Use of PIT Tags to Monitor Survival and Dispersal of Freshwater Mussels Relocated to the Big South Fork National River and Recreational Area Kentucky and Tennessee

A Proposal

By:

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For:

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Purpose and Need

The National Park Service (NPS) is cooperating with the U.S. Fish and Wildlife Service (FWS) to pursue conservation actions to maintain and restore freshwater mussel populations to the free-flowing reach of the Big South Fork of the Cumberland River (BSF) within the BSF National River and Recreation Area (NRRA) and further the recovery of federally endangered mussels. Restoration activities include relocation, augmentation through captive propagation, and monitoring (Biggins et al. 2001). This effort is consistent with NPS Management Policies (2003) to restore extirpated native species to historical habitat and recover all endangered species that belong in the Park unit.

The BSF and its tributaries contain at least 25 mussel species, five of which are federally endangered. Historically the BSF contained 55 species, about half of which apparently have been extirpated from the watershed (NPS 2003; Ahlstedt et al. 2005). However, since the BSF was continuous with the rich mussel fauna of the Cumberland River, the BSF presumably contained as many as 70 mussel species (Gordon and Layzer 1989, Ahlstedt et al. 2005). After designation as a NRRA in 1974, the BSF was surveyed to assess aquatic biota. The impact of natural resource extraction was believed to be so severe that no mussels survived (Ahlstedt et al. 2005). Extraction of the area's natural resources increased pollutants in overland runoff, such as acid mine drainage and sediment, which are typically detrimental to mussel populations (Rikard et al. 1986). Ahlstedt et al. (2005) thoroughly surveyed the BSF and its tributaries for mussels from 1998 to 2002, and reported a total of 26 species. This survey and previous others have shown that even though this river is isolated from the main channel of the Cumberland River by a reservoir, the BSF is one of the best remaining refugia for freshwater mussels in the Cumberland River system (Ahlstedt et al. 2005).

Among the species found in the BSF are five federally endangered mussels: tan riffleshell (*Epioblasma florentina walkeri*), Cumberlandian combshell (*E. brevidens*), Cumberland bean (*Villosa trabalis*), Cumberland elktoe (*Alasmidonta atropurpurea*), and littlewing pearlymussel (*Pegias fabula*). All of these endangered species also occur in portions of the Tennessee River system, except the Cumberland elktoe, which is a Cumberland River endemic. Populations of the five endangered mussels in the BSF represent some of the largest remaining in the Cumberland system (Ahlstedt et al. 2005).

The NRRA is one of the first NPS holdings to be actively involved in freshwater mussel recovery. Plans are in place to reintroduce mussel populations in the NRRA, which involves relocating adults for release. If no adults of target species can be found within the BSF, adult mussels may be relocated from relatively stable populations elsewhere in the Cumberland or Tennessee rivers (Biggins et al. 2001). Successful monitoring of relocated mussels is essential for accurate assessment of reintroduction 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 and reintroduced 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 have been used in shallow waters to assess spatial distributions of local fish populations, fine-scale 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.

We propose to design 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 reintroductions of listed and non-listed mussel species into historical habitats of the Big South Fork of the Cumberland River. The objectives of our study are to evaluate the external PIT tagging method, retention, and post-tagging survival of freshwater mussels and to determine the effectiveness of PIT-tag technology for monitoring translocation success.

Kurth et al. (2007) studied the retention of external PIT tags and recapture of mussels in the field. Their recapture rates using PIT-pack searches with visual confirmation exceeded recaptures from visual searches alone at all study sites. They released mussels with external PIT tags at 3 study sites and recaptured them with a PIT pack 8 to 10 mo and 21 to 23 mo after release. Numbers of recaptured mussels differed among study sites; however, they 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 post-translocation monitoring. Retention of tags completely encapsulated with cement ranged from 89.5 to 100% (Kurth et al. 2007). Previous studies assessed external freshwater mussels tagging methods with visual searches to relocate mussels marked with numbered tags (Lemarie´ 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 the Kurth et al. (2007) 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. Additionally, if the water is turbid, PIT tags can locate burrowed mussels and mussels that would have been overlooked had the sites been searched only visually (Kurth et al. 2007).

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 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 non-detection 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 (Zydlewski, personal comment 2007). 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.

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) cost ~$10,000 to construct and is designed for research on a variety of organisms such as fish, mussels, and amphibians (Kurth et al. 2007). On the other hand, the percentage of tagged mussels recaptured using PIT tags can far exceed the percentage recaptured during visual searches (Kurth et al. 2007). 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 (Zydlewski personal comment 2007).

In conclusion, PIT tags permit repeated, nondestructive sampling of individuals with little disturbance, last indefinitely, and have negligible effects on short-term survival of freshwater mussels. The need for freshwater mussel translocations and reintroductions to protect and conserve threatened and endangered mussel species will increase as aquatic habitat restoration 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 relocated mussels and trends in population re-establishment at sites of concern, especially for endangered or threatened species (Kurth et al. 2007).

Methods and Materials

We will determine the efficacy of recapturing PIT-tagged mussels that are reintroduced within the Big South Fork NRRA using current technology. We will place external PIT tags on approximately 10,000 mussels (various federally listed and non-listed species) collected during 2008, 2009, and 2010 from populations outside the BSF. We will choose the outside water bodies because they have naturally occurring populations of targeted mussel species, and because, based on neutral markers, populations of these mussels are genetically similar to the species that once existed in the BSF (Biggins et al. 2001). We will tag mussels by cementing a PIT tag to the posterior end of the right valve, and completely encapsulate the PIT tag in dental cement to increase tag retention. We will place tagged mussels in water before the cement is fully cured (5 min after application) to avoid overdrying and cracking of the cement. We will be able to tag ~60 mussels/h with this method, and use 20-mm or 12-mm tags at all sites (Kurth et al. 2007).

We will estimate survival among treatments: mussels reintroduced from water bodies outside of BSF, those within the BSF, and within site of origin (control). We will measure, tag, and move mussels to 1 X 2-m plots or replace them where they are found in site. We will mark the corners of the plots with stakes with flagging, and record Global Positioning System (GPS) locations for each reintroduction plot and for each of the

tagged mussels that are returned to their original location. Adjusted χ^2 analysis will be used for small sample sizes. We will calculate recapture rates by dividing the number of mussels recaptured at each site by the number tagged (Strayer and Smith 2003).

We will recapture externally PIT-tagged mussels with a mobile PIT detection unit (PIT pack). The PIT pack Destron Fearing FS1001A DC-powered, full duplex transceivers and custom-designed portable antennas will be used. When a PIT tag is within range of an antenna (~0.5 m), the tag will emit a 134.2-kHz (ISO standard frequency) radio frequency, which is transmitted back to the receiver for decoding. The antennas, enclosed in an airtight PVC wand and attached to the transceiver, consist of several wraps of 12- to 18-gauge wire, with inductance values ranging from 325 to 375 lH and a set of capacitors (Hill et al. 2006). The capacitors are attached to an antenna lead cable from the transceiver, fixing the capacitance between 33 and 44 nF. The fixed capacitance is 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 will tune the adjustable capacitor while antennas are submerged, and conduct field experiments with the PIT pack tuned to phase 0 to 2%, signal 1 to 20%, and current 2.5 to 5.0 amps (Kurth et al. 2007).

The reintroduction sites for externally PIT-tagged mussels will be searched 30 d after tagging and visually confirm recaptures with snorkeling. If the PIT-tag reader registers a tag but no mussel is observed, we will assume the mussel has burrowed into the substrate. To minimize substrate disturbance, we will not excavate burrowed mussels preparing to overwinter. These data will not be used in the calculations of recapture success because the signals may be from detached tags. During 2009 and 2010, we will search again for PIT-tagged mussels at the reintroduction sites, beginning at the last location recorded with GPS during 2008. In 2009, we will conduct searches of the reintroduction sites using PIT-pack searches with visual confirmation and excavation to confirm recaptures (3–4 d/site). In 2010, we will repeat the PIT pack searches with visual confirmation (3-4 d/site).

Water clarity or flow levels may restrict visual searches in the BSF. If the PIT pack detects a tagged mussel, but we do not see the mussel, we will excavate the area within 0.5 m of the signal to 15 to 45 cm deep to determine if the signal is coming from a burrowed mussel or an unattached tag. If we find no tagged mussel after excavation, we will assume the tag has become detached. We will search (with snorkeling and the PIT pack) the sites at BSF 4 times each to at least 10 m beyond the perimeter of the original study area to detect mussels that may have moved. We will also search the shorelines for valves of dead mussels. Extensive scouring and spring flooding may substantially reconfigure the substrate at the BSF sites, so in addition to searching the study area plus 10 m beyond the perimeter, we will also sweep the antenna bank to bank downstream of the site for 100 m over a total of 3 d.

Expected Results

This monitoring effort will promote recovery of federally endangered mussel species in the Big South Fork of the Cumberland River, a unit of the National Park Service and a refugium for Cumberland River mussel fauna. The project additionally supports the Memorandum of Agreement between the USFWS, USGS, and the states of Tennessee and Alabama, and is in direct support of the USFWS and NPS recovery goals

for the species found within the BSF National Park and the Big South Fork river system in TN and KY.

Technology/Information Transfer

The information obtained from this project will aid conservation efforts with other NPS researchers, Tennessee Wildlife Resources Agency, Kentucky Fish and Wildlife, as well as mussel recovery activities of USGS, USFWS, and the Virginia Department of Game and Inland Fisheries. The knowledge obtained will be available to other researchers in annual and final reports, a technically refereed publication, and biologists working with related taxa would greatly benefit from this research.

Work Schedule

Total project duration is 36 months; February 1, 2008 – December 31, 2010

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Budget

Option 1: **TOTAL BUDGET: PIT Tags = 10,000**

PERFORMANCE PERIOD: 36 MONTHS

PERSONNEL		
Name/Position		Period
Melissa Petty/Research Specialist		$
TOTAL PERSONNEL SALARIES	$	
FRINGE BENEFITS		
Reg Fac 31.25% - Res Fac 32.25%	$0	
SMR/Wages 8.25%		$0
GRA 2.5%		
Classified 36.75%		$
TOTAL FRINGE BENEFITS	$	
TOTAL SALARIES & FRINGES	**$**	
TRAVEL		
Fieldwork		$
Conference/Symposium	x3	$
TOTAL TRAVEL COSTS	**$**	
MATERIALS & SUPPLIES		
FS1001A Reader/Transceiver	x2	$17,000
PIT pack, antennas, accessories	x2	$1,200
PIT tags ($3.85/ea)	x10,000	$38,500
TOTAL MATERIALS & SUPPLIES	**$56,700**	
TECHNICAL SERVICES		
Summer Field Technician	x1	$
	$	
TOTAL COSTS	**$**	
INDIRECT COSTS		
Overhead 50%	**$**	
FINAL TOTAL COSTS	**$**	

Option 2: **TOTAL BUDGET: PIT Tags = 7,000** and **Magnetic Tags = 3,000**

PERFORMANCE PERIOD: 36 Months

PERSONNEL		
Name/Position		Period
Melissa Petty/Research Specialist		$
TOTAL PERSONNEL SALARIES	$	
FRINGE BENEFITS		
Reg Fac 31.25% - Res Fac 32.25%	$0	
SMR/Wages 8.25%		$0
GRA 2.5%		
Classified 36.75%		$
TOTAL FRINGE BENEFITS	$	
TOTAL SALARIES & FRINGES	**$**	
TRAVEL		
Fieldwork		$
Conference/Symposium	x3	$
TOTAL TRAVEL COSTS	**$**	
MATERIALS & SUPPLIES		
FS1001A Reader/Transceiver	x2	$17,000
PIT pack, antennas, accessories	x2	$1,200
PIT tags ($4.25/ea)	x7,000	$29,750
Magnets ($0.10/ea)	x3,000	$300
Magnet Reader/Gradiometer	x2	$1,780
TOTAL MATERIALS & SUPPLIES	**$50,030**	
TECHNICAL SERVICES		
Summer Field Technicians	x3	$
	$	
TOTAL COSTS	**$**	
INDIRECT COSTS		
Overhead 50%	**$**	
FINAL TOTAL COSTS	**$**	

Year 1: **ANNUAL BUDGET: PIT Tags = 2,000** and **Magnetic Tags = 1,000**

PERFORMANCE PERIOD: 12 Months

PERSONNEL		
Name/Position		Period
Melissa Petty/Research Specialist		$
TOTAL PERSONNEL SALARIES	$	
FRINGE BENEFITS		
Reg Fac 31.25% - Res Fac 32.25%	$0	
SMR/Wages 8.25%		$0
GRA 2.5%		
Classified 36.75%		$
TOTAL FRINGE BENEFITS	$	
TOTAL SALARIES & FRINGES	**$**	
TRAVEL		
Fieldwork		$
Conference/Symposium	x1	$
TOTAL TRAVEL COSTS	**$**	
MATERIALS & SUPPLIES		
FS1001A Reader/Transceiver	x2	$17,000
PIT pack, antennas, accessories	x2	$1,200
PIT tags ($4.25/ea)	x2,000	$8,500
Magnets ($0.12/ea)	x1,000	$120
Magnet Reader/Gradiometer	x2	$1,780
TOTAL MATERIALS & SUPPLIES	**$28,600**	
TECHNICAL SERVICES		
Summer Field Technicians	x1	$
	$	
TOTAL COSTS	**$**	
INDIRECT COSTS		
Overhead 50%	**$**	
FINAL TOTAL COSTS	**$**	

Year 2: **ANNUAL BUDGET: PIT Tags = 5,000** and **Magnetic Tags = 1,000**

PERFORMANCE PERIOD: 12 Months

PERSONNEL		
Name/Position		Period
Melissa Petty/Research Specialist		$
TOTAL PERSONNEL SALARIES	$	
FRINGE BENEFITS		
Reg Fac 31.25% - Res Fac 32.25%	$0	
SMR/Wages 8.25%		$0
GRA 2.5%		
Classified 36.75%		$
TOTAL FRINGE BENEFITS	$	
TOTAL SALARIES & FRINGES	**$**	
TRAVEL		
Fieldwork		$
Conference/Symposium	x1	$
TOTAL TRAVEL COSTS	**$**	
MATERIALS & SUPPLIES		
PIT tags ($4.25/ea)	x5,000	$21,250
Magnets ($0.12/ea)	x1,000	$120
TOTAL MATERIALS & SUPPLIES	**$21,370**	
TECHNICAL SERVICES		
Summer Field Technicians	x1	$
	$	
TOTAL COSTS	**$**	
INDIRECT COSTS		
Overhead 50%	**$**	
FINAL TOTAL COSTS	**$**	

Year 3: **ANNUAL BUDGET: Magnetic Tags = 1,000**

PERFORMANCE PERIOD: 12 Months

PERSONNEL		
Name/Position		Period
Melissa Petty/Research Specialist		$
TOTAL PERSONNEL SALARIES	$	
FRINGE BENEFITS		
Reg Fac 31.25% - Res Fac 32.25%	$0	
SMR/Wages 8.25%		$0
GRA 2.5%		
Classified 36.75%		$
TOTAL FRINGE BENEFITS	$	
TOTAL SALARIES & FRINGES	**$**	
TRAVEL		
Fieldwork		$
Conference/Symposium	x1	$
TOTAL TRAVEL COSTS	**$**	
MATERIALS & SUPPLIES		
Magnets ($0.12/ea)	x1,000	$120
TOTAL MATERIALS & SUPPLIES	**$120**	
TECHNICAL SERVICES		
Summer Field Technicians	x1	$
	$	
TOTAL COSTS	**$**	
INDIRECT COSTS		
Overhead 50%	**$**	
FINAL TOTAL COSTS	**$**	