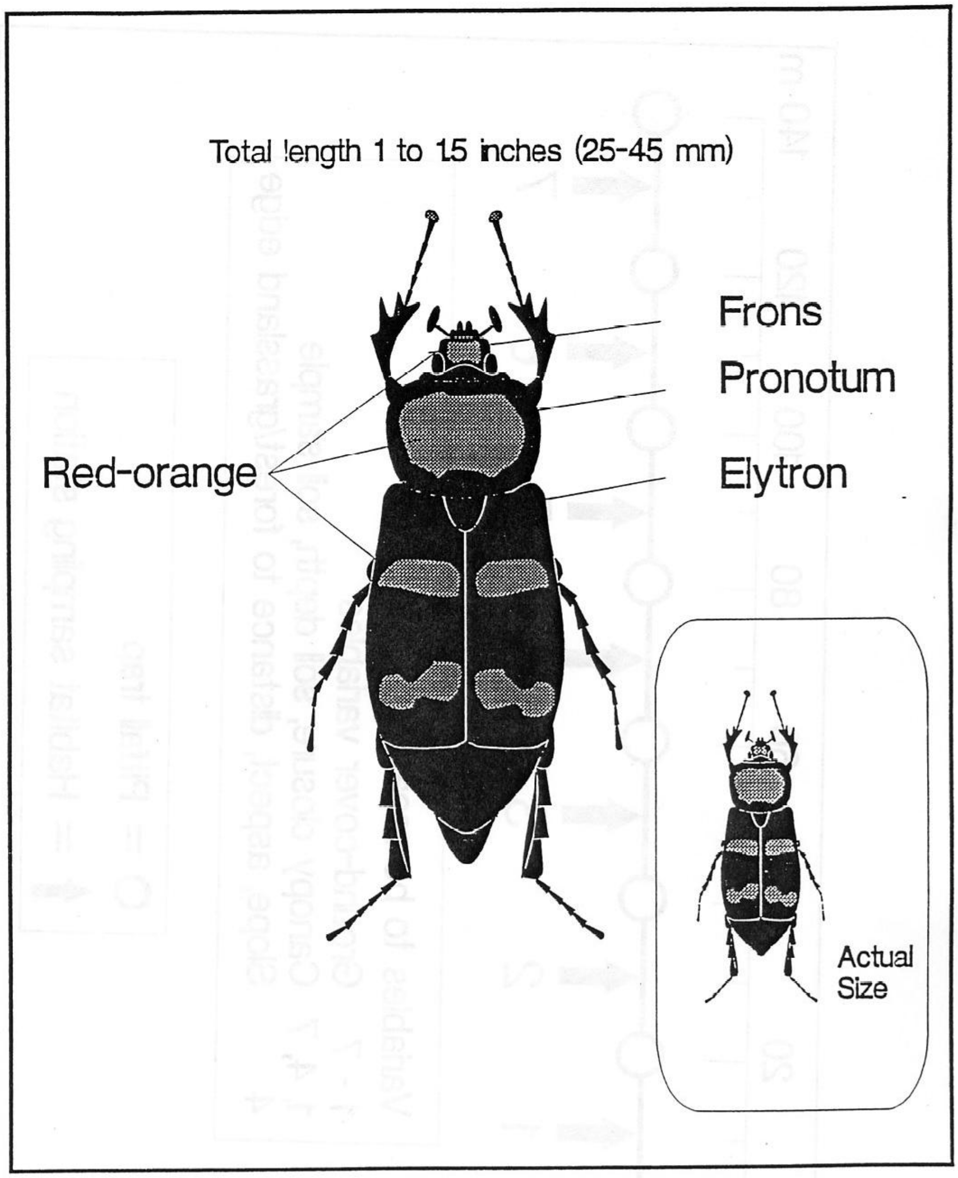


Figure 1. The Amerian burying beetle.

United States Department of the Interior

FISH AND WILDLIFE SERVICE
Division of Ecological Services
9014 East 21st Street
Tulsa, Oklahoma 74129-1428
918/581-7458 / (FAX) 918/581-7467

American Burying Beetle *Nicrophorus Americanus*
Baiting Away Guidance for Oklahoma

Updated May 20, 2009

Introduction
The goal of this document is to provide guidance in implement baiting away actions for the ABB as a means of complying with section 7 and 9 of the Endangered Species Act (ESA). Section 9 of the ESA prohibits all persons from the taking of federally listed species. Take includes harming, harassing, or killing. Section 7 of the ESA requires federal agencies to consult with the Service if a project they authorize, fund, or carry out may adversely affect a federally listed species. Baiting away is a technique to remove ABBs from a given area prior to soil disturbance without handling the ABB or physically relocating them to another area. Implementing other temporary relocation measures, not recommend by the Service, may not result in avoidance of impacts or take of the ABB.

Time
Bait away should be implemented during the ABB's prime active period in Oklahoma, May 20 to September 20. Refer to the Services "ABB Survey Guidance" for additional information.

Timing
Bait away should be implemented during the ABB's prime active period in Oklahoma, May 20 to September 20. Although the capture rates of ABBs are known to be higher during certain dates within their prime active period, the Service is not recommending restricting bait away implementation to specific dates during this prime active period. Refer to the Services "ABB Survey Guidance" for additional information.

Minimum Bait Away Effort
To have confidence in avoiding impacts to ABBs, a minimum of 3 consecutive days of baiting away must occur prior to soil disturbance. Additional baiting away may be necessary if soil disturbance does not commence or is not completed the day immediately following the conclusion of baiting away (day 4).

*Timeframe a Baiting Away Effort is Valid**
Bait Away Conducted for Projects Implemented During the **Active Period**:
The Service recommends bait away commence at least 3 days prior to the disturbance of soil. This bait away effort is only valid for the present. After the 3 days of baiting away, soil disturbance must commence on the 4th day. If soil disturbance is not concluded for the entire project area by day 4, then additional days of baiting away are needed until all the areas that are to be disturbed are removed of topsoil. The Service's definition of completed soil disturbance is when all the topsoil in the project area has been removed or all the vegetation has been removed leaving only soil. If project construction will occur in phases and therefore soil disturbance will occur in phases, then baiting away can also occur in phases. However, any newly disturbed areas will still need to be baited away 3 days prior to soil disturbance.

Bait Away Conducted for Projects Implemented During the **Inactive Period:**
During the ABB's inactive period, September 21 to May 19, ABBs bury in the soil to overwinter. Projects to be implemented during the ABBs inactive season should plan to address the ABB just prior to the onset of their inactive season since baiting away cannot be implemented at the time of the proposed project disturbance. The objective of implementing bait away just prior to the ABB's inactive period is to lure ABBs to a disturbance free area where they will then bury in the soil and overwinter safely. Luring ABBs to overwinter outside the project area allows the commencement of project construction during the inactive period. Baiting away must commence

on September 14 and continue for 6 days.

*Bait Away Effective Radius**
Bait away efforts are only valid for the specific project site where ABBs are being lured from. The effective radius of a bait away effort is dependent on the number of bait stations deployed. Specific spacing guidelines are explained below.

Minimum Project Size
Projects with a radius equal to or less than 0.5 mile from the center to any given point along the perimeter are suitable for baiting away to be implemented.

Projects with a radius greater than 0.5 mile from the center to any given point along the perimeter are not suitable for baiting away to be implemented. Project areas greater than 0.5 miles from the center to any point along the perimeter are too large to deploy bait stations outside the project boundary and be effective at luring ABBs. Trapping and relocating should be implemented. Please refer to the Service's May 20, 2009 "*ABB Trap and Relocation Guidance*".

The average nightly movements of ABBs are around 0.5 miles. So, to err on the side of the species a maximum project radius size of 0.5 miles is necessary to ensure ABBs are effectively removed from the proposed disturbance area.

Weather Requirements
An additional night of baiting away is required when the temperature falls below 60ºF between 7:00 PM and 7:00 AM or when rainfall greater than ½ inch occurs between 7:00 PM and 7:00 AM. If any additional nights of baiting away are required and conducted because of weather, this needs to be noted and explained in the "*ABB Bait Away Form*". Refer to the Services "ABB Survey Guidance" for additional information.

Weather data for all surveys should be collected using the Oklahoma Mesonet website, www.mesonet.org. Directions for using the Oklahoma Mesonet website are provided below.

Oklahoma Mesonet:

1. At www.mesonet.org, click on "Past Data and Files" under "Mesonet Data" (Left side of the screen)
2. Click on the Mesonet station closest to the survey site. Then under the "Station Monthly Summary" heading select the month and year of the survey. Then click "Get summary."

   Temperature:
   - In "summary report" of this Mesonet page, find the date of the survey. Daily Mesonet data is measured from midnight to midnight, so if traps are set on June 3rd, temperature data from both the 3rd and the 4th will be needed to address the entire trapping night, which is between 7:00 PM to 7:00 AM, and because the nightly low temperature most often occur past midnight.

   Rainfall:
   - In "summary report" of this Mesonet page, find the date of the survey. Once again, if the traps are set on June 3rd, Mesonet data from the both the 3rd and 4th need to be reviewed to address the entire trapping night, which is between 7:00 PM to 7:00 AM. Rainfall over ½ inches during a trap night requires further analysis and reporting. The time of the rainfall needs to be determined. This Mesonet page only reports the entire rainfall that occurred in a 24 hour period (midnight to midnight). To determine when during a 24 hour period rainfall events occurred and how much rain fell, proceed to the next paragraph for instructions to navigate through Mesonet to the proper page.

   - Click on "Past Data and Files". Then under the heading "Data Files" on the bottom right of screen click on "Mesonet MTS Files". Then click on the relevant date. Then select the Mesonet station nearest to the survey area. Rain totals are given in 5 minute increments on this Mesonet page. Remember that daily Mesonet data is provided for a 24 hour period, beginning at 12:00 AM and ending at 12:00 AM. So the date the traps were set and the following date need to be reviewed to determine the rainfall for the entire trapnight.

Baiting Location

For nonlinear projects bait stations should be deployed at 1,000 foot intervals and 500 feet outside the project perimeter.

For linear projects with a width from 0.35 to 0.5 mile, bait stations should be deployed along both long sides of the project at 1,000 foot intervals and 500 feet outside the project perimeter. For projects with a smaller width, less than 0.35 mile, bait stations should be deployed alternately along both sides of the projects long boundaries at 1,000 foot intervals. (Meaning one bait station should be deployed on side A and then another bait station deployed 1,000 linear feet away on side B, and so on.)

Projects with a width greater than 0.5 mile or a diameter greater than 1 mile will need to implement trap and relocation.

Ants

Bait stations should not be placed within 23 feet (7 meters) of ant hills. If ants are discovered on bait it should be relocated at least 23 feet away. Ants can swarm and kill an ABB (Creighton *et al*,. 1993 and Bedick *et al*,. 2004).

Bait

Bait roughly the size of a whole chicken (3-5 pounds) needs to be used at each station. Bedick *et al*. (2004) found higher capture rates of ABBs when larger bait was utilized.

Bait Enclosure and Cover

Bait needs to be enclosed, secured to the ground, and covered. The enclosure and cover must allow ABBs access to bait, permit ABBs to easily exit, allow ABBs access to soil under bait, allow bait odor to escape, and protect ABBs from desiccation but prevent access by other non-target scavengers. Enclosures and covers need to be secured to the ground with rebar, stakes, or other such item to prevent removal by vertebrate scavengers. All materials used to enclose and cover bait, and to secure the enclosure and cover to the ground must be able to withstand weather conditions and vertebrate scavengers. Enclosures and covers must allow for visual inspection of the bait to determine replacement needs.

Some enclosure examples are: Havahart traps, wire mesh, expanded metal, and metal baskets. Enclosures must contain holes large enough to allow ABBs access to bait but prevent access by other non-target vertebrate scavengers. Typically ABBs fly to an area, land and then crawl to carrion. So, bait enclosures need to have holes at ground level to ensure ABBs will have access to the bait. ABBs have been reported to remain under the bait or in the soil under the bait throughout the daytime. Preliminary data suggest that *Nicrophorus* species could be adversely affected or killed via desiccation as a result of exposure to temperature extremes from remaining with the bait and not being able to bury in the soil (Hoback 2007, personal communication). Consequently, enclosure bottoms must permit ABBs access to the soil. The ABB is vulnerable to desiccation due to overexposure to heat and direct sun. Enclosed bait placed in a dense, forested habitat type where shade is provided the entire day will not require any additional protective covering for shade. However, enclosed bait that is not shaded by dense, forested vegetation will require additional covering for shade. The covers must not prevent ABBs from readily accessing the bait or exiting the enclosure, or prohibit the escape of bait odor.

Checking Bait Stations and Disturbed Bait

Each bait station must be daily. Any bait that no longer emits a pungent odor, has desiccated, has been scavenged or otherwise not effective needs to be replaced. When 20 percent or more of the bait stations are missing bait, an additional night of baiting away is needed prior to soil disturbance.

Reporting

The Service has prepared a standard "*Bait Away Reporting Form*" (Appendix A). Use of this form ensures that all of the needed data is recorded. This form must be completed and submitted to the Oklahoma Service Field

Office within 30 days of completion of each bait away effort.

This form is to be completed for each bait away effort for each night during the bait away effort. The "*Bait Away Reporting Form*" must be completed in Excel. This is to be submitted electronically in excel file format to ABBcontact@fws.gov. The Service will then review the form and provide a response, via electronic mail, regarding our acceptance or non-acceptance of the bait away effort as sufficient.

If bait away effort is conducted in compliance to the Endangered Species Act or the National Environmental Protection Act, project names and numbers need to correctly correspond. Each row in the spreadsheet should represent an individual bait station. All latitude and longitude data should be reported in decimal degrees and the coordinate system/projection should be in NAD 83. Only complete and accurate forms will be accepted. Incomplete and/or inaccurate forms will be returned and the bait away effort will be considered invalid until the forms are corrected and/or properly completed, and submitted. When sending corrected forms, indicate that it is a correction, what specifically has been corrected, and the project name.

Protocols and Forms
All protocols and forms can be downloaded from the Oklahoma Ecological Services Field Office's website http://www.fws.gov/southwest/es/oklahoma/beetle1.htm

Bait away radius and validity, and placement and spacing are more restrictive for "ABB Baiting Away Guidance" than the Service's "ABB Survey Guidance" because ABB surveys are only aimed at determining the presence or absence of ABBs. Baiting away is aimed at removing all ABBs from the project area.

Portions of this guidance were developed from the U.S. Fish and Wildlife Service's July 14, 2005, "ABB Survey Guidance" and a U.S. Fish and Wildlife Service Working Group on May 6, 2004, and other meetings between Service personnel and permittees in March and April 2009. The Oklahoma Ecological Services Field Office, in coordination with other Field Offices, update this protocol as necessary due to new findings. The purpose of this guidance is to streamline and update American burying beetle bait away recommendations among the Arkansas, Oklahoma, Kansas, and Arlington, Texas Field Offices. However, each state protocol may be different in some manners due to the land use and actions that occur in the different states. Each state Service office should be contacted for their most current protocols.

American Burying Beetle Bait Away Hardcopy Reporting Form

This form is to be submitted to the Oklahoma Ecological Services Field Office electronically within 30 days of each bait away effort.
The data for each bait station should be summarized and entered in the report below

Project Name	Project Description	Month	Day	Year	Nights Baited	Project Soil Disturbance Date	BA Company	BA Surveyor	Land owner	Project Proponent	County	General Location	State

Section	Township	Range	Lat	Long	Coord Sys	Bait Type	Bait Size (lbs)	Bait Spacing (ft)	Perimeter Distance (ft)	# Bait Station	Project Size	Bait Checked	Bait Replaced	ABBs Present
					NAD 83									

Other Nicrophorus Present

United States Department of the Interior

FISH AND WILDLIFE SERVICE
Division of Ecological Services
9014 East 21st Street
Tulsa, Oklahoma 74129-1428
918/581-7458 / (FAX) 918/581-7467

American Burying Beetle *Nicrophorus Americanus*
Trapping and Relocating Guidance In Oklahoma

Updated May 20, 2009

Introduction

The goal of this document is to provide guidance in designing and conducting trapping and relocation efforts for the ABB as a means of complying with section 7 and 9 of the Endangered Species Act (ESA). Section 9 of the ESA prohibits all persons from the taking of federally listed species. Take includes harming, harassing, or killing. Section 7 of the ESA requires federal agencies to consult with the Service if a project they authorize, fund, or carry out may adversely affect a federally listed species. Trapping and relocating is a technique to remove ABBs from a given area prior to soil disturbance. Implementing other relocation measures, not recommend by the Service, may not result in avoidance of impacts or take of the ABB. Trapping and relocating methods primarily follow the Service's "ABB Survey Guidance", but any captured ABBs are relocated elsewhere. The "ABB Survey Guidance" is available at http://www.fws.gov/southwest/es/oklahoma/beetle1.htm The following "Trapping and Relocating Guidance" takes precedence over conflicting guidance in the "ABB Survey Guidance".

Time

Trapping and relocating should be implemented during the ABB's prime active period in Oklahoma, May 20 to September 20. Refer to the Services "ABB Survey Guidance" for additional information.

Timing

Trapping and relocating should be implemented during the ABB's prime active period in Oklahoma, May 20 to September 20. Although the capture rates of ABBs are known to be higher during certain dates within their prime active period, the Service is not recommending restricting bait away implementation to specific dates during this prime active period. Refer to the Services "ABB Survey Guidance" for additional information.

Minimum Trap and Relocate Effort

To have confidence in avoiding impacts to ABBs, a minimum of 3 consecutive nights of trapping where no ABBs are captured is needed. Trapping and relocation more than 4 nights may be required if ABBs continued to be captured. Once 3 consecutive night's pass in which no ABBs are captured, the project/soil disturbance can commence. Once the area is disturbed (*i.e*., topsoil and vegetation cleared) it is assumed that the ABB would not be attracted to the disturbed area.

*Timeframe a Trap and Relocate Effort is Valid**

Trapping and Relocating Conducted for Projects Implemented During the **Active Period**:
Trapping and relocation efforts conducted during the ABBs active period are only valid for 5 days after the 3rd night of trapping in which no ABBs are captured. After 5 days have passed and the project soil disturbance has not commence additional trapping and relocation will be needed.

Trapping and Relocating Conducted for Projects Implemented During the **Inactive Period**:
As stated above, 3 consecutive trapping nights where no ABBs are captured is needed to avoid impacts to the ABB. The Service does not recommend conducting trap and relocation at the end of the ABBs active season as a means of removing ABBs from a project site so soil disturbance can commence during the ABBs inactive season. We do not recommend this because it is impossible to predict when or if 3 consecutive nights of trapping with no ABB captures will occur. It is possible that the ABBs inactive period will commence before 3 consecutive nights of no ABB captures is reached.

*Trap and Relocate Effective Radius**

The effective radius of a trap and relocate effort is dependent on the number of transects deployed. Each transect has an effective trapping radius of approximately 0.5 mile. Refer to the Services "ABB Survey Guidance" for additional information.

*Transect Spacing and Placement **
Transects should be spaced 0.5 mile apart on all projects and about 500 feet outside the perimeter of the project for both linear and non-linear, irregardless of the project size. Transects should be placed in a configuration that best represents the different habitat types present in the survey area and on the highest spots in the survey area.

Nonlinear projects with a radius equal to or less than 0.5 mile from the center to any given point along the perimeter should deploy transects outside the project boundary so ABBs are not lured into the project site.

For linear projects with a width equal to or less than 0.5 mile, transects should be deployed alternately along both sides of the projects long boundaries at 0.5 mile intervals. (Meaning one transect should be deployed on side A and then another transect deployed 0.5 linear mile away on side B, and so on). For linear projects with a width between 0.5 and 1 mile, transects should be deployed length-wise along both long sides of the project at 0.5 mile intervals.

There are some projects where the rights-of-way or areas outside the project boundary are unavailable or too small for transect deployment. In these cases, transects can be deployed at 0.5 mile intervals inside the project boundary so the entire project area is within the effective trapping area of at least one transect. Transects must be deployed in areas where no soil disturbance will occur or where soil disturbance has already occurred and will not be disturbed again.

If none of the above can be applied, then additional coordination with the Service is recommended.

Transect
Same as the Service's "ABB Survey Guidance".

Traps
Same as the Service's "ABB Survey Guidance".

Bait
Same as the Service's "ABB Survey Guidance".

Setting and Checking Traps
Same as the Service's "ABB Survey Guidance".

Disturbed bait or traps
Same as the Service's "ABB Survey Guidance".

Processing ABBs
All ABBs captured and relocated must be marked with a numbered, colored bee tag. No other type of marking is allowed unless specifically authorized by the Service, this include clipping of the elytra. Bee tags should be attached to the elytra with superglue gel (not liquid, this is too runny). Tagged ABBs must be monitored until glue dries (about 5 minutes) to ensure wings are not glued together and they are able to fly.

Holding and Transporting ABBs
ABBs to be held for transport must be confined in a hard plastic container. The container must contain a damp paper towel, meal worms for food, and puncture holes for air. Containers must be placed in a cooler with sufficient coolant to keep the temperature at approximately 60 to 65 degrees Fahrenheit. Each ABB should have 6 square inches of surface area. Each ABB should be kept in an individual container. Keep coolers out of the sun while in the field and during transport. During transport the cooler should be in air-conditioned vehicle. ABBs can only be held in this manner for 3 hours.

ABB Release
All relocations of ABBs must be coordinated and approved by the Service prior to initiating any trap and relocation effort. Release locations must have documented current occurrences of ABBs. Prior written approval from the

landowner must be obtained before ABBs can be released. Release ABBs should be provisioned with carrion at the release site. If the release occurs May or June the a male and female ABB should be paired and placed on a 200 gram carcass. If the release occurs after June then each individual ABB should be provided a piece of carrion. The size of carrion can be as small as 5-7 oz during this time period.

Reporting

The Service has prepared a standard '*Trapping and Relocating Form'* (Appendix 1). Use of this form ensures that all of the needed data is recorded. This form must be completed and submitted to the Oklahoma Service Field Office within 30 days of completing the relocation effort.

This form is to be completed for each trap and relocation effort for each night during the trap and relocation effort. The "*Trapping and Relocating Form*" must be completed in Excel. This is to be submitted electronically in excel file format to ABBcontact@fws.gov. The Service will then review the form and provide a response, via electronic mail, regarding our acceptance or non-acceptance of the bait away effort as sufficient.

If trap and relocation effort is conducted in compliance to the Endangered Species Act or the National Environmental Protection Act, project names and numbers need to correctly correspond. Each row in the spreadsheet should represent an individual bait station. All latitude and longitude data should be reported in decimal degrees and the coordinate system/projection should be in NAD 83. Only complete and accurate forms will be accepted. Incomplete and/or inaccurate forms will be returned and the trap and relocation effort will be considered invalid until the forms are corrected and/or properly completed, and submitted. When sending corrected forms, indicate that it is a correction, what specifically has been corrected, and the project name.

Accidental Death

Same as the Service's "ABB Survey Guidance".

Protocols and Forms

All forms can be downloaded from the Oklahoma Ecological Services Field Office's website <http://www.fws.gov/southwest/es/oklahoma/beetle1.htm>.

*Trap and relocation radius and validity, and transect placement and spacing are more restrictive for "ABB Trapping and Relocating Guidance" than the Service's "ABB Survey Guidance" because ABB surveys are only aimed at determining the presence or absence of ABBs. Whereas, trapping and relocating is aimed at removing all ABBs from the project area.*Transects and trap design should follow the Services "ABB Survey Guidance" dated April 6, 2005.

This guidance was developed from the U.S. Fish and Wildlife Service's July 14, 2005, "ABB Survey Guidance" and a U.S. Fish and Wildlife Service Working Group on May 6, 2004, and other meetings between Service personnel and permittees in March and April 2009. The Oklahoma Ecological Services Field Office, in coordination with other Field Offices, update this protocol as necessary due to new findings. The purpose of this guidance is to streamline and update American burying beetle trap and relocate recommendations among the Arkansas, Oklahoma, Kansas, and Arlington, Texas Field Offices. However, each state protocol may be different in some manners due to the land use and actions that occur in the different states. Each state Service office should be contacted for their most current protocols.

AMERICAN BURYING BEETLE RELOCATION DATA REPORTING FORM (Electr

PERMITTE INFORMATION			PROJECT INFORMATION:		
Endangered Species Permit #	Permittee Company:	Permittee:	Project Company:	Project Name:	Fish & Wildlife Section 7 Consultation #:

*Weather data refers to current conditions.
Old=breeding adult, born the previous year; Young=newly eclosed adult; Unknown=age or sex cannot be determi
Tag# refer to color and number of bee tag.

Updated 5-20-09

ronic)

	SUMMARY INFORMATION							
Project Size:	Trap and Relocation Start Date:	Trap and Relocation End Date:	3 Days With No ABB Captures, Start Date:	3 Days With No ABB Captures, End Date:	Date:	State:	County:	Landowner

ined.

CAPTURE DATA				CAPTURE
General Location:	Legal Description (township, range, section to quarter):		Lat-Decimal Degrees:	Long-Decimal Degrees:

DATA	CAPTURE DATA			
Projection:	Dominant Vegetative Species:	Primary Soil Type (Refer to County Soil Survey) :	Temp* (ºF):	Wind* (mph):
NAD83				

Cloud Cover* (%): Rain: yes or no	Date: State: County: Landowner: General Location:

RELEASE DATA	RELEASE DATA				RI
Legal Description (township, range, section to quarter):	Lat-Decimal Degrees:	Long-Decimal Degrees:	Projection:	Dominant Vegetative Species:	
			NAD83		

ELEASE DATA					LIST EACH ABB CA
					Tag# * Sex* Age*
Primary Soil Type (Refer to County Soil Survey) :	Temp* (ºF):	Wind* (mph):	Cloud Cover* (%):	Rain: yes or no	

PTURED AND TRANSLOCATED		
Time Captured	Time Released	Death (Y/N)