



Threatened and Endangered Fish Translocation to and from Abrams Creek, Citico Creek and Tellico River, Great Smoky Mountains National Park and Cherokee National Forest

Implementation and Genetic Monitoring Plan

Natural Resource Report NPS/GRSM/NRR—2015/930



**ON THIS PAGE**

Supervisory Fishery Biologist Matt Kulp performing snorkel surveys for Citico Darter in Abrams Creek, Great Smoky Mountains National Park

Photo by: Caleb Abramson, National Park Service.

ON THE COVER

Citico darter in Abrams Creek, Great Smoky Mountains National Park

Photograph by: Caleb Abramson, National Park Service.

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Executive Summary

The construction of dams for hydroelectric power fragmented the lower Little Tennessee River and its tributaries beginning in 1919. Several small fish, now protected under the Endangered Species Act (ESA), are known from lower Abrams Creek and Citico Creek, tributaries to the Little Tennessee River, and the Tellico River. Although the new Federal Energy Regulatory Commission (FERC) license mandated a Fish Passage Plan to facilitate genetic mixing of Citico darter, Smoky Madtom, Yellowfin Madtom and Spotfin Chub among Citico Creek, Abrams Creek and Tellico River, the plan did not provide procedural details or logistical considerations to implement the plan. This Implementation and Monitoring plan will provide more detailed roles and responsibilities of those conducting and overseeing implementation of the Fish Passage Translocation Plan, including translocation logistics, responsibilities, timing, target sizes and numbers for each species, prophylactic disease prevention protocols, annual costs and guidance on evaluating translocation success over time.

The goals of this Implementation and Monitoring Plan are to ensure an empirical basis for fish passage for four federally listed fishes within the Little Tennessee River watershed that will allow for their present and future preservation. Second is to outline more detailed translocation procedures for the Citico Darter, Smoky Madtom, Yellowfin Madtom and Spotfin Chub in order to: 1) implement the fishway outlined in the Tapoco Project's Fish Passage Translocation Plan and 2) conserve and augment genetic diversity for each target species. The project Licensee is responsible for funding translocations between Abrams and Citico Creek. Translocations are generally performed by contract using the guidelines outlined herein.

Genetic analysis of the Abrams and Citico Creek populations indicate that approximately a 5% migration rate per generation (e.g., $0.05 \times$ an effective population size (N_e) of $75 \approx$ four individuals per generation) is necessary to offset the influence of genetic drift over the course of 50 generations (approximately 100-150 years given the species of concern). Therefore, in order to successfully achieve the goal of genetic mixing for Citico and Abrams Creek populations, the introduction of at least two adult Smoky Madtoms, two adult Citico Darters, and one adult Yellowfin Madtom annually will be necessary. The 5% migration rate per generation is a genetic target that will be monitored over time. Other genetic targets include estimation of the number of loci or sample size (N) necessary to provide accurate estimation of effective population size (N_e) for each population, and establishment of baseline genetic data for Tellico populations of each species. Genetic goals will be reevaluated over time and an adaptive management approach will be used to determine what changes can be made to successfully meet the 5% migration rate.

The formalization of this multi-agency plan ensures that all state, federal and private entities have a common set of goals and objectives. The plan also outlines a uniform set of procedures to ensure the species translocation not only meet plan goals but also adhere to NPS and USFWS policies and mandates.

Acknowledgments

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Introduction/Background

History of Little Tennessee River System Impoundments: The construction of dams for hydroelectric power has fragmented the lower Little Tennessee River and its tributaries since 1919. Several small fish, now protected under the Endangered Species Act (ESA), are known from lower Abrams Creek and Citico Creek, tributaries to the Little Tennessee River, and the Tellico River (Figure 1). Fish populations in these areas are now separated by several dams and reservoirs. Prior to the construction of reservoirs on the main stem of the Little Tennessee River, no physical barriers prevented the movement of these fishes among Abrams Creek, Citico Creek and the Tellico River. Although some species have a naturally small range, the extent of this range is generally dictated by natural barriers, extreme habitat specialization, inter-specific competition, body size, or a combination of these factors. Most stream fish species likely evolved within a larger natural range that exchanged individuals, even if only periodically (Jenkins and Burkhead 1994).

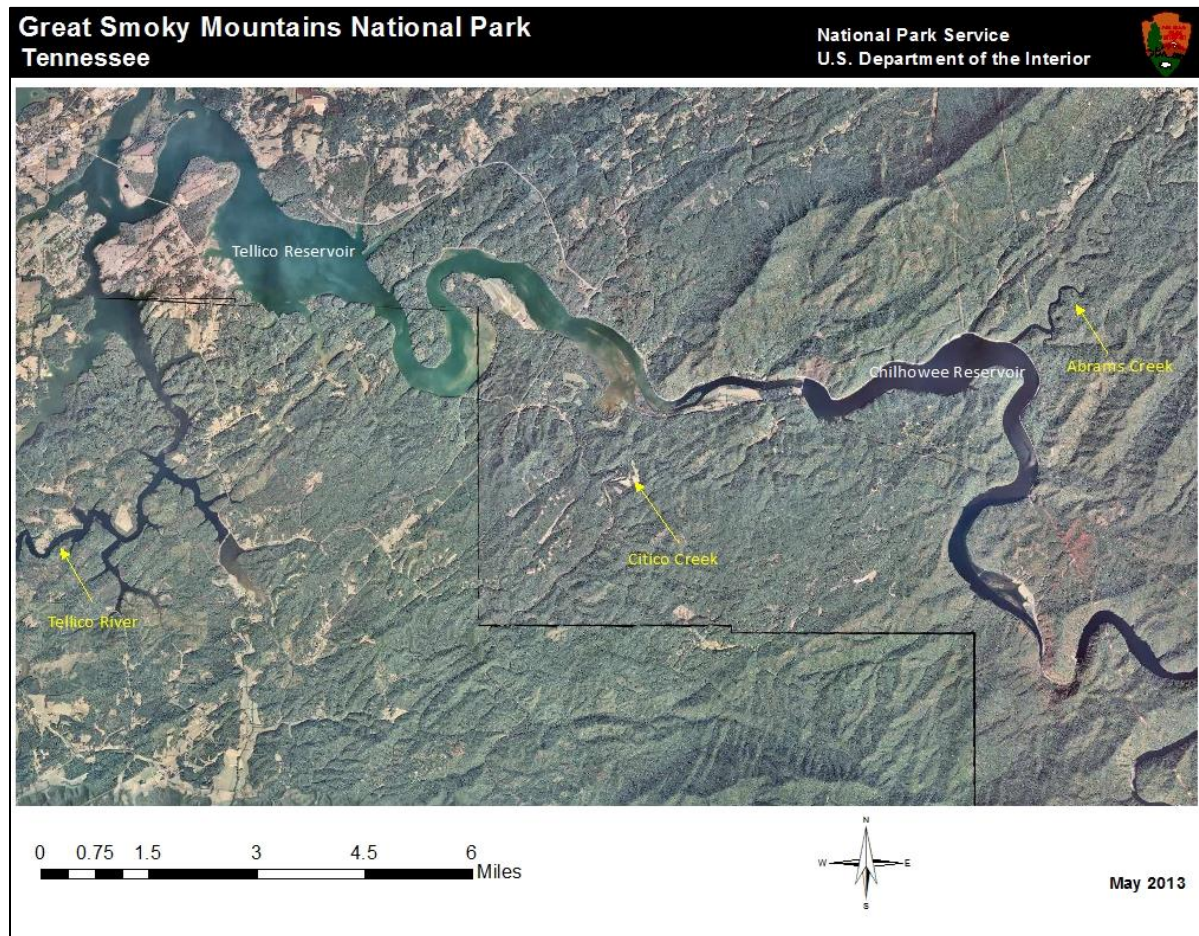
The remaining undammed and unregulated reaches above impoundments within the system include three large tributaries; Abrams Creek (19.4 km), Citico Creek (14.5 km) and Tellico River (22.4 km) (Figure 1). Abrams Creek lies entirely within Great Smoky Mountains National Park (GRSM), while Tellico River and Citico Creek are found within the Cherokee National Forest. The Tellico River is a Little Tennessee River tributary just downstream from the mouths of Abrams and Citico Creeks (all four fishes historically occurred in these creeks) and all three streams drain the same physiographic provinces (Blue Ridge and Ridge and Valley). These reaches are important focal areas for the recovery of the endangered Smoky Madtom (*Noturus baileyi*), endangered Duskytail Darter (*Etheostoma percnurum*) (renamed Citico Darter *Etheostoma sitikuense*¹), threatened Yellowfin Madtom (*Noturus flavipinnis*), and threatened Spotfin Chub (*Erimonax monachus*).

In 1957, U.S. Fish and Wildlife Service (USFWS) biologists, in cooperation with several state and federal agencies, utilized rotenone to extirpate 46 fish species, including four species now federally listed (see *History of Fish Restoration* below), from the lower 23.5 kilometers (km) of Abrams Creek. The rotenone treatment, in conjunction with impoundment of the Little Tennessee River by Chilhowee Dam, was an attempt to establish a trophy Rainbow Trout (*Oncorhynchus mykiss*) fishery (Lennon and Parker 1959). Application of the piscicide removed all native fish from the main stem of lower Abrams Creek (Lennon and Parker 1959), formerly the most diverse stream in GRSM (Simbeck 1990). The creation of Chilhowee Reservoir effectively isolated Abrams Creek from

¹ Taxonomic Note: Blanton and Jenkins [Blanton, R.E. and R.E. Jenkins. 2008. Three new darter species of the *Etheostoma percnurum* species complex (Percidae, subgenus *Catonotus*) from the Tennessee and Cumberland river drainages *Zootaxa* 1963:1-24.] analyzed variation in morphology including meristics, morphometrics, and pigmentation among the four extant populations and limited specimens from the two extirpated populations of the federally endangered Duskytail Darter, (*Etheostoma percnurum*). These analyses found each of the extant populations is morphologically diagnosable. The Citico Creek, Abrams Creek, and Tellico River (Tennessee River system) were described as *Etheostoma sitikuense*, the Citico Darter. So, fish previously referred as the duskytail darter (*Etheostoma percnurum*) at Citico Creek, Abrams Creek, and Tellico River in the vicinity of the Tapoco Hydroelectric Project, have now been re-described as the Citico darter (*Etheostoma sitikuense*).

recolonization by many naturally occurring species including Spotfin Chubs, Citico (Duskytail) Darter, Smoky and Yellowfin madtoms. Afterwards, Smoky Madtom were presumed to be extinct until discovery in 1980 of a population in nearby Citico Creek, Monroe County, TN (Bauer *et al.* 1983). The Citico Creek population is still the only known naturally occurring population for Smoky Madtom. The Smoky Madtom was added to the endangered species list, under the ESA, in October 1984 (49 FR 43065) and the Yellowfin Madtom was added to the threatened list in 1977 (42 FR 45527).

Figure 1. Map of Little Tennessee study area including three tributary streams (i.e. Tellico River, Citico Creek and Abrams Creek) included in fish translocation plan.



Initial Steps Towards Restoration: As required by the ESA, the USFWS developed federal recovery plans for Citico Darter (Biggins and Shute 1994), Spotfin Chub (Boles 1983), Smoky Madtom (Biggins 1985) and Yellowfin Madtom (Biggins 1983) to outline actions needed to remove the species' from the endangered species list. A recovery objective of each of the four plans was to establish viable populations of each species within their historical range, including Abrams Creek, Citico Creek and Tellico River.

Abrams Creek Fish Restoration: Beginning in 1986, the USFWS and Tennessee Wildlife Resources Agency (TWRA) contracted with Conservation Fisheries, Inc. (CFI) of Knoxville, Tennessee, to

propagate Smoky Madtom, Yellowfin Madtom and Citico Darters (beginning in 1992) for reintroduction to lower Abrams Creek using Citico Creek source stock (Rakes and Shute 2007). From 1994 through 2001, Spotfin Chub adults were collected from various sources, propagated at CFI and transplanted into Abrams Creek (Shute et al. 2005). Efforts to restore Citico Darters continued until 2001 and restoration stockings of both madtom species continued through 2002 (Shute et al. 2005). During these years, egg masses of both madtom species were collected from Citico Creek, propagated and grown out to sub-adult size at the CFI propagation facility (Rakes et al. 1999). Once at a desirable size, the sub-adults were transplanted into various release sites deemed to have good habitat along Abrams Creek. Citico Darters were captured from Citico Creek and propagated at the CFI facility, but the bulk of production was from nest eggs collected from the creek and reared at CFI (Shute et al. 2005). Total numbers of Smoky Madtom, Yellowfin Madtom, Citico Darters and Spotfin Chub propagated and stocked into Abrams Creek since 1986 were 3,425, 1,789, 3,732 and 10,730 respectively (Table 1) (CFI unpublished data). All Citico Darters, Smoky and Yellowfin Madtoms stocked into Abrams Creek after 2002 were for research (Miller 2011) and/or fish passage (Smet 2005) purposes.

Table 1. Number of individual fish of various species stocked into Abram Creek, Great Smoky Mountains National Park between 1986 and 2012 by Conservation Fisheries, Inc.

Year	Smoky Madtoms	Yellowfin Madtoms	Citico Darters	Spotfin Chubs
1986	0	18		
1987	92	115		
1988	118	155		
1989	174	90		
1990	151	0		
1991	134	0		
1992	0	0		
1993	52	0	85	
1994	38	26	51	700
1995	166	94	118	1,200
1996	116	0	667	0
1997	438	0	396	0
1998	116	61	216	3,500
1999	369	247	203	3,350
2000	644	365	0	500
2001	266	87	1,694	1,480
2002	315	286	0	
2003	16	32	0	
2004	15	0	0	
2005	49	56	0	
2006	8	8	0	
2007	0	0	0	
2008	0	0	0	
2009	100	100	204	
2010	48	49	98	
2012	0	0	0	
Total:	3,425	1,789	3,732	10,730

Recent studies (Rakes and Shute 2007a; Gibbs 2008; Throneberry 2008; Miller 2011) indicate self-sustaining (viable), naturally reproducing populations of Citico Darter, Smoky Madtom and Yellowfin Madtom have been established in portions of their historical range in lower Abrams Creek. Spotfin Chub were not observed during recent studies within lower Abrams Creek and have been determined to be extirpated (Gibbs 2008; Miller 2011). Gibbs (2008) determined that Abrams Creek cannot support a viable population of Spotfin Chub due to: 1) the loss of downstream connectivity to a larger mainstem river system, and 2) a drainage area (225 km²) well below the range of other higher order watersheds supporting Spotfin Chub populations (626 - 1973 km²). Therefore, attempts to transplant Spotfin Chub back into Abrams Creek were terminated in 2008.

Throneberry (2008) estimated recovery (or carrying capacity) of Smoky and Yellowfin Madtom populations within Abrams Creek to be 50% and 58% based upon occupancy of stream reaches with sufficient macrohabitat. Smoky and Yellowfin Madtoms are known to populate the lowest 17.4 km of Abrams Creek (Throneberry 2008; Miller 2011). The Citico darter was determined to be 100% recovered based upon occupancy of habitat with sufficient macrohabitat within sampled reaches of Abrams Creek, but the species only inhabits a 4-km section the creek, which begins 9.6 km upstream from Chilhowee Reservoir (Gibbs 2009).

Citico Creek Restoration: The Cherokee National Forest, USFWS, and TWRA have contracted with CFI to monitor Smoky Madtom and Yellowfin Madtom since 1986 and Citico darter since 1992 (Shute *et al.* 2005). While conducting population monitoring, CFI has collected nests to propagate these three species for releases into Abrams Creek and Tellico River. CFI has augmented (released young reared from harvested nests) Yellowfin Madtom and Citico Darters in Citico Creek to facilitate passage around a lowhead dam in the creek (CFI unpublished data) (Table 2). The effort to expand the range of the Yellowfin Madtom below the lowhead dam was deemed unsuccessful (CFI unpublished data).

Table 2. Number of individual fish of various species stocked into Citico Creek, Cherokee National Forest between 1990 and 2012 by Conservation Fisheries, Inc.

Year	Yellowfin Madtoms	Citico Darters
1990	74	
1993	101	
1996	106	16
1999	78	
2000	40	
2010		321
2011		150
2012		112
Total:	399	599

Tellico River Restoration: Based upon the success in Abrams Creek and the suitability of available habitat, on August 12, 2002, the USFWS published a Final rule, pursuant to 50 CFR Part 17, that established "Nonessential Experimental Population Status" and reintroduction of four fishes (Smoky Madtom, Yellowfin Madtom, Citico Darter, and Spotfin Chub) in the Tellico River (USFWS FR

67:52420-52428). These reestablished populations are classified as nonessential experimental populations (NEPs) in accordance with section 10(j) of the ESA, as amended. Based on an evaluation by species experts, none of these species were known to exist in this river reach or its tributaries (Etnier and Starnes 1993). These reintroductions are recovery actions and are part of a series of reintroductions and other recovery actions that the USFWS, Federal and State agencies, and other partners are considering and conducting throughout the species' historic ranges. This rule provides a plan for establishing the NEPs and provides for limited allowable legal taking of the four fishes within the defined NEP area.

Reintroduction methods for the four Tellico River fishes were similar to those noted above for Abrams Creek (Table 3) (CFI unpublished data). The USFWS and TWRA contracted with CFI to propagate for reintroduction to Tellico River using Citico Creek source stock (Petty *et al.* 2013). Recent studies (Petty *et al.* 2013) indicate naturally reproducing populations of all four species have been established in portions of Tellico River.

Table 3. Number of individual fish of various species stocked into Tellico River, Cherokee National Forest between 2002 and 2012 by Conservation Fisheries, Inc.

Year	Smoky Madtoms	Yellowfin Madtoms	Citico Darters	Spotfin Chubs
2002	0	0	0	2,780
2003	226	154	490	1,261
2004	196	204	317	1,520
2005	414	186	480	602
2006	148	84	490	3,017
2007	277	419	510	1,460
2008	312	379	603	2,400
2009	351	413	365	2,524
2010	201	96	292	698
2011	291	156	325	977
2012	130	130	701	1,246
Total:	2,546	2,221	4,573	18,485

Ramifications of Isolation: Isolation of local populations may pose threats to the long-term viability of a species, including: 1) increased probability of local extinctions due to demographic and environmental stochasticity (Levins 1969), 2) loss of gene flow and genetic variability, with an accompanying risk of genetic erosion (Scudder 1989, Riddell 1993), 3) loss of large numbers of immigrants from reproductive source areas that sustain reproductive sinks (Sheldon 1987, Dennis *et al.* 1991, Rieman and McIntyre 1995), 4) loss of overall range and outlying reaches that may contain refugia or other resources (Larimore *et al.* 1959, Cunjak 1988, Fausch and Young 1995, Matheney and Rabeni 1995), and 5) barriers to re-colonization after local extinctions have occurred (Sheldon 1987, Dennis *et al.* 1991, Rieman and McIntyre 1995; Fausch *et al.* 2009). In fragmented stream systems, it is critical to re-establish or enhance existing connectivity between sub-populations to increase the likelihood of species viability (Hanski and Gilpin 1991, Schlosser and Angermeier 1995).

The establishment of Chilhowee Reservoir has impeded the natural movement of the four target species between Citico and Abrams Creek, thereby eliminating the exchange of genetic material between populations since 1959. Due to the lack of exchange genetic of material between Abrams Creek and Citico Creek, in 2005, Alcoa Power Generating Inc. (APGI) was required by the Federal Energy Regulatory Commission (FERC) to develop a *Fish Passage Translocation Plan* (Smet 2005) to mitigate the exchange of genetic material. The plan recommends that for each of the three target species, “*one effective genome per generation*” should be “passed” between the Citico and Abrams Creek (and eventually Tellico River) populations (recommended 100 per generation for spotfin chub). In order to facilitate this genetic exchange, the APGI (now purchased and FERC responsibilities assumed by Brookfield Smoky Mountain Hydropower, LLC) provides \$10,000 annually for federal and state agencies and NGO’s working to restore these fish species (Smet 2005). These funds have been used to propagate individuals of each species from Citico Creek stock, continue reintroductions into Tellico River and facilitate gene flow between Citico and Abrams Creeks from 2005 to present. This effort is anticipated to continue for the duration of the FERC license through year 2043. Periodic transplants of each species into Abrams Creek will continue until new genetic data provides guidance for alternative management, methodology or policies.

In addition to concerns regarding the lack of gene flow between systems, the loss of genetic diversity due to artificial propagation practices are also of concern. It is important to ensure that translocation of the newly established Abrams Creek and Tellico River populations have and continue to have similar levels of genetic diversity when compared to the donor population (in this case Citico Creek; see Miller and Kapuscinski (2003) for review). To ensure that genetic diversity is maintained over time and is similar among populations of each species, genetic monitoring will be conducted so that genetic changes can be detected in a timely manner permitting adaptive management practices to avoid or minimize further loss of genetic variation in each population.

FERC License & Translocation Plan

The Cheoah, Calderwood, and Chilhowee dams on the Little Tennessee River are developments of the Brookfield Smoky Mountain Hydro Project (formerly Tapoco) and are privately owned and licensed by FERC to produce hydroelectric power. A new license for the project is in effect from 2005 - 2045, subject to conditions for operation, maintenance, and mitigation of environmental effects. The license for the Tapoco Hydroelectric Project, issued originally to APGI, was transferred to Brookfield Smoky Mountain Hydropower LLC (SMH) on November 15,

2012. Brookfield accepted all the terms and conditions of the License to maintain and operate Project No. P-2169. The Tapoco Hydroelectric Project is now known as Brookfield Smoky Mountain Hydro (BSMH) (143 FERC ¶ 62,156).

Statutory Authority: Section 18 of the Federal Power Act (FPA), 16 USC § 811, states in part: “*The Commission shall require the construction, maintenance, and operation by a licensee., such fishways as may be prescribed by the Secretary of Commerce or the Secretary of Interior.*”

Section 1701(b) of the National Energy Policy Act of 1992, P.L. 102-486, Title XVII, § 1701(b), 106 StaL 3008, states: “*The items which may constitute a 'fishway' under section 18 [16 USC § 811] for*

the safe and timely upstream and downstream passage of fish shall be limited to physical structures, facilities, or devices necessary to maintain all life stages of such fish, and project operations and measures related to such structures, facilities, or devices that are necessary to ensure the effectiveness of such structures, facilities, or devices for such fish."

In 2005, the Prescription for Fishways at the SMH Project was issued under authority delegated to the Southeast Regional Director of the USFWS from the Secretary of the Interior; the Assistant Secretary for Fish, Wildlife and Parks; and the Director of the USFWS pursuant to section 18 of the FPA.

FERC Issues a New License: On January 25, 2005, the FERC issued an order approving settlement and a new 40 year license for the hydroelectric project (FERC No. 2169, 110 FERC ¶ 61,056). Fifty years after the construction of Chilhowee Dam, a new license was issued for continued operation. Under the FPA, USFWS prescribed the construction, operation, and maintenance of a fishway by the licensee. The Secretary of Interior, through the USFWS, developed the fishway prescription for the Tapoco Project, which includes specific measures for passage of the four federally-listed fish species discussed above at the Chilhowee Development. These measures are also consistent with the terms of a larger settlement agreement that resolved some environmental and other issues for operation of the project dams for a 40-year term. The terms of the fishway prescription are in effect through the license term of February 28, 2045. Article 401 of the license required the Licensee to file a Fish Passage Translocation Plan (FPTP) for four specific "target species" that now occur in the vicinity of the Project's Chilhowee Development within 6 months of the effective date of the license (or September 1, 2005). Article 401 of the license states that it: *"requires the Licensee to prepare a plan in accordance with the U.S. Department of the Interior's prescription [Prescription] of fishways under Section 18 of the Federal Power Act."* By order dated August 22, 2006, FERC modified and approved the FPTP, which is a requirement of the license (110 FERC ¶ 61,056 USA, FERC).

Tapoco Hydroelectric Project (FERC No. 2169) Fishway Prescription - General Terms and Conditions for Fishways: To ensure the immediate and timely contribution of any fishway to the Upper Tennessee River fish restoration effort, the following measures were included and shall be incorporated into the License to ensure the effectiveness of the fishway pursuant to Section 1701(b) of the 1992 National Energy Policy Act (P.L. 102-0486, Title XVII, 106 Stat. 3008).

- a) A fishway shall be developed, operated, and maintained to provide effective (safe, timely, convenient) passage for Spotfin Chub (*Erimonax* (formerly *Cyprinella*) *monachus*), Yellowfin Madtom (*Noturus flavipinnis*), Smoky Madtom (*Noturus baileyi*), and Citico Darter (*Etheostoma sitikiense*) between Citico Creek and Abrams Creek, and between Tellico River and Abrams Creek.
- b) The populations to be passed are those occurring at tributaries to the Little Tennessee River, including Abrams Creek (trib. at LT RM 37), Citico Creek (trib. at LT RM 31.8) and Tellico River (trib. at LT RM 19.2). The population of spotfin chubs at the Little Tennessee River (>LT RM 88.5) is also a source for augmentation of the populations.

- c) The design population to be “passed” between each of the three designated rivers for each target species is:

Target Species	Fishway Exchange
Spotfin Chub	100 per generation
Yellowfin Madtom	1 effective genome/generation
Smoky Madtom	1 effective genome/generation
Duskytail Darter	effective genome/generation

- d) The fishway identified in Article 401, as set forth in Part II (2) below, shall be fully operational at the Chilhowee Development as soon as possible but no later than 6 months after the effective date of the new license so that continuing impacts of the Project may be mitigated and benefits of passage realized as soon as practicable.

The Licensee has developed a Fish Translocation Plan (appendix A), on file with FERC, as required by the License. The plan included translocation of target species’ (individuals) to/from Citico and Abrams Creeks with the intent that genetic mixing (i.e., connectivity and gene flow) between populations would occur. If genetic mixing was occurring on a per generation basis, then we would expect a high level of genetic similarity between Citico and Abrams populations for each species.

The National Park Service (NPS) has taken the initiative in developing this plan in order to ensure that NEPA guidelines outlining the review of major actions on NPS lands were being met. The formalization of this multi-agency plan ensures that all state, federal and private entities have a common set of goals and objectives. The plan also outlines a uniform set of procedures to ensure the species translocation not only meet plan goals, but also adhere to NPS and USFWS policies and mandates.

There are two goals to this plan. First, is to ensure an empirical basis for fish passage for four federally listed fishes within the Little Tennessee River watershed that will allow for their present and future preservation. Second is to outline a detailed translocation implementation plan for the Citico Darter, Smoky Madtom and Yellowfin Madtom in order to: 1) implement the fishway outlined in the Tapoco Project’s Fish Passage Translocation Plan and 2) conserve and augment genetic diversity for each target species.

Study Areas

Abrams Creek is a fifth order tributary of the Little Tennessee River (enters at river mile (rmi) 37, river kilometer (rkm) 59) that flows almost entirely within the western portion of GRSM, Blount County, TN (Figure 1). Abrams Creek drains a watershed of 198 km² that encompasses approximately 348 km of streams (Parker and Pipes 1990) and terminates at Chilhowee Reservoir, an impoundment of the Little Tennessee River. The lower 24 km reach of Abrams Creek extends from Abrams Falls to an embayment of Chilhowee Reservoir. Due to its fertility, size, and low gradient, Abrams Creek was once the most diverse stream in GRSM, containing a fish community including about half of the 68 GRSM fish species (Simbeck 1990).

Citico Creek is a fourth order tributary entering the Little Tennessee River at rmi 31.8 (rkm 51.2). The Citico Creek watershed drains an area of roughly 186 km², most within the Cherokee National Forest (CNF) in Monroe County, TN. Much of the headwaters of the Citico Creek watershed are designated Wilderness. In 1985, a 10.5 km reach of Citico Creek was designated a critical habitat for Smoky madtoms (49 FR 43065; Biggins 1985), as organisms existing over such a small range are considered to be at increased risk of extinction. Citico Creek is geographically isolated from Abrams Creek by Chilhowee and Tellico Reservoir's, as well as Chilhowee Dam, which was completed in conjunction with the Abrams Creek reclamation project. The physical barrier of Chilhowee Dam and the alteration of the Little Tennessee River from riverine to reservoir habitat between Citico and Abrams Creeks, eliminated connectivity between the two locations, preventing natural recolonization.

Tellico River is a fourth order tributary of the Little Tennessee River at rmi 19.2 (rkm 30.9) that originates in Cherokee County, NC and flows almost entirely within Monroe County, TN. Most of the study area is found within the CNF upstream of TN Highway 360. Tellico River drains a watershed of 738 km² into the impounded Tellico Reservoir (Figure 1).

Baseline Genetic Results to Date

Assessment of Genetic Diversity and Differentiation: Estimates of genetic diversity (i.e., allelic richness and expected heterozygosity) were similar between Abrams and Citico Creek populations for each species and suggest that each species' reintroduction program has been successful in the preservation of genetic diversity between source and founding populations (Moyer and Williams 2012). Furthermore, Moyer and Williams (2012) determined that the amount of genetic differentiation between populations for each species was minimal, indicating that the fish passage strategy is capturing and maintaining a large portion of the neutral genetic variation observed in each species from each population (Table 4).

Table 4. A table listing a selection of descriptive genetics values for yellowfin madtom, Smoky madtom and Citico darters within Abrams Creek from Moyer and Williams 2013 (Appendix B).

Species	Allelic Richness (A _r)	Average Observed Heterozygosity (H _o)	Average Expected Heterozygosity (H _e)	Estimated Effective Population Size (N _e)
Yellowfin Madtom	1.51	0.106	0.108	75 (15-infinity)
Smoky Madtom	2.08	0.178	0.177	72 (29-691)
Citico Darter	2.62	0.227	0.225	46 (19-291)

Assessment of the One Migrant Per Generation Rule: Upon initial implementation of the fishway passage strategy, no genetic information existed to quantify the rate of exchange of each focal species between Abrams and Citico Creeks; therefore, a target objective of one effective genome (migrant) per generation was established. The one-migrant-per-generation rule has been applied widely to species conservation plans (Mills and Allendorf 1996); however many of the assumptions of this model are often unrealistic and violated, drawing into question its interpretation and implementation in a conservation context (Vucetich and Waite 2000; Wang 2004). In order to more adequately address this rate of exchange between Abrams and Citico populations of concern, Moyer and Williams (2012) assessed the level of two-way migration necessary to impede significant

population divergence over 50 generations (given the current levels of genetic diversity found in our focal species) (Appendix B).

Moyer and Williams (2012) simulation approach indicated an approximate 5% migration rate per generation (e.g., $0.05 \times$ an effective population size (N_e) of $75 \approx$ four individuals per generation) was necessary to offset the influence of genetic drift over the course of 50 generations (approximately 100-150 years given the species of concern) (Appendix B). The generation time of Spotfin Chub is two to three years (Etnier and Starnes 1991), Yellowfin Madtom is three to four years (Dinkins and Shute 1996), whereas, it is two years for the Smoky Madtom and Citico Darter (Layman 1991; Dinkins and Shute 1996).

It is important to note that an estimated value of four migrants per generation (approximately two migrants per year for a species living two years) is the number of individuals that successfully migrate between populations, reproduce and recruit into the local population resulting in gene flow. Thus, to successfully achieve the goal of genetic mixing for Citico and Abrams creek populations, the introduction of at least four adults of each species will be necessary – how much more is dependent on a clearer understanding of average survival rates and reproductive success of transplanted individuals inhabiting Abrams Creek and should be an area of future research. Also of importance is a better understanding of N_e for Abrams and Citico Creek populations. Current simulations assumed each population (for each species) had an N_e of 75 because insufficient data were available to estimate N_e for each population. Accuracy of N_e estimation (and subsequent migration simulations) relies on either increasing sample sizes or the number of molecular markers (Waples and Do 2010). The former may be hard to accomplish given the difficulties of sampling these species; however, new genomic approaches may offer a feasible way to increase the number of markers (Hohenlohe et al. 2011). In doing so, a better estimate of the effective number of migrants will be achieved for each species.

Genetic Recommendations:

1. Estimation of the number of loci or sample size necessary to provide accurate estimation of effective population size for each population.
2. Establishment of baseline genetic data for Tellico populations of each species.

Translocation Procedures

Timing and Size of Fish Collected

Adults will be collected using small dip nets between August and September. Visual Implant Elastomer (VIE) tags or fin clips may be used for ensuring there are no recaptures and for archiving genetic material. Wild adults will be collected for translocations because in some cases hatchery reared fishes have been shown to have negative effects when released back to nature; thus compromising the success of conservation efforts (Heath et al. 2003; Araki et al. 2009; Theriault et al. 2011).

Number of Fish and Frequency of Collections

Suggested Fish Passage Rate: As described above, the generation time of yellowfin madtom is three to four years (Dinkins and Shute 1996), whereas, it is two years for the Smoky madtom and duskytail darter (Layman 1991; Dinkins and Shute 1996). If we aim for a 5% migration rate, we can hope that greater than a 2% migration rate would actually occur, which should minimize genetic differentiation overtime. For yellowfin madtom using this scenario, we would transfer one adult per year from Citico Creek to Abrams Creek and one adult per year from Abrams Creek to Citico Creek (Table 5). For the Smoky madtom and duskytail darter, we would transfer two adults per year from Citico Creek to Abrams Creek and two adults per year from Abrams Creek to Citico Creek. Note that the actual number of effective migrants per generation will be unknown (i.e. the individuals that survive and reproduce); however, we will be applying an adaptive management strategy reliant on genetic monitoring to assess if the number of migrants prescribed above is effective at accomplishing the goal of minimizing the genetic differentiation between populations for each species. If the populations start diverging more than observed from our initial assessment, we will reevaluate the number of effective migrants necessary to avoid genetic differentiation.

Table 5. Summary of published generation ranges and recommended number of adults to translocate annually in order to meet and/or exceed translocation goals for Abrams and Citico Creek's.

Species	Generation Range (years)	Minimum Number of Adults Translocated (annually)
Citico Darter	2	2
Smoky Madtom	2	2
Yellowfin Madtom	3-4	1

Prophylactic Disease Mitigation

Given the logistical complexity of the prescribed translocation efforts, there is potential that diseases and/or parasites and non-indigenous species (if present) could be transferred to the receiving body of water via previously infected sampling gear (i.e. wading boots, wetsuits), direct translocations from source stream water or specimens themselves. In order to minimize the potential for transfer of undesirable organisms from one stream to the other, the following disinfection protocols will be followed:

1. **Sampling Gear (including waders, wading boots, wetsuits):** Any sampling gear that has not been disinfected and completely dry for at least 48 hours will be disinfected prior to any

translocation efforts. Acceptable disinfection procedures include a dip in 2-4% bleach solution for at least 1 minute, a dip in 2% Virkon[®] disinfectant for at least 1 minute or the use of another Aquatic Nuisance Species (ANS) approved disinfection product. Ideally, all gear should also be completely dried for 48 hours prior to use (whenever possible). Any gear that will be used in the stream or streamside at the receiving water body should also be disinfected prior to entering the water body.

2. ***Specimens and Water from Source Stream:*** All specimens collected from the source stream will be visually inspected for parasites and/or signs of disease. Only healthy and robust individuals will be selected for translocation. Specimens deemed healthy and fit for translocation will be dipped into a prophylactic bath of antihelminth praziquantel for approximately 1-2 minutes in order to eliminate any diseases and/or parasites associated with the individual(s). During transport, specimens will be placed in bags in coolers, surrounded with enough ice to maintain temperature and acclimated using a 5% formalin solution to eliminate external parasites and reduce stress.

Logistics of Translocation

The project Licensee is responsible for funding translocations between Abrams and Citico Creeks related to the fishway prescription. Translocations are generally performed by contract per using these guidelines. The contractor will coordinate the effort and obtain all necessary permits from the respective federal agencies (i.e. USFWS, USFS and NPS) and other land managers. Translocations will be contingent upon annual funding to support these efforts. This document does not obligate any party to undertake specific actions and may not represent the views, official positions, or approval of any individuals or agencies involved.

Monitoring for Short and Long Term Success

Abrams Creek Population Monitoring

The NPS, in cooperation with federal, state and private cooperators, will monitor the three Abrams Creek target species annually using visual assessments conducted between August and September. Permanent monitoring sites were established in 2012 within three zones along Abrams Creek (Figure 2) in order to evaluate each species throughout their range. The sampling protocols (Kulp and Moore 2013) provide density estimates for each zone and well as periodic evaluation of species distribution changes over time. Currently, these protocols have only been implemented on Abrams Creek, however the working group would like to initiate these protocols on all three study streams to improve sampling efficiency and comparability.

Surveys conducted in 2012 indicate Smoky madtoms were most abundant in zone-1 (site density range 0-25.4 fish/100 square meter (m^2), less abundant in zone-2 (site density range 0-3.3 fish/100 m^2) and were not observed in zone-3 sites (Figure 3). Yellowfin madtom were present in all three zones, but most prevalent in zone-2 (site density range 0.8-11.8 fish/100 m^2). Citico darters were found in zones 2 and 3 but were most prevalent in zone 3 (site density range 1.1-24.1 fish/100 m^2). Reproduction was documented for yellowfin and Smoky madtom in zone-1, all three species in zone-2 and Citico darters and yellowfin madtom in zone-3.

The purpose of population monitoring is to document long-term demographic trends of each species. For successful restoration, we expect that each population will reach and maintain their respective carrying capacity for the stream and have a stable (or increasing) population density over time. If population densities (Figure 3) are not maintained or increasing over time, then potential mitigating factors and the need for translocation (both numbers of individuals and frequency of translocation) will be evaluated using an adaptive management approach.

Citico Creek and Tellico River Population Monitoring

Since 1986, annual monitoring surveys have been conducted by CFI on Citico Creek using day and night visual snorkeling surveys and the catch-per-unit-effort (CPUE) methodology. Surveys were typically conducted annually between August and October; however annual effort, sample sites and timing of surveys (e.g., day vs. night and month) were not standardized. Since 2002, CFI has utilized similar annual monitoring techniques on sites throughout the Tellico River restoration area.

Future monitoring goals are to implement the Abrams Creek sampling protocols (Kulp and Moore 2013) on both Citico Creek and the Tellico River. Discussions are currently underway among the USFWS, TWRA and CFI to determine if the Abrams Creek protocols will be used to monitor the Citico and Tellico populations. Standardized protocols would not only provide comparable results within streams over years, but also among streams over years.

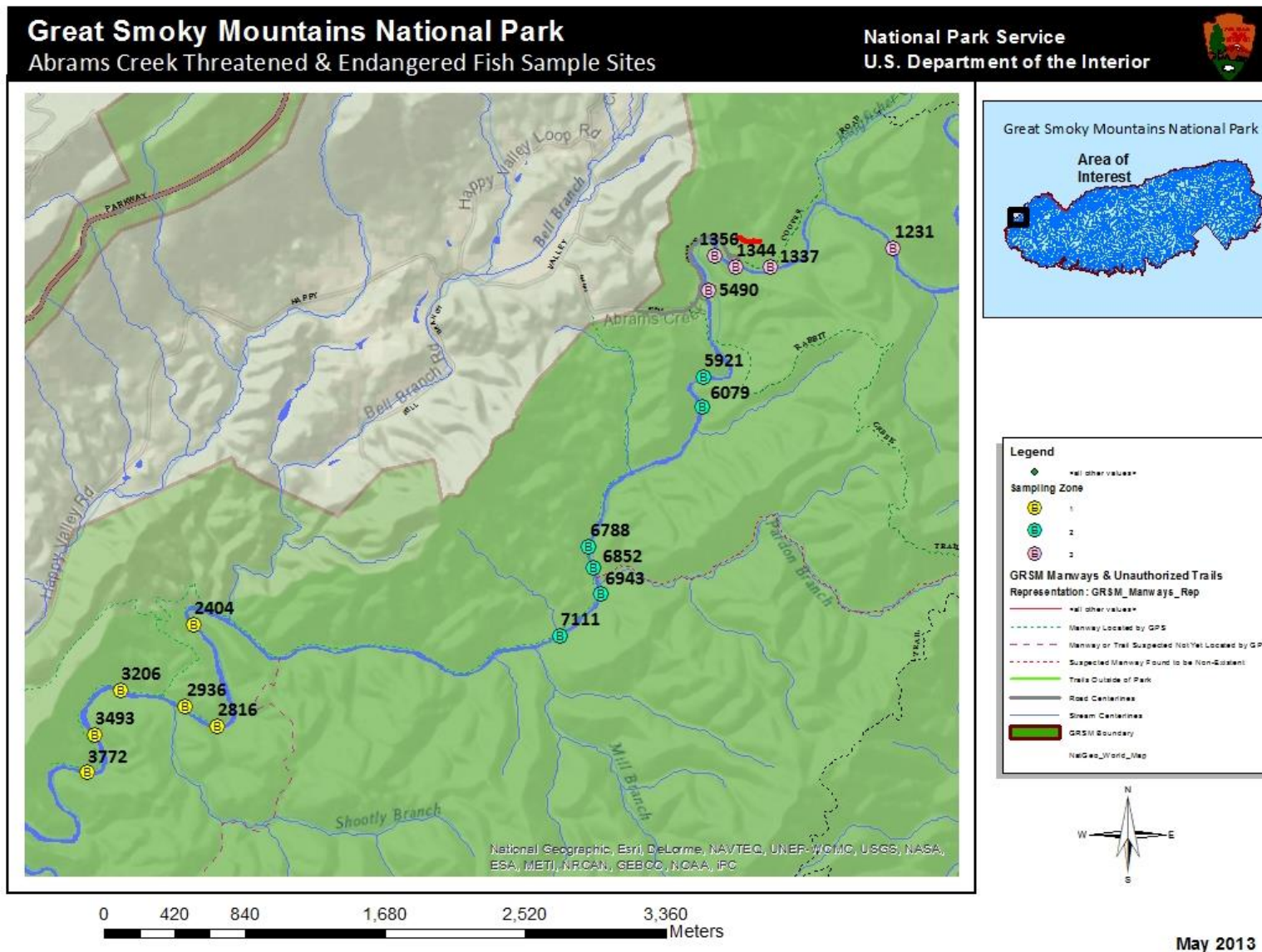


Figure 2. Map of Abrams Creek study area bordered by Abrams Falls on the upstream end and Chilhowee Reservoir on the downstream end.

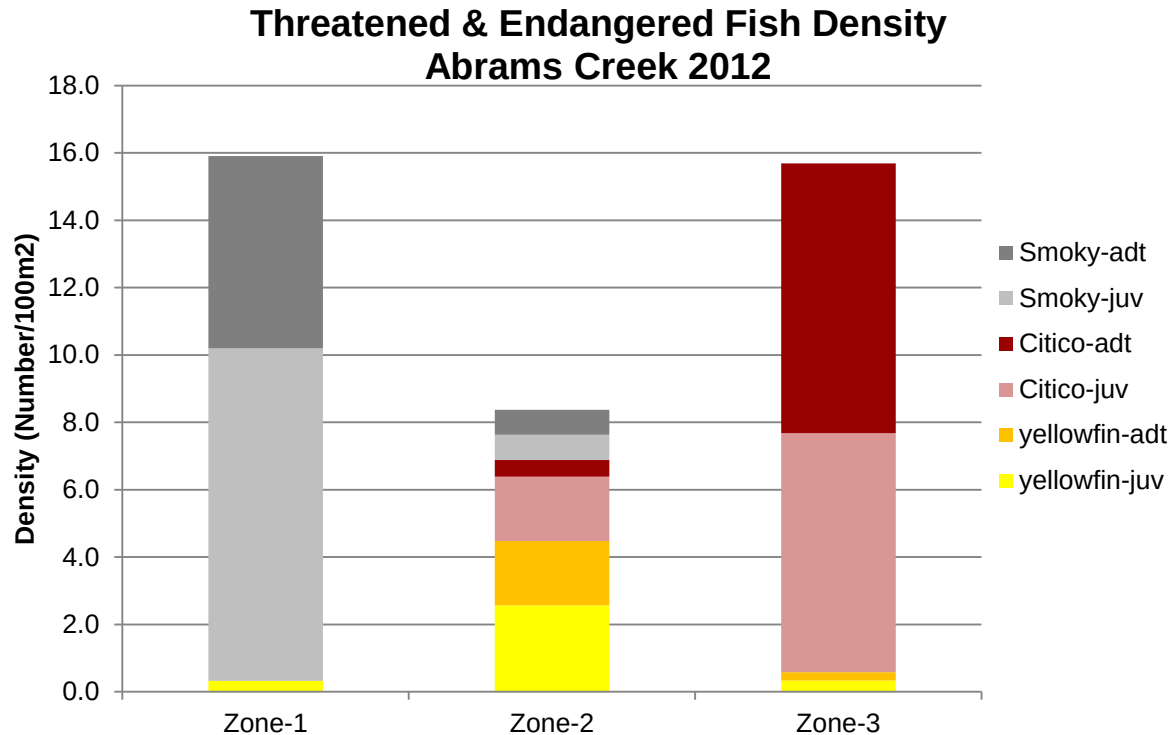


Figure 3. Summary of threatened and endangered mean fish densities within three zones of Abrams Creek sampled in 2012, Great Smoky Mountains National Park. Note density values represent the mean of all sites sampled within the respective zone.

Genetic Monitoring

Baseline estimates of genetic diversity (allelic richness, observed heterozygosity, effective population size) and genetic differentiation have been estimated for each species and population (Table 3) (Moyer and Williams 2012). Monitoring estimates of genetic diversity will assist in documenting long-term trends in genetic diversity of wild populations and in informing captive rearing protocols or habitat restoration efforts to maximize retention of remaining genetic diversity. The purpose of monitoring estimates of genetic differentiation between populations is to document long-term genetic trends and to evaluate the success of the translocation initiative. Over the course of genetic monitoring, if genetic analyses indicate that populations are significantly diverging from one another, then the translocation initiative will be deemed unsuccessful and need to be further evaluated. For successful restoration, we expect that each population will reach and maintain their respective carrying capacity for the stream and have a stable (or increasing) level of genetic diversity over time. If these levels (Table 3) are not maintained or increasing over time, then the need for translocation (both numbers of individuals and frequency of translocation) should be reevaluated using an adaptive management approach.

Sampling for genetic monitoring will occur between August and November every three years. Fin clips will be obtained from 30-50 individuals from each population. Optimally, samples should be obtained from multiple locations and habitats from within each population.

Data Management

Translocation Data, Genetics Monitoring, Population Monitoring

This section describes the procedures for data handling, analysis and report development. Project information management may be best understood as an ongoing or cyclic process, as shown in Figure 4.

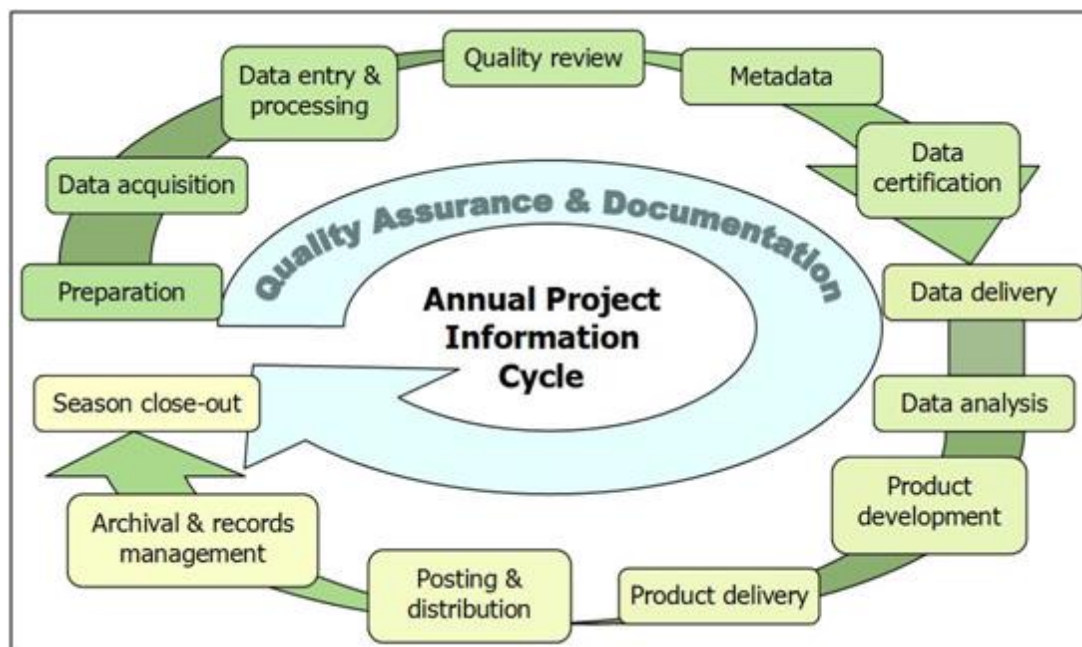


Figure 4. Idealized flow schematic of the cyclical stages of project information management, from pre-season preparation to season close-out.

The stages of this cycle can be briefly summarized as follows:

- *Preparation* – This step includes training, logistics planning and printing forms and maps. To facilitate data recording during inclement weather, data sheets should be printed on waterproof paper. To ensure that all field data for the NPS are collected and recorded in a useable manner, data are recorded in the units specified on the data sheets.
- *Data acquisition* – All data recorded during field trips are reviewed at the sampling site and the contractor Crew Leader reviews and initials all data sheets prior to departure from the site. Legible copies of data sheets and/or electronic PDF versions are provided to the NPS data manager (DM) on an approximately bi-weekly basis during sampling. Once the Crew Leaders have submitted legible copies of data sheets to the DM, the quality control (QC) officer examines the sheets and records potential errors, documents and corrects discrepancies, and periodically alerts Crew Leaders to prevent similar errors in the future.
- *Data entry & processing* – All data collected on field forms must be entered into the project database (MS Access or SQL Server). Data entry should take place as soon as possible, ideally no longer than one week after the data are collected, and should be done by one of the

field crew. After the data have been entered into the project database it should be reviewed by someone other than the person who entered the data. If two people are involved in the data entry process (e.g. one person dictating the information from the data sheet and the second entering the data into the database), data verification can take place almost at the same time that the data is entered. Both parties must be involved in reviewing the data and it is still imperative that the two people involved review each record and compare it to the data sheet to ensure proper data entry. Once the data have been checked, the reviewer should indicate on the data sheet as well as in the database that the data has been verified and by whom. Any data entry errors should be corrected in the database and the name of the person who made the correction as well as the date of the change should also be indicated. This information can be entered in the metadata fields on the main Event Information tab in the database under “Updated by.”

- *Quality review* – All data are reviewed for quality and logical consistency. Data should be reviewed to identify validation errors but specific queries can be designed to catch validation errors that are not captured during the data entry process. These queries, which will evolve over time, are intended to summarize data fields and identify those data points that might be questionable. Development of these queries requires input from all those on the project team.
- *Metadata* – Metadata is essential for data managers and data users to track and understand the content, quality and standards relating to each data set. All data sets, both spatial and non-spatial (e.g. tabular data sets) need to be fully documented with Federal Geographic Data Committee (FGDC) compliant metadata. A metadata template is available, but the monitoring may rely on contractors to assist in the completion of metadata because of the knowledge they have regarding data collection and analysis.
- *Data certification* – Once field data are certified as complete by the agency and/or contractor for the period of record, they should be sent to NPS for upload to the master database. Data should be certified and transferred at least once annually.
- *Data delivery* – Certified data and metadata are delivered for archiving and upload to the master project database. Only the back-end data file associated with the project data base should be transferred. This file should be sent to the NPS project manager, Matt Kulp (matt_kulp@nps.gov). If the file is too large to e-mail (e.g. greater than 6 MB) and compressing the data file into a ZIP format does not help, data can be posted to the NPS public FTP site.
- *Data analysis* – Translocation data will be summarized and reported to the GRSM data manager without any formal analysis requirement. Final genetics and population monitoring data will be analyzed and reported by the USFWS (genetics) and NPS (population monitoring).
- *Product development* – Reports, maps, and other products for translocation, genetics and population monitoring will be developed by the contractor, USFWS and NPS.

- *Product delivery, posting and distribution* – Reports and other products for posting and archiving will be sent to the IRMA data portal (<https://irma.nps.gov/App/Portal>) by the end of the respective collection year.
- *Archiving and records management* – Review analog and digital files for retention (or destruction) according to NPS Director’s Order 19. Retained files are renamed and stored as needed.
- *Season close-out* – Review and document needed improvements to project procedures or infrastructure, complete administrative reports, and develop work plans for the coming season. Needed improvements should be noted in the annual report and incorporated into future work efforts.

Reporting

Fish Translocation and Genetics Report: A summary report of fish translocations will be completed annually by the Licensee or its contractor following the Natural Resource Data Series (NRDS) format as described at: <http://www.nature.nps.gov/publications/nrpm/> . The report may be a standalone report, or as part of a more comprehensive report covering all aspects of the Abrams, Citico and Tellico River population and genetics monitoring and translocations. Once completed and approved by the contracting agency, the report will be uploaded to the NPS Integrated Resource Management Application (IRMA) (<https://irma.nps.gov/App/Portal>). The report will outline the number of fish removed from each study stream, disease prevention treatments and the number/origin of fish successfully translocated to each study stream. Beginning in 2017 and continuing every five years thereafter, the annual report, or a separate standalone report issued by the USFWS, will include a summarization of the genetic sampling results, including estimates of genetic diversity and differentiation and their respective trajectories over time. Also provided will be an update on the current carrying capacity estimate. These data will be used to evaluate population recovery and progress towards genetic goals.

Roles & Responsibilities

There are distinct, as well as shared roles and responsibilities, of the parties for implementation and monitoring of fish translocation under this plan (Table 6). The translocation of fish for passage requires an integration of many moving parts, bringing the authorities and roles of multiple federal agencies, landowners and managers, the hydroelectric licensee, and non-profit organizations into a coordinated effort for conservation of rare fishes.

The responsibility for administration, protection, and development of GRSM is exercised by the NPS, under the direction of the Secretary of the Interior by the NPS, subject to the provisions of sections of Pub. L. 113-21 (36 CFR, Title 36), as amended, including provisions for permits (§ 1.6). Pub. L. 108-343, Oct. 18, 2004, 118 Stat. 1372, known as the “*Tapoco Project Licensing Act of 2004*”, authorized land exchange in GRSM between the Secretary of the Interior and private corporation, and provided that FERC had jurisdiction to license Tapoco Hydroelectric Project on lands transferred by the Secretary.

The primary purpose of the Endangered Species Act (§2(b)) is “*to provide a means whereby the ecosystems upon which endangered or threatened species depend may be conserved...*” Under the Endangered Species Act, 50 CFR Part 17, the USFWS has responsibility for development and administration of §4 Recovery Plans, while §7(a)(1) obligates all Federal agencies to utilize their authorities to further the purposes of the Act by carrying out programs for the conservation of endangered and threatened species.

Section 18 of the Federal Power Act, 16 USC § 811, states in part:

"The Commission shall require the construction, maintenance, and operation by a licensee..., such fishways as may be prescribed by the Secretary of Commerce or the Secretary of Interior."

Section 1701(b) of the National Energy Policy Act of 1992, P.L. 102-486, Title XVII, § 1701(b), 106 StatL 3008, states:

"The items which may constitute a 'fishway' under section 18 [16 USC § 811] for the safe and timely upstream and downstream passage of fish shall be limited to physical structures, facilities, or devices necessary to maintain all life stages of such fish, and project operations and measures related to such structures, facilities, or devices that are necessary to ensure the effectiveness of such structures, facilities, or devices for such fish."

The Prescription for Fishways at the Tapoco Project was issued under authority delegated to the Southeast Regional Director of the USFWS from the Secretary of the Interior; the Assistant Secretary for Fish, Wildlife and Parks; and the Director of the U.S. Fish and Wildlife Service pursuant to section 18 of the FPA.

Table 6. Table outlining partner agencies, agency contact(s) and roles/responsibilities of each contact relative to the fish translocation implementation plan.

Party	Role	Point of Contact
USFWS	FPA §18 Prescription ESA Recovery	mark_a_cantrell@fws.gov stephanie_chance@fws.gov bob_butler@fws.gov
USNPS	Land Manager	matt_kulp@nps.gov
USFS	Land Manager	jim_herig@fs.us.gov
SMH	Licensee, funding	myra.hair@brookfieldrenewable.com
CFI	Contractor	xenisma@gmail.com noturus@aol.com

Translocation and Monitoring Costs Estimates

Translocation: Fish translocation from one stream to another is estimated to take one day per stream with the average cost per day to be roughly \$1,600-\$2,000 (Pat Rakes, CFI, personal communication). Therefore, the cost will be between \$3,200-\$4,000 annually to move fish to and from Abrams Creek and Citico Creek. When Tellico River is added to the schedule, three days will be necessary and costs will increase to roughly \$6,000 per year due to the extra time needed to collect additional fish to transfer to two locations.

Genetics Monitoring: Evaluation of number of genetic markers will require one week (40hrs) of USFWS GS-13 time (\$2,446). Genetic monitoring costs are contingent upon the number of molecular markers necessary (Table 7).

Table 7. A table representing the estimated annual genetic monitoring costs for collecting T&E fish from Abrams Creek. These are annual costs accrued each time the genetic monitoring is implemented.

Lab protocol	No. Samples	Price/Sample	No. Markers	Total
DNA Extraction	100	\$2.68	NA	\$268.00
PCR for genotyping	100	\$0.28	30	\$840.00
Genotyping	100	\$0.99	30	\$2,970.00
ABI service contract (25% of chemical costs)				\$1,019.50
Salary (GS 9 @ 26.12/hr or 1045/wk)	21week			\$21,945.00
Benefits (39%)				\$8,558.55
Overhead (22%)				\$7,832.23
Annual Total				\$43,433.28

Population Monitoring: Fish monitoring protocols require three surveyors to survey each sample site (Kulp and Moore 2013). Multiple sample sites can be sampled daily, however based upon previous sampling efforts, it takes a minimum of six days to sample all sample sites within all three zones. Therefore, we will plan on 8 days (one pay period [ppd]) in order to prepare/clean gear and complete the sampling (Table 8).

Table 8. Table representing the estimated annual T&E fish population monitoring costs.

Resource	Count	Unit	Cost	TOTAL
NPS GS-11 Fish Biologist	1	ppd	\$3,873	\$3,873
NPS GS-5 Bioscience Tech	2	ppd	\$1,353	\$2,706
Contract Fish Biologist	1	ppd	\$4,000	\$4,000
Contract Fish Technician	2	ppd	\$3,200	\$6,400
Supplies/Equipment (snorkels, masks, wetsuits, boots)	Annual	ea	\$ 400	\$ 400
Annual Total				\$17,379

NEPA Compliance

The National Environmental Policy Act (NEPA) and FERC regulations (18 CFR §§ 4.38 and 16.8) require that license applicants consult with state and federal agencies, Native American tribes, and other entities prior to filing license applications with FERC. Pre-filing consultation must be completed and documented according to FERC regulations. In March 1999, APGI initiated NEPA (National Environmental Policy Act) “scoping” with the relicensing Participants, which included the U.S. Fish and Wildlife Service, National Park Service, Tennessee Department of Environment and Conservation and several other state, federal and NGO’s (FERC 2004).

In a letter dated September 19, 2003, Interior requested reservation of authority to prescribe fishways under Section 18 for the Chilhowee Development that generally included: (1) developing a plan for the Chilhowee Development that would translocate Spotfin Chub, Yellowfin Madtom, Smoky Madtom, and Duskytail Darter between Abrams Creek and Citico Creek and between Abrams Creek and the Tellico River using trap and truck methods; and (2) developing a study plan that evaluates the presence and status of potomadromous and diadromous fishes in the upper end of the Tellico Reservoir in the vicinity of the Chilhowee Dam tailrace.

FERC issued the draft EA (DEA) on March 15, 2004, for a 30-day public comment period. On April 8, 2004, the 30-day comment deadline was extended by an additional 30 days until May 14, 2004. Comments were addressed in the Draft EA, which can be reviewed at FERC (2004).

FERC issued a Final Environmental Assessment (EA) For Hydropower License Tapoco Hydroelectric Project, Project No. 2169-020 North Carolina and Tennessee (Federal Energy Regulatory Commission Office of Energy Projects Division of Hydropower - Environment and Engineering, 888 First Street, NE Washington, D.C. 20426) (FERC 2004). The Final EA (FERC 2004) considered various aspects of issuance of the new license for the hydroelectric project, including analyses of the provisions for translocation of fishes pursuant to the fishway prescribed by Interior. The Final EA (FERC 2004) also included a Finding of No Significant Impact (FONSI) based upon the independent analysis which concluded that licensing the project(s) with the recommended measures would not result in major federal actions significantly affecting the quality of the human environment.

The GRSM Resource Management and Science interdisciplinary team has reviewed the Final EA (FERC 2004), which prescribed the fish passage mitigation outlined within the translocation plan

titled Threatened and Endangered *Fish Translocation to and from Abrams Creek and Citico Creek, Great Smoky Mountains National Park and Cherokee National Forest*. The team determined:

1. The fish translocation prescription outlined within the FERC Final EA (FERC 2004) is not significantly different from the protocols outlined within this plan,
2. The FERC Final EA (FERC 2004) provided sufficient time for review and public comment on the proposed action(s), and
3. The FERC Final EA (FERC 2004) analysis concludes that licensing the project(s) with the recommended measures would not be major federal actions significantly affecting the quality of the human environment.

Therefore, it is the opinion of the GRSM Resource Management and Science interdisciplinary NEPA team that all NEPA requirements for this plan have been met and/or exceeded.

Endangered Species Act (ESA): Activities associated with the implementation and monitoring of this Plan is authorized under ESA section 10(a)(1)(A) permits . The latest programmatic Biological Opinion for ESA section 10(a)(1)(A) permits for fish in the Southeast Region was signed July 26, 2013 and is applicable to this plan.

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Appendix A

Alcoa Power Generating Inc. Tapoco Division

Tapoco Project (FERC No. 2169) Fish Passage Translocation Plan

Alcoa Power Generating Inc. Tapoco Division

Tapoco Project (FERC No. 2169)

Fish Passage Translocation Plan

August 2005

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List of Terms (or similar - Glossary, Acronyms, etc.)

APGI	Alcoa Power Generating Inc.
CFI	Conservation Fisheries, Inc.
FERC	Federal Energy Regulatory Commission (also Commission)
Licensee	Alcoa Power Generating Inc.
NPS	National Park Service
NCWRC	North Carolina Wildlife Resources Commission
Plan	Fish Passage Translocation Plan
Project	Tapoco Hydroelectric Project
RSA	Relicensing Settlement Agreement
TVA	Tennessee Valley Authority
TWRA	Tennessee Wildlife Resources Agency
USFS	United States Forest Service
USFWS	United States Fish and Wildlife Service (also Service)
UT	University of Tennessee
YOY	Young-of-year

Introduction/Background

On January 25, 2005 the Federal Energy Regulatory Commission (FERC) issued an order approving settlement and issuing Alcoa Power Generating Inc. (APGI, Licensee) a new 40-year license for the Tapoco Hydroelectric Project (FERC No. 2169) (Project). Article 401 of the License requires APGI to file a Fish Passage Translocation Plan (Plan) for four specific “target species” that now occur in the vicinity of the Project’s Chilhowee Development within six months of the effective date of the license (or September 1, 2005). The License Article reads as follows:

Article 401. Requirement to File Plans for Commission Approval and Requirement to Consult. Appendix D requires the Licensee to prepare a plan in accordance with the U.S. Department of the Interior’s prescription of fishways under Section 18 of the Federal Power Act. The plan shall also be submitted to the Commission for approval and shall include an implementation schedule. This plan is listed below.

Fishway Prescription	Plan	Due Date
Part I.	Fish Passage Translocation Plan	Within six months of the effective date of the License.

The Licensee shall prepare the plan after consultation with the U.S. Fish and Wildlife Service. The Licensee shall include with the plan, documentation of its consultation, copies of comments and recommendations made in connection with the plan, and a description of how the plan accommodates the comments and recommendations. If the Licensee does not adopt a recommendation, the filing shall include the Licensee’s reasons, based on project-specific information. The Commission reserves the right to make changes to any plan submitted. Upon Commission approval, the plan becomes a requirement of the License, and the Licensee shall implement the plan, including any changes required by the Commission.

The License includes and incorporates by reference the U.S. Fish and Wildlife Service (USFWS) Section 18 Prescription of a Fishway for the Chilhowee Development (the Prescription is Appendix D to the License). The Prescription was issued under authority to the Southeast Regional Director from the Secretary of the Interior; the Assistant Secretary for Fish, Wildlife, and Parks; and the Director of the USFWS pursuant to Section 18 of the Federal Power Act and filed with FERC on September 19, 2003. The Prescription includes the following license article language:

*Within six (6) months of the effective date of the new license, the Licensee shall develop and file with the Commission a plan for fish passage at the Chilhowee Development. The plan shall be prepared in consultation with the U.S. Fish and Wildlife Service and shall provide for fish passage at the Chilhowee Development for four target fish species, the Spotfin Chub (*Erimonax monachus*), Yellowfin Madtom (*Noturus flavipinnis*), Smoky Madtom (*Noturus baileyi*), and Duskytail Darter (*Etheostoma percnurum*). Fish passage will entail annual funding by the Licensee for the trapping and relocation of certain numbers of each target fish species, each season. Actual numbers of fish species will be determined annually in*

consultation with the U.S. Fish and Wildlife Service. Annual funding shall be used first to accomplish the primary fish passage objective of moving a certain number of each of the target fish species between Abrams Creek and Citico Creek, and between Abrams Creek and the Tellico River. Funding will be used secondarily to conduct associated sampling, marking and genetics testing to help to demonstrate that the Service's goal of genetic mixing between the sub- populations of the four fish species is being met. Funding can also be used to trap and transport fish between Tellico River and Citico Creek, to the extent that such efforts may also enhance the overall genetic health of the Abrams Creek populations. The Licensee shall develop in consultation with and submit for approval by the Service, all functional and final fishway plans, schedules, and effectiveness studies for the fishway described herein.

The USFWS Prescription also defines the “General Terms and Conditions for “Fishways” at the Chilhowee Development, which are the basis of this plan.

The January 25, 2005 FERC order also approved a Relicensing Settlement Agreement (RSA) for the Tapoco Project. Section 5.1, Appendix A of the RSA (proposed license article FP-1) is similar to the requirements of the Tapoco License and the Section 18 Prescription. These requirements include the provisions of the fish passage plan such as compliance date, consultation requirements, target species to be passed, and funding priorities. FP-1 also states that annual funding for the fish passage is estimated to be no more than \$10,000 per year.

History of Fish Restoration

The four target fish species, the Spotfin Chub; the Yellowfin Madtom; the Smoky Madtom; and the Duskytail Darter, were previously extirpated from Abrams Creek as a result of a 1957 Rotenone treatment aimed at enhancing sport fish populations as mitigation for the original Project license. According to accounts, the Rotenone was entrained through the Calderwood Powerhouse draft tubes into the Little Tennessee River and injected at Abrams Falls on Abrams Creek. Many of the lower Abrams Creek fishes had no refuge and recolonization was limited to those species that also occurred upstream of the falls or in Panther Creek. Coincidentally, the construction of Chilhowee Reservoir on the mainstem of the Little Tennessee River prevented the movement of these fishes among Abrams Creek, Citico Creek, and the Tellico River.

Since the mid-1980s, Conservation Fisheries, Inc. (CFI), a private organization, has been working with the USFWS, the Tennessee Wildlife Resources Agency (TWRA), U.S. Forest Service (USFS), National Park Service (NPS), the University of Tennessee (UT), and the North Carolina Wildlife Resources Commission (NCWRC) to reintroduce the four target fish species into Abrams Creek, within the Great Smoky Mountains National Park. Years after the first reintroduction of the fishes into Abrams Creek, natural reproduction of all four species has been documented in Abrams Creek. Though, the reproduction and recruitment of Spotfin Chub has been very low in recent years. The agencies plan to continue working with CFI until all four species are more widespread throughout lower Abrams Creek, and populations of all four are self- sustaining and relatively stable over 10 years. Additionally, CFI has reintroduced the four target fish species into the Tellico River, which

also has ideal habitat for the reintroduction of the four fishes. The fish translocations described in this Plan will complement the ongoing reintroduction efforts.

Location and Setting

The Project is located approximately 15 miles south of Maryville, Tennessee, and approximately 90 miles northeast of Chattanooga, Tennessee. The Project is in the western portion of the Little Tennessee Watershed on the Little Tennessee and Cheoah rivers in Graham and Swain counties in North Carolina and Blount and Monroe counties in Tennessee. The Project includes four hydroelectric developments, the Santeetlah, Cheoah, Calderwood, and Chilhowee developments. The 1,734-acre (surface area) Chilhowee Development is located on the Little Tennessee River between river miles 33 and 34, downstream of the Calderwood Development in Blount and Monroe counties. The Tennessee Valley Authority's (TVA) Tellico Dam is located downstream of the Chilhowee Development and the headwaters of Tellico Reservoir at full pond extend to the Chilhowee tailwater.

The fish populations to be passed are those occurring in tributaries to the Little Tennessee River, including Abrams Creek (tributary to Little Tennessee River – Mile 37), Citico Creek (tributary to the Little Tennessee River at Mile 31.8), and the Tellico River (tributary to the Little Tennessee River at Mile 19.2) (Figure 1). The population of Spotfin C hub in the Little Tennessee River (between Mile 88.5 and Mile 113.2) is also a source for augmentation of the populations. Abrams Creek is a tributary that feeds directly into Chilhowee Reservoir approximately three miles upstream of Chilhowee Dam. Both Citico Creek and the Tellico River are tributaries to the Little Tennessee that feed directly into Tellico Reservoir. Citico Creek is located approximately two miles downstream of Chilhowee Dam and the Tellico River is approximately 15 miles downstream of Chilhowee Dam.

Goals and Objectives

The goals of the Tapoco Project Fish Passage Translocation Plan are to:

1. Develop, operate, and maintain passage at the Chilhowee Development in Blount and Monroe counties, Tennessee for the identified target fish species, and;
2. Ensure that the requirements of License Article 401, the Section 18 Prescription of a Fishway for the Chilhowee Development, and the Tapoco Project Relicensing Settlement Agreement are met.

Within these broad goals, the three principal objectives of the Tapoco Project Fish Passage Translocation Plan are:

1. Enhancement of the overall genetic health of the Abrams Creek, Citico Creek, and Tellico River fish populations, particularly the four target species;
2. Genetic mixing between the sub-populations of the four target fish species, and;

3. Safe, timely, and convenient fish passage for populations of four identified fish species: Spotfin Chub, Yellowfin Madtom, Smoky Madtom, and Duskytail Darter between Citico Creek and Abrams Creek, and between Tellico River and Abrams Creek.

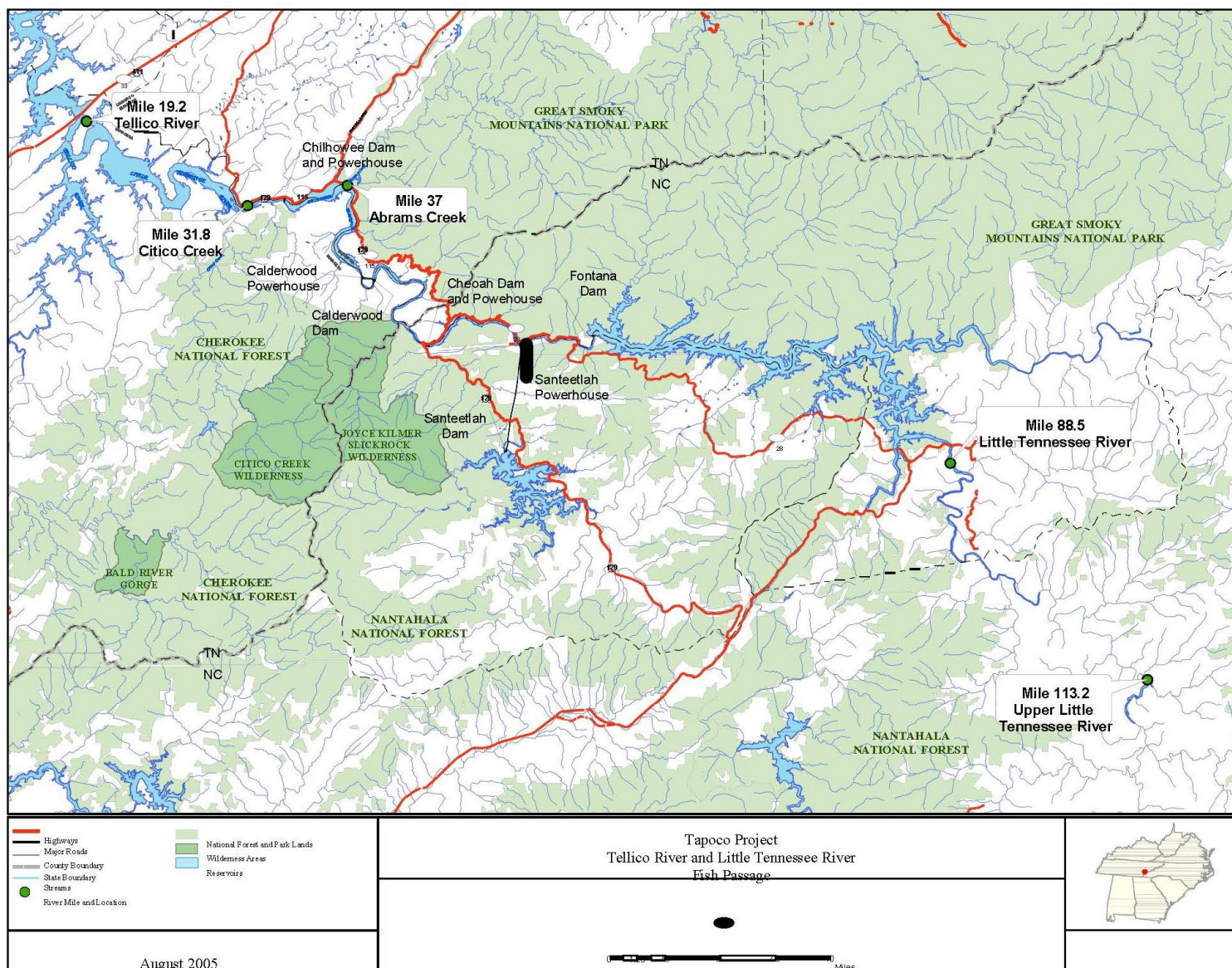


Figure 1. Tapoco Project Tellico River and Little Tennessee River Fish Passage

Fish Passage at Chilhowee

Target Fish Species and Design Population

The fish species targeted for translocation are the Spotfin Chub; the Yellowfin Madtom; the Smoky Madtom; and the Duskytail Darter. Each of these species is federally listed as either endangered or threatened range-wide by the USFWS. The target species are all native to and currently occurring within the Tennessee River Basin although in nearly all instances their populations are fragmented. The species now occur primarily upstream of the Project boundary, but because they have been reintroduced and suitable habitat for each of the species has been identified within the Project boundary, they may occasionally occur within the boundary.

Spotfin Chub

The Spotfin Chub, also known as Turquoise Shiner, is federally listed as threatened by the USFWS (September 9, 1977). At the time the species was listed as threatened in

1977, the USFWS also designated a portion of the Little Tennessee River in Macon and Swain counties, from the backwaters of Fontana Reservoir upstream to the North Carolina-Georgia state line as critical habitat. The USFWS published a Spotfin Chub Recovery Plan for the species, approved in November 1983. The goal of the Recovery Plan is to restore viable populations of Spotfin Chub to a significant portion of its historic range and remove it from the federal endangered species list. This species is native to the Tennessee River drainage and has recently been found in tributaries to the mainstem Little Tennessee River in both North Carolina and Tennessee (including Citico Creek, a tributary to the Little Tennessee River downstream of the Tapoco Project).

Yellowfin Madtom

The Yellowfin Madtom is federally listed as threatened (September 9, 1977). The USFWS published a Yellowfin Madtom Recovery Plan for the species, approved in June 1983. The goal of the Recovery Plan is to restore viable populations of the Yellowfin Madtom to a significant portion of its historic range and remove it from the federal endangered species list. The Yellowfin Madtom is an inhabitant of pools and backwaters of small to moderate-sized streams in the upper Tennessee River drainage. The madtom has been collected from six streams in the Tennessee River Basin: Chickamauga Creek, Hines Creek, North Fork Holston River, Copper Creek, Powell River, and Citico Creek.

Smoky Madtom

The USFWS listed the Smoky Madtom as an endangered species on October 26, 1984. Concurrent with this listing, the USFWS also designated Citico Creek from the Cherokee National Forest boundary at upper Citico Creek Bridge on Mountain Settlement Road upstream to the confluence of Citico Creek with Barkcamp Branch as critical habitat. The USFWS published a Smoky Madtom Recovery Plan for the species, approved in August 1985. The goal of the Recovery Plan is to restore four viable populations of the Smoky Madtom and to protect the species and its habitat to such a degree that the species no longer qualifies for protection under the Endangered Species Act (ESA). The Smoky Madtom was present historically in the Little Tennessee River at the Calderwood Bypass. Currently, it occurs in Citico Creek, which joins the Little Tennessee River downstream of the Project.

Duskytail Darter

The Duskytail Darter was listed as endangered by the USFWS on April 27, 1993. The USFWS published a Duskytail Darter Recovery Plan for the species, approved in March 1994. The goal of the Recovery Plan is to restore viable populations of the Duskytail Darter to a significant portion of its historic range and remove the species from the Federal List of Endangered and Threatened Wildlife and Plants. Historically, the Duskytail Darter was likely widespread in the middle reaches of the Cumberland River and the upper reaches of the Tennessee River. However, it presently has a very fragmented distribution.

Installation and Operation of the Chilhowee Fishway

Section 1701(b) of the National Energy Policy Act of 1992, P.L. 102-486, Title XVII, §1701(b), 106 Stat. 3008, states, “The items which may constitute a ‘fishway’ under

Section 18 for the safe and timely upstream and downstream passage of fish shall be limited to physical structures, facilities, or devices necessary to maintain all life stages of such fish, and project operations and measures related to such structures, facilities, or devices that are necessary to ensure the effectiveness of such structures, facilities, or devices for such fish.” Consistent with the Section 18 Prescription and Administrative Record filed by the USFWS with FERC on September 19, 2003, fish passage at the Chilhowee Development will entail annual funding by APGI for the trapping and relocation of certain numbers of the target fish species, each season for the purpose of facilitating the exchange of individuals and their genetic material between the Citico Creek, Abrams Creek, and Tellico River populations of these fish. Such translocation or “passage” is needed since the existence of Chilhowee Dam and Reservoir serve to block the natural movement of fish between these populations and other forms of fishways are not feasible given the lack of habitat and the extent of the reservoir.

Annual funding will be used to first accomplish the primary fish passage objective of moving a certain number of target fish species between Abrams Creek and Citico Creek, and between Abrams Creek and the Tellico River. Funding will be used secondarily to conduct associated sampling, marking and genetics testing to help to demonstrate that the Service’s goal of genetic mixing between sub-populations of the four fish species is being met. Funding may also be used to trap and transport fish between Tellico River and Citico Creek, to the extent that such efforts may also enhance the overall genetic health of the Abrams Creek populations.

APGI and the USFWS will meet annually each winter to discuss the specific numbers of each fish species to be passed, the timing and method of translocation, and the disbursement of funds.

Table 1: Generally, the USFWS recommended that the design population to be “passed” between each of the three designated rivers for each target species be one of the following.

Target Species	Fishway Exchange
Spotfin Chub	100 per generation
Yellowfin Madtom	1 effective genome/generation
Smoky Madtom	1 effective genome/generation
Duskytail Darter	1 effective genome/generation

This exchange rate is the Service's current assessment of the exchange rate that would likely have occurred naturally; and represents the exchange of a few individuals per decade moving successfully between these populations. The USFWS has further concluded that historically, the movement of individuals probably comprised young-of- year (YOY) dispersal movements during fall and winter, therefore translocations scheduled during this same time frame (August – May) would most closely mimic natural dispersal.

Implementation

The Service's Section 18 Prescription requires that the fishway "be fully operational at the Chilhowee Development as soon as possible but no later than six months after the effective date of the new license [September 1, 2005] so that continuing impacts of the Project may be mitigated and benefits of passage realized as soon as practicable." By e-mail dated August 29, 2005, the USFWS stated that it considers the fishway operational upon the filing of this Plan with FERC.

The initial disbursement of funds will be made available to the USFWS upon their request and no later than December 31, 2005. Thereafter, APGI will provide funding to the USFWS annually for the term of the new license (through 2045). APGI and the USFWS anticipate that the first translocations will occur in 2005-2006, following the first annual meeting.

Effectiveness Monitoring

The USFWS, in cooperation with other state and federal fishery agencies and CFI, will conduct monitoring to measure the effectiveness of this plan in meeting the goals and objectives described in Section 1.4. Monitoring may be funded in part or total out of the \$10,000 annual funding provided by APGI.

Appendix B

Genetic assessment of Abrams Creek reintroduction program for the federally threatened yellowfin madtom (*Noturus flavipinnis*), and endangered Smoky madtom (*Noturus baileyi*) and Citico darter (*Etheostoma sitikuense*)

Genetic assessment of Abrams Creek reintroduction program for the federally threatened yellowfin madtom (*Noturus flavipinnis*), and endangered Smoky madtom (*Noturus baileyi*) and Citico darter (*Etheostoma sitikuense*)

Report generated by USFWS Conservation Genetics Lab

15 February 2012

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Summary of major findings

1. Low levels of genetic diversity were observed for each species
2. Comparisons of genetic diversity were significantly different between Citico and Abrams Creek populations for each species
3. These differences were attributed to variance in reproductive success (either in the hatchery or wild) and not as having used too few brood for the reintroduction program
4. Simulations indicated that at least four effective migrants per generation are necessary to minimize genetic differentiation between Citico and Abrams Creek populations for each species
5. Low levels of genetic diversity in Citico creek species were attributed to wholesale deforestation of the surrounding watershed in the 1900s and highlight the importance of protecting these species from further genetic and demographic bottlenecks.

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Introduction/Background

The southeastern portion of the United States has been identified as a region of high ichthyofaunal diversity as well as a region that harbors the greatest number of imperiled freshwater fishes (Warren et al. 2000; Jelks et al. 2008);. Of the nearly 700 fish species found in southeastern United States waters, more than 25% are considered imperiled. Disproportionately represented among these imperiled fishes are madtom catfishes of the ictalurid genus *Noturus* and darters of the percid genus *Etheostoma* (Warren et al. 2000; Jelks et al. 2008). While principal causes of freshwater fish imperilment in the southeastern United States are often associated with habitat loss and degradation from impoundments, urbanization, agriculture, deforestation, erosion, and pollution, (Moyle and Leidy 1992), the aforementioned ictalurid and percid genera are often at greater risk due to their specialization for lotic, benthic habitats (Angermeier 1995), and because many are geographically and/or genetically isolated (Warren et al. 2000). One of the most notable and poignant examples of the wholesale loss of a native fish community due to human induced changes was that of Abrams Creek, TN (Fig. 1). In the summer of 1957 coinciding with the closing of Chilhowee Dam, an ichthyocide was applied to a 14 mile stretch of the creek in order to create a recreation trout fishery. As a result, at least 20 species of fish were extirpated including the now federally threatened Yellowfin Madtom (*Noturus flavipinnis*), and endangered Smoky Madtom (*N. baileyi*) and Citico Darter (*Etheostoma sitikuense*).

Until a relatively recent discovery of *N. baileyi* in Citico Creek, TN (Bauer et al. 1983) and *N. flavipinnis* in the Powell River, TN (Taylor et al. 1971), both madtom species were believed extinct. Currently *N. baileyi* is known from only a 13.8 km portion of Citico Creek but has been reintroduced into Abrams Creek and Tellico River (Fig. 1; Dinkins and Shute 1996; Shute et al. 2005). *N. flavipinnis* was thought to have been wide spread throughout the upper Tennessee River drainage system (Taylor 1969), but now has a disjunct distribution inhabiting portions of Citico Creek and Clinch and Powell rivers (Etnier and Starnes 1993; Dinkins and Shute 1996). Both *Noturus* species often seek shelter under bedrock crevices making detection and collection difficult (Shute et al. 2005; Davis et al. 2011). Like many other *Noturus* species, *N. flavipinnis* and *N. baileyi* deposit eggs (average clutch size approximates 55 and 36 eggs/nest, respectively) in a cavity and are guarded by the male (Dinkins and Shute 1996). Sexual maturity for both males and females is reached at 1-2 years of age for both species with an average generation time of two for *N. baileyi* and three for *N. flavipinnis* (Etnier and Starnes 1993; Dinkins and Shute 1996). Individuals attain lengths of 68.9mm SL for *N. baileyi* and 96 mm SL for *N. flavipinnis* (Dinkins and Shute 1996).

The federally endangered *E. sitikuense*, is a small (28-64 mm SL) benthic darter species that until recently, was a member of the *Etheostoma percnurum* species complex. It was elevated to species status based on meristic, morphometric, and pigmentation differences among other members of the *E. percnurum* complex (Blanton and Jenkins 2008). The distribution of *E. sitikuense* is confined to a 3.5 km stretch of Citico Creek, TN, but has been reintroduced to Abrams Creek and Tellico River (Shute et al. 2005). Like both madtom species, *E. sitikuense* is nocturnal seeking shelter beneath cobble and boulder substrate. Females lay adhesive eggs (19-44) in nesting cavities created and guarded by males beneath rocks and are capable of producing multiple clutches as suggested by

varied egg counts ($n = 23-200$) found on the undersides of rocks (Layman 1991). It is not known when *E. sitikuense* reaches sexual maturity in the wild (sexual maturity of age-1 fish has been observed under hatchery conditions; P Rakes, Conservation Fisheries, Inc, pers. comm.), but appreciable mortality of age 2 adults occurs soon after spawning suggesting that the average generation time is no greater than two. Note that most of the known life history of *E. sitikuense* is from specimens collected from the Little River, TN and that these specimens are now considered as *E. marmopinum* (Blanton and Jenkins 2008). Thus slight variation in the life history may exist between *E. marmopinum* and *E. sitikuense*.

In part due to the rarity of each of these species (both in terms of abundance and distribution), a captive propagation and reintroduction program was initiated by a multi-agency team (in accordance with United States Fish and Wildlife Service recovery plans) in an effort to restore the extirpated Abrams Creek population for each species. Reintroductions began in 1986 for *N. baileyi* and *N. flavipinnis* and 1993 for *E. sitikuense*, and entailed the annual removal of nests from Citico Creek for hatchery grow out and subsequent stocking of offspring in Abrams Creek (see Shute et al 2005 for details). Furthermore, in the spring of 2004, a relicensing settlement agreement for the Tapoco Hydroelectric Project (Federal Energy Regulatory Commission No. 2169) established a fish passage plan for the continued passage of *N. flavipinnis*, *N. baileyi*, and *E. sitikuense* from Citico to Abrams Creek. The rationale by the United States Fish and Wildlife Service was that historic migration occurred between Citico and Abrams populations (prior to the construction of Chilhowee Dam) and that this migration rate should be mimicked and maintained via the reintroduction effort. The objective of this plan, thus, was to move a certain number of each of the targeted fish species' nests (*N. flavipinnis*, *N. baileyi*, and *E. sitikuense*) from Citico Creek to Abrams Creek in an effort to maintain USFWS's goal of one effective genome per generation that was deemed sufficient to obtain genetic mixing between the populations for each species of concern.

Although each species' reintroduction effort appears demographically successful (i.e., the observed occurrence of natural reproduction and multiple age classes; Shute et al. 2005), perceived genetic risks (i.e., loss of genetic diversity due to inbreeding or genetic drift) should be evaluated. For example, the source population generally should have a high degree of genetic diversity and genetic similarity to that of the new or recipient population to offset the potential decrease in average fitness associated with inbreeding and/or a loss of genetic variation (Miller and Kapuscinski 2003). Furthermore, the USFWS recommendation of one effective genome per generation as a measure of fish passage success should be evaluated because this theoretical expectation is based on many simplifying assumptions of which many are routinely violated (Mills and Allendorf 1996; Vucetich and Waite 2000). Finally, an understanding of past and present processes shaping present levels of genetic variation is also critical to management and conservation planning because information gleaned from conservation genetics can assist in the proper design, implementation, and monitoring of management and conservation strategies for imperiled species. For example, populations or species that have undergone population bottlenecks throughout their evolutionary history may have reduced genetic load (i.e., a reduction in mean fitness of a populations resulting from detrimental variation) and be less prone to have inbreeding depression during subsequent population bottlenecks (Hedrick 1994; 2001). As a consequence, such a population may have increased viability and be

more likely to recover from near-extinction/extirpation than a population lacking such a history (Hedrick 2001).

The objective of this study was to assess putative genetic risks associated with the Abrams Creek reintroduction effort for *N. flavipinnis*, *N. baileyi*, and *E. sitikuense* as well as provide base-line genetic data for genetic risk assessment and monitoring of each species inhabiting Citico Creek. These objectives were accomplished by 1) estimating and comparing levels of genetic diversity and divergence between Citico and Abrams Creek populations for each species, 2) estimating effective population size for each species, 3) estimating the level of migration necessary to minimize genetic differentiation between Abrams and Citico creek populations for each species, and 4) understanding the processes that have shaped present estimates of genetic diversity in Citico Creek populations for each species. In doing so, the effectiveness of each reintroduction program and the Tapoco Hydroelectric Project fishway passage strategy can be quantitatively evaluated.

Materials and Methods

Sampling Design

Tissue collections were conducted by Conservation Fisheries, Inc. via mask and snorkel (Dinkins and Shute 1996). Sampling was performed from 2008-2010, encompassed the known range of each fish species in Citico and Abrams creeks, and entailed noninvasive pelvic or caudal fin collections (Table 1). All tissue samples were placed in 95% non-denature ethanol and archived at to the United States Fish and Wildlife Service Conservation Genetics Lab in Warm Springs GA. Genomic DNA was extracted from each fin clip using the DNeasy Blood and Tissue kit (QIAGEN, Inc., Valencia, California) protocol.

Molecular Methods Polymerase chain reaction (PCR) amplification conditions for *N. flavipinnis* and *N. baileyi* followed that of previously outlined protocols (Williams and Moyer In press). Primer information for *N. flavipinnis* and *N. baileyi* can be found in Tables 2 and 3, respectively. For *E. sitikuense*, we used a suite of nine microsatellite markers (Table 4) known to amplify in other *Etheostoma* species (Tonniss 2006; Beneteau et al. 2007; Gabel et al. 2008). PCRs were performed in 8 µL reaction volumes consisting of 30–100 ng of template DNA, 1× Taq reaction buffer (Applied Biosystems Inc.), 3.25 mM MgCl₂, 0.375 mM of each dNTP, 0.50 µM of each primer (Tables 2-4), and 0.0875 U Taq polymerase (Applied Biosystems, Inc.). PCR conditions for Ebl1, Ebl2, Ebl4, Ebl6, Ebl8 were an initial denaturation at 94 °C (10 min.), followed by a touchdown procedure involving 33 cycles and consisting of denaturing (94 °C, 30 s), annealing, and extension (74 °C, 30 s) cycles, where the initial annealing temperature was initiated at 56 °C (30 s), and decreased by 0.2 °C/cycle. For Eca11EPA and Eca13EPA PCR conditions were an initial denaturation at 94 °C (10 min.), then 27 cycles each at 94 °C for 30 s, 64 °C for 30 s and 72 °C for 30 s cycles, followed by an extension step at 72 °C for 10 min. Finally, Esc26b and Esc187 PCR conditions were an initial denaturation at 94 °C (10 min.), then 33 cycles each at 94 °C for 30 s, 54 °C for 30 s and 72 °C for 30 s cycles, followed by an extension step at 72 °C for 10 min.

Prior to electrophoresis, 2 μ L of a 1:100 dilution of PCR product was mixed with a 8 μ L solution containing 97% formamide and 3% Genescan LIZ 500 size standard (Applied Biosystems, Inc.). Microsatellite reactions were visualized with an ABI 3130 genetic analyzer (Applied Biosystems, Inc.) using fluorescently labeled forward primers and analyzed using GeneMapper software v3.7 (Applied Biosystems, Inc.).

Estimation of genetic differentiation Tests for gametic disequilibrium (all pairs of loci per population, where the population was either Abrams or Citico creeks) and locus conformance to Hardy–Weinberg equilibrium (HWE; for each locus in the population) were implemented using GENEPOP v4.0.10 (Raymond and Rousset 1995) for each species. Significance levels for all simultaneous tests were adjusted using a sequential Bonferroni correction (Rice 1989).

We compared basic estimators of genetic diversity for Abrams and Citico creek populations for each species. Specifically, we tested for homogeneity in average allelic richness, observed heterozygosity, expected heterozygosity, and fixation index between populations. The fixation index along with genetic diversity in the form of per locus observed and expected heterozygosity were calculated using the computer program GenAIEx v6.4 (Peakall and Smouse 2006). The program HP-RARE (Kalinowski 2005) was used to estimate allelic richness. Tests for significance were conducted using the Wilcoxon rank-sum test (Sokal and Rohlf 1995) as implemented in S-Plus v7.0 (Insightful Corporation).

To assess the degree of genetic differentiation between Abrams and Citico creek populations for each species, we first compared per locus genic frequency distributions between populations using the genic differentiation option in GENEPOP v4.0.10 (Raymond and Rousset 1995) with default parameter settings. We also calculated DEST and FST, which are measures of population differentiation based on genetic polymorphism data (Jost 2008; Meirmans and Hedrick 2011), between creeks using the programs DEMETics (Gerlach et al. 2010) and Arlequin v3.5 (Excoffier and Lischer 2010), respectively. Confidence in DEST was assessed via bootstrap resampling (500 replicates as implemented in DEMETics). Analysis of population structure was performed using a Bayesian-based clustering algorithm implemented in the program STRUCTURE v2.3.3 (Pritchard et al. 2000; Falush et al. 2003). The program STRUCTURE assumed no a priori sampling information; rather, individuals were probabilistically assigned to groups in such a way as to achieve Hardy–Weinberg and gametic equilibria. The program STRUCTURE was run with three independent replicates for K (i.e., distinct populations or gene pools), with K set from one to eight. The burn-in period was 50,000 replicates followed by 500,000 Monte Carlo simulations run under a model that assumed no admixture and independent allele frequencies.

Estimation of effective population size The effective population size (N_e) for each population was estimated for each species using the linkage disequilibrium (LD) method (Hill 1981). The measure of LD was that of Burrow's composite measure (Campton 1987) and was estimated for each species using the program LDNe (Waples and Do 2008). Allele frequencies close to zero can affect estimates of N_e (Waples 2006); therefore, we excluded alleles with frequencies less than 0.02 (Waples and Do 2010). Parametric 95% confidence intervals were also calculated using LDNe (Waples and Do 2008; Waples and Do 2010). Note that we first attempted to estimate N_e for each

population but our point estimate in each case was infinity -- an indication that sample size was limited). Note also that we sampled multiple cohorts to obtain our estimate of N_e for each species (a violation of LDNe assumptions), but as pointed out by Waples and Do (2010), a reasonable conjecture is that if the number of cohorts represented in a sample is roughly equal to the generation time for each species, then the LD estimate should roughly correspond to N_e for a generation. Until this conjecture is found true, the estimates of N_e for each species should be treated with caution. Note that the generation time approximates a value of two for *N. baileyi* and *E. sitikuense* and three for *N. flavipinnis* (Etnier and Starnes 1993; Dinkins and Shute 1996).

Testing alternate evolutionary scenarios We tested whether the lack of genetic diversity in Citico Creek species (see below) was attributed to a recent or more historical bottleneck by testing competing hypothesis (Fig. 2) in an approximate Bayesian computation (ABC) framework (Beaumont et al. 2002), as implemented by the program DIY ABC v1.0.1.34beta (Cornuet et al. 2008). This approach was employed to model evolutionary scenarios given a distribution of values for each parameter (discussed below) and summary statistics based on the observed microsatellite data. Summary statistics included average number of alleles, expected heterozygosity, allele size variance across loci, and M-index (Garza and Williamson 2001). The ABC method entailed generating simulated data sets (based on the microsatellite data for each species collected from Citico Creek), selecting simulated data sets closest to observed data set, and estimating posterior distributions of parameters through a local linear regression procedure (Beaumont et al. 2002; Cornuet et al. 2008). In doing so, this approach provided a way to quantitatively compare alternative evolutionary scenarios.

The ABC approach relied on prior knowledge of the following four parameters: ancestral effective population size (N_{ea}), contemporary effective population size (N_e), effective population size during a bottleneck (N_{eb}) and time of bottleneck (T , in generations). The parameter N_{ea} was modeled as having a distribution bounded by 436-2180 for each species. This parameter was unknown for each species; therefore, we chose to model it using a very broad uniform distribution. The upper end of this bound was based on tripling the upper 95% confidence interval estimate of the census size for *N. flavipinnis* in Citico Creek (1453 adults; Dinkins and Shute 1996) and an understanding that the effective size is often 10-50% of the census size (i.e., $1453 \times 3 \times 0.10 \approx 436$; $1453 \times 3 \times 0.50 \approx 2180$; (Palstra and Ruzzante 2008). Our observed distribution for N_e for each species was based on the LDNe 95% confidence interval estimate of this parameter (below). Note that LDNe estimated the upper confidence interval of N_e for *N. flavipinnis* as infinity, so we arbitrarily set the upper bound for the N_e distribution in the DIY ABC analysis as 50% of the current census estimate of 1453 (note we used the upper 95% CI census estimate found by Dinkins and Shute to incorporate any measure of uncertainty). The parameter T took on differing values depending on the evolutionary scenario. For the first two scenarios (A and B; Table 8) we model either a gradual decrease in genetic diversity (scenario A) or an historic (Pleistocene) bottleneck. For these scenarios we chose to set T to 3333 generations for *N. flavipinnis* and 5000 for *E. sitikuense* and *N. baileyi*, which was representative of the end of the most recent glacial event approximately 10,000 years ago (i.e., 10,000 years divided by a three or two year generation time; Etnier and Starnes 1993; Dinkins and Shute 1996). Scenario C was modeled as a bottleneck occurring during the construction of a concrete dam in 1973; thus T was

set to 12 generations for *N. flavipinnis* and 18 for *E. sitikuense* and *N. baileyi* (2009-1973 = 36 years divided by a two or three year generation time). Finally, scenario D described a more historical event – the intensive land use in 1900 that left much of the region deforested (T set to 36 or 55 depending on generation time). After the initial time of the bottleneck, Neb was assumed to be between a value of 2 and 50 and the duration of the bottleneck was modeled as having a uniform distribution from either [1-4999], [1-3332], [1-17], [1-12], [1-35], or [1-54] generations depending on the evolutionary scenario and generation time of each species.

We simulated 1,000,000 datasets per scenario for each species (via DIY ABC) to produce reference datasets using uniform priors for each parameter (Table 8). Prior information regarding the mutation rate and model for microsatellites was taken as default values in DIY ABC. The posterior distribution of each scenario was estimated using local linear regression on logit transformed data for the 10,000 simulated datasets closest to the observed dataset (Cornuet et al. 2008). The exact posterior probability of each scenario was reliant on the model that generated the posterior probability distribution; therefore, poor model fit could lead to inaccurate estimation of the models posterior distribution and subsequent model choice (Cornuet et al. 2010). As recommended by Cornuet et al. (2010), we employed the model checking function of DIY ABC to assess the goodness-of-fit between each model parameter posterior combination and the observed dataset by using different summary statistics for parameter estimation and model discrimination. The parameter estimation summary statistics used were M-index and allele size variance, while the model discrimination summary statistics were average number of alleles and average expected heterozygosity.

Assessment of one genome per generation strategy We assessed the effectiveness of the pre-specified management recommendation that one genome per generation be exchanged between populations via coalescent simulations. Ten microsatellite loci were simulated ($n = 100$ simulations) for two populations using SIMCOAL2 v2.1.2 (Laval and Excoffier 2004) to assess the amount of migration necessary to maintain current levels of genetic diversity and minimize population differentiation. We assumed a diploid model for which two populations diverged either 10, 30, or 50 generations ago. For each divergence date we assumed either a one-way (simulating the movement of genes from Citico to Abrams) or two-way migration model and assessed the degree of expected genetic differentiation (as estimated by F_{ST}) for four migration rates (0.00, 0.01, 0.02, and 0.05). Input values for effective population and sample sizes for each population were 75 and 30 (respectively) and were similar to observed values (see below). For each simulated dataset, F_{ST} was estimated using ARLEQUIN v3.5.

Results indicated that a migration rate of 0.05 was necessary to minimize genetic differentiation among populations; however, the model assumed constant population size. To assess the effects of population growth on genetic differentiation, we evaluated three differing growth rates (0.009, 0.09, and 0.18) over 10, 30, and 50 generations using a migration rate of 0.05 (all other parameters and assumptions are as above). The program SIMCOAL2 uses a continuous exponential growth model with a growth parameter (r) to simulate growth; thus the growth rates used in our study correspond to discrete growth parameter (λ) values of 1.01, 1.1, and 1.2, respectively.

Results

Estimation of genetic differentiation -- *N. flavipinnis*. A total of 59 *N. flavipinnis* were analyzed using 21 microsatellite markers (n = 30 Citico Creek; n = 29 Abrams Creek; Tables 1 and 2). For each population, all loci conformed to per locus HWE after sequential Bonferroni corrections (all $P > 0.17$) except Nfl D146. Microsatellite marker Nfl D146 was monomorphic for allele 234 except two individuals from Citico Creek had genotype 246/254. There was no evidence of gametic disequilibrium after sequential Bonferroni correction (all $P > 0.006$; n = 43 comparisons per population for an $\alpha = 0.001$).

A comparison of genetic diversity between Citico and Abrams Creek samples (Table 5) showed no significant differences in average allelic richness (1.507 vs. 1.506; $P = 0.90$), average observed heterozygosity (0.110 vs. 0.106, $P = 0.51$), or average expected heterozygosity (0.111 vs. 0.108, $P = 0.70$). There was also no significant difference in the average fixation index (0.077 vs. 0.045, $P = 0.61$).

Genic differentiation between Citico and Abrams creeks was significant ($P > 0.001$) with three (Nfl C143, Nfl C145 and Nfl D139) of 21 loci causing the significance. The value of DEST (averaged across loci) between Citico and Abrams creeks was 0.012 and significantly different ($P = 0.001$) from zero. As above, loci driving this significance were Nfl C143, Nfl C145, and Nfl D139. The value of F_{ST} was 0.018 and not significant ($P = 0.198$). The program STRUCTURE revealed that the most probable number of groups was one, as the proportion of sampled individuals to each sampling site was symmetrical for all K-values 2-4 (data not shown) -- an indication that Abrams and Citico creeks are essentially the same population (Evanno et al. 2005).

Estimation of genetic differentiation -- *N. baileyi*. A total of 87 *N. baileyi* were analyzed using ten microsatellite markers (n = 64 Citico Creek; n = 23 Abrams Creek; Tables 1 and 3). For Abrams Creek, all loci conformed to per locus HWE after sequential Bonferroni corrections (all $P > 0.07$). Quite the opposite was found for Citico Creek samples -- four (Nfl D109, Nfl A12, Nfl C120, and Nfl C135) of ten loci deviated significantly from HWE. Deviations from HWE are presumably due to a high degree of relatedness among a large portion of individuals (i.e., 38 captive individuals whose tissues were preserved on the same date were collected after the juveniles had been through several tank moves and no record was kept to be able to elucidate sibships; P. Rakes, CFI., pers. comm.). There was no evidence of gametic disequilibrium after sequential Bonferroni correction (all $P > 0.004$; n = 28 comparisons per locality for an $\alpha = 0.002$).

A comparison of genetic diversity between Citico and Abrams Creek samples (Table 6) showed no significant differences in average allelic richness (2.59 vs. 2.08; $P = 0.38$), average observed heterozygosity (0.162 vs. 0.178, $P = 0.63$), or average expected heterozygosity (0.195 vs. 0.177, $P = 0.73$). While the average fixation index was greater for Citico Creek samples, presumably due sampling related individuals, the disagreement was not significant (0.255 vs. 0.054, $P = 0.08$).

Significant differences ($P > 0.001$) in allelic distributions were found for two of ten loci (Nfl D109 and Nfl C120). The value of DEST (averaged across loci) between Citico and Abrams samples was

0.035 and significantly different ($P = 0.001$) from zero. The value of F_{ST} was 0.060 and significant ($P < 0.001$). As above, the loci driving this significance were Nfl D109 and Nfl C120. The program STRUCTURE revealed that the most probable number of groups was one.

Estimation of genetic differentiation -- *E. sitikuense*. A total of 56 *E. sitikuense* were analyzed using nine microsatellite markers ($n = 25$ Citico Creek; $n = 31$ Abrams Creek; Tables 1 and 4). All loci conformed to per locus HWE for each locality (all $P > 0.08$). There was no evidence of gametic disequilibrium after sequential Bonferroni correction (all $P > 0.01$; $n = 15$ comparisons per locality for an $\alpha = 0.003$).

A comparison of genetic diversity between Citico and Abrams Creek samples (Table 7) showed no significant differences in average allelic richness (2.67 vs. 2.62; $P = 1.00$), average observed heterozygosity (0.246 vs. 0.227, $P = 0.86$), or average expected heterozygosity (0.228 vs. 0.225, $P = 0.93$). There was also no significant difference in the average fixation index (0.066 vs. 0.092, $P = 0.61$).

There were significant differences in allelic distributions between Citico and Abrams samples ($P = 0.005$). Four (Ebl 6, Eca 13EPA, Ebl 4, and Esc 26B) of nine loci appeared to be causing the significance. The value of DE_{ST} (averaged across loci) between Citico and Abrams samples was 0.025 and significantly different ($P = 0.004$) from zero. The value of F_{ST} was 0.020 and significant ($P = 0.027$). The loci driving this significance were the same as that in the allelic distribution test. The program STRUCTURE revealed that the most probable number of groups was one.

Estimation of effective population size Estimates of N_e via LDN_e for *N. flavipinnis*, *N. baileyi*, and *E. sitikuense* were 75 (15-infinity), 72 (29-691), and 46 (19-291), respectively (95% confidence interval in parentheses). Note that negative estimates of N_e occur when the genetic results can be explained entirely by sampling error without invoking any genetic drift, so the biological interpretation of a negative value is an N_e of infinity (Waples and Do 2010).

Testing alternate evolutionary scenarios We were interested in testing whether the observed genetic variation present in Citico Creek populations for each species could be attributed anthropogenic events (e.g., the construction of an impoundment); therefore, we tested four alternative evolutionary scenarios that might explain the observed genetic variation (Table 8, Fig. 2). Scenario D (model depicting deforestation during the 1900s) had the greatest posterior probability for two of three species; however, no scenario produced a significant posterior probability (i.e., $>95\%$) when compared to other competing evolutionary scenarios (Table 8). Our assessment of model misfit indicated that several test quantities had low tail-area probabilities when applied to scenarios A-C for each species (Table 8) casting serious doubts on the adequacy of the tested model-posterior combination. For example, scenario B for *N. flavipinnis* had the greatest posterior probability among competing scenarios; yet, both test quantities (no. alleles and heterozygosity) were significantly different from observed values suggesting that this scenario inadequately explained the observed pattern of genetic diversity found in this species. Finally, scenario D had the greatest posterior probability for each species when comparing it only to scenario C (i.e., recent dam construction vs. 1900 deforestation; Table 8).

Assessment of one genome per generation strategy Results of computer simulations for one-way migration assuming no population growth in both populations are summarized in Figure 3. As expected with no migration, our two populations diverged significantly at 30 generations and beyond. A similar pattern was observed for migration rates of 0.01 and 0.02 (Fig 3A). In contrast, a migration rate of 0.05 maintained a value of F_{ST} that was non-significant among 10, 30, and 50 generations (Fig. 3A). Incorporating population growth in our one-way migration model resulted in values of F_{ST} that were less than that of assuming no growth (except for $r = 0.18$ for 30 generation model; Fig 3B). In all cases, however, the average value of F_{ST} was not significantly different (i.e., overlapping 95% confidence intervals) than the estimated value assuming no growth (Fig 3B).

Results for two-way migration assuming no population growth in both populations are summarized in Figure 4. A migration rate of 0.05 maintained a value of F_{ST} that was non-significant among 10, 30, and 50 generations (Fig. 4A). Incorporating population growth in our two-way migration model resulted in values of F_{ST} that were similar to or less than that of assuming no growth (Fig. 4B).

Discussion

Abrams Creek reintroduction program

Metapopulation theory (Levins 1969) emphasizes the importance of connectivity between seemingly isolated wild populations. No single population may be able to guarantee the long-term survival of a given species, but the combined effect of many populations may be able to do this. In accordance with metapopulation theory, the United States Department of Interior's prescription for fishway pursuant to section 18 of the Federal Power Act for the Tapoco Project (P-2169) adopted a fishway passage strategy for *N. flavipinnis*, *N. baileyi*, and *E. sitikuense*. This strategy included translocation of target species' nests (individuals) from Citico to Abrams Creek with the intent that genetic mixing (i.e., connectivity) between populations would transpire.

If genetic mixing was occurring on a per generation basis, then we would expect a high level of genetic similarity between Citico and Abrams populations for each species. Estimates of genetic diversity (i.e., allelic richness and expected heterozygosity) were similar between each population for each species and suggest that each species' reintroduction program has been successful in the preservation of genetic diversity between source and founding populations. However, these indices provide little information about the degree of differentiation between populations. For example, each population could have the same number of alleles, but the identity of each allele may perhaps be different between the populations indicating significant differentiation between populations. Allelic distribution tests, the measure of DEST or FST, and STRUCTURE results should provide an understanding of these patterns and provide a better measure of differentiation between populations.

Measures of population differentiation produced somewhat conflicting results. Allelic distribution tests and estimates of DEST or FST (except for *N. flavipinnis*) indicated significant differentiation between Abrams and Citico creek populations whereas our STRUCTURE result, which assigned individuals to groups in such a way that Hardy Weinberg and genotypic equilibrium were achieved, indicated no significant population structure between samples from Abrams and Citico creeks. The interpretation of significant genetic differentiation must be viewed with caution. While allelic frequency distributions and DEST estimates were found to be significant between populations, the amount of differentiation inferred by DEST was minimal (DEST scales from zero to one with zero being no differentiation and one being complete differentiation) calling in to question whether this level of differentiation is biologically meaningful. Furthermore, the loss of alleles (which was minimal in our study) is potentially much more detrimental to a population than allele frequency differences between populations because lost alleles can be recovered only by migration or mutation. Thus, while genetic differences were observed between Abrams and Citico populations for each species, the fish passage strategy appears to be capturing a large portion of the neutral genetic variation observed in each species inhabiting Citico Creek.

The cause of such genetic differentiation between populations arises due to the sampling phenomenon of genetic drift. Genetic drift is defined as the random changes in allele frequencies of a population between generations due to the binomial sampling of genes during meiosis (Allendorf and Luikart 2007); thus, genetic drift is more pronounced in small populations. Minimizing genetic

drift is a primary concern when attempting to reestablish a population because utilizing too few brood stock (termed a founder effect and is a special case of genetic drift; Allendorf and Luikart 2007) or observing large differences in reproductive success among brood stock will cause changes in allele frequencies (if not loss of genetic diversity) between founder and source populations. To avoid potential founder effects, a reasonable estimate of 30-50 individuals has often been recommended to capture most allele frequencies in the source population (Miller and Kapuscinski 2003); however, often as many as 100-200 parents are recommended in order to capture multiple low frequency alleles (Miller and Kapuscinski 2003; Allendorf and Luikart 2007); in the population. The total number of nests sampled over the years for the reintroduction program has been greater than 30 for each species (approximately 48, 150, and 40 nest for *N. flavipinnis*, *N. baileyi* and *E. sitikuense*; pers. comm. P. Rakes, Conservation Fishes Inc.). These values equate to a minimum of 96, 300 and 80 brood parents (respectively) assuming one female and one male contributed to the nest. Thus sampling effects due to too few brood stock should have been minimized for this reintroduction effort and cannot explain the observed frequency differences between species in Abram and Citico creeks. Yet, many of these collected nests did not produce offspring (pers. comm. P. Rakes, Conservation Fishes Inc); thus greater than expected variance in reproductive success (i.e., larger than binomial variance of family size) may be attributing to the observed differentiation in allele frequencies between Abram and Citico creek populations. In conclusion, while there appeared to be significant genetic differentiation between the source and introduce population, much of the observed differences were likely explained by a greater than expected variance in reproductive success. Future monitoring efforts should therefore focus on assessing the degree of differential reproductive success that might be occurring in the hatchery and if necessary, take steps to equalize family contributions in order to minimize variance in reproductive success (Allendorf 1993).

Genetic variation of Citico Creek species

Genetic variation is important in maintaining the adaptive potential of species/populations and the fitness of individuals to help ensure their survival (Frankel and Soule 1981; Frankham 2005; Laikre 2010); its importance is reflected by the International Union for Conservation and Nature's recognition that genetic diversity is an essential component of biodiversity (McNeely et al. 1990). The observed low level of genetic diversity found in *N. flavipinnis*, *N. baileyi*, and *E. sitikuense* from Citico Creek is an indication that past processes have greatly influenced current levels. A simple comparison of average number of alleles and observed heterozygosity values summarized in other freshwater fishes (9.1 and 0.54, respectively; (DeWoody and Avise 2000) suggest that species in our study have undergone a past bottleneck event. The low level of heterozygosity found in each species was quite intriguing because heterozygosity is relatively insensitive to the effects of bottlenecks (Allendorf 1986); thus the bottleneck must have been extremely intense and/or have occurred for a long duration. We chose to model differing evolutionary scenarios to help explain this discrepancy. Results indicated that the observed lack of genetic diversity was better explained by contemporary rather than more historic processes. Specifically, only scenario D (i.e., modeled during the 1900s and a period of significant deforestation around Citico Creek) had a relatively high posterior probability of support and had all simulated test quantities equaling that of observed – a pattern consistent for each examined species. In contrast, Pleistocene events or a gradual reduction in genetic diversity since the Pleistocene often had posterior probabilities less than that of scenario D,

as well as test quantities that were significantly different from observed values. These findings indicated that anthropogenic events during the 1900s attributed to the lack of genetic variation seen today for *N. flavipinnis*, *N. baileyi*, and *E. sitikuense* from Citico Creek and highlighted the need to protect these species and their respective habitat from future demographic bottlenecks as witnessed in the 1900s.

Management recommendations

Abrams Creek population

Our findings indicated observed allele frequency differences between Citico and Abrams creek populations of *N. flavipinnis*, *N. baileyi*, and *E. sitikuense* that were likely the result of larger than binomial variance of family size occurring either in the hatchery or wild (or both). Minimizing this variance (if it is occurring in the hatchery) can be achieved to some degree by implementing hatchery protocols that attempt to better maintain and monitor equal family contributions prior to release. A hatchery protocol that rears each family/nest separately is the simplest method to maintain and monitor variance in family size; however, if tank space is a limiting factor then all individuals should be marked in such a way as to distinguish family of origin. When combining families is deemed necessary, family contributions should be equalized but only after significant periods of mortality have passed (often after the critical early life history stages). Families could be combined incrementally as space dictated. Ideally, the numbers of offspring to rear per family should be determined a priori based on expected survival rates during incubation and rearing so that a target stocking number is attained with all families contributing equally throughout the entire period of stocking. However, equalization of family sizes ($\pm 5\%$) at stocking does not necessitate reduction of all families to the size of the smallest annual production group. Doing so could unduly compromise the intended demographic benefits of the effort. Instead, offspring from those families that are below the target number will simply be underrepresented and will likely necessitate the rearing of additional families in future years to meet propagation targets. Further, the numbers stocked from other families should not be increased to make up for this shortfall but should be kept as targeted originally. In all cases, hatchery rearing protocols should be assessed and refined so that documentation of individual family sizes upon stocking are recorded to monitor and assess the variance in annual family contribution of hatchery reared individuals. In doing so, genetic drift and the loss of genetic diversity via hatchery reintroductions should be minimized in Abrams Creek species of concern.

The intent of the fish passage strategy adopted by the Federal Energy Regulatory Commission relicensing agreement was to provide genetic mixing (i.e., connectivity) between Abrams and Citico creek for populations of *N. flavipinnis*, *N. baileyi*, and *E. sitikuense*. Upon implementation of the fishway passage strategy, no genetic information existed to quantify the rate of exchange of each focal species between Abrams and Citico; therefore, a target objective of one effective genome (migrant) per generation was established. The one-migrant-per-generation rule has been applied widely to species conservation plans (Mills and Allendorf 1996); however many of the assumptions of this model are often unrealistic and violated, drawing into question its interpretation and implementation in a conservation context (Vucetich and Waite 2000; Wang 2004). In an effort to more adequately address this rate of exchange between Abrams and Citico populations of concern,

we assessed the level of migration necessary to impede significant population divergence over 50 generations (given the current levels of genetic diversity found in our focal species). Our simulation approach revealed that approximately a 5% migration rate per generation (or $0.05 \times$ an N_e of 75 $\approx$ four individuals per generation) was necessary to offset the influence of genetic drift over the course of 50 generations (approximately 100-150 years given the species of concern). It is important to note that the estimated value of four migrants per generation (approximately two migrants per year) is the number of individuals that successfully migrate between populations and reproduce, resulting in gene flow. Thus, to successfully achieve the goal of genetic mixing for Citico and Abrams creek populations, the introduction of offspring from more than four nests per generation will be necessary – how much more is dependent on a clearer understanding of average survival rates for hatchery reared individuals inhabiting Abrams Creek and should be an area of future research. Also of importance is a better understanding of N_e for Abrams and Citico creek populations. Current simulations assumed both populations (for each species) had an N_e of 75 because insufficient data were available to estimate N_e for each population. Accuracy of N_e estimation (and subsequent migration simulations) relies on either increasing sample sizes or the number of molecular markers (Waples and Do 2010). The former may be hard to accomplish given the difficulties of sampling these species; however, new genomic approaches may offer a feasible way to increase the number of markers (Hohenlohe et al. 2011). In doing so, a better estimate of the effective number of migrants will be achieved for each species.

Citico Creek population

Noturus baileyi and *E. sitikuense* present a difficult and somewhat unique conundrum – except for the introduced Abrams and Tellico Creek populations, they are only found in Citico Creek (in 13.8 and 9.6 river km stretch, respectively), are in relatively low numbers, exhibit low levels of neutral genetic variation, and this variation has been lacking for at least 50-100 generations. We must be reminded that extinction is a demographic process and protection of these species from human-induced habitat loss and habitat modification should be of high priority. Fortunately, reintroduction efforts in Abrams and Tellico creeks appear successful (i.e., increasing population size and similar levels of genetic diversity; Shute et al 2005, this study), which eases the risk of extinction for the abovementioned species. However, small populations are often vulnerable to random fluctuations in demographic, environmental, and genetic processes -- all of which are not mutually exclusive and can influence the rate of extinction (Gilpin and Soule 1986; Reed 2010). Our study was concerned with assessing the risk of genetic conditions (inbreeding depression, loss of genetic variation, genetic load) likely to influence population persistence. General conservation goals based on genetic considerations are frequently established at an $N_e = 50$ to minimize inbreeding depression and an $N_e = 500$ to maintain sufficient evolutionary potential (Franklin 1980; Franklin and Frankham 1998). The empirical point estimate of N_e for each species in this study approximated the critical threshold level for inbreeding, but it was less than other estimates of N_e for populations of conservation concern (Palstra and Ruzzante 2008). These findings suggest that inbreeding depression could be of immediate importance to the persistence of Citico Creek species that we examined. Yet, the relative risk of inbreeding depression for each species appears lower if we consider the demographic history of the organisms. Our ABC analyses suggest that species inhabiting Citico Creek have been isolated and confined at small population sizes for 50-100 generations. Most detrimental variants of medium

and large effect have presumably been purged over the course of 50-100 generations. Such populations in theory should show limited lowered fitness upon inbreeding (Hedrick 2001; Glémin 2003); thus the risk of extinction/extirpation due to inbreeding depression appears low (note that there still is a risk of inbreeding depression because alleles with minor effects can still persist in the populations). In contrast, the risk of extinction from low genetic diversity and genetic load (i.e., a reduction in mean fitness resulting from detrimental variation for a population when compared to other populations; Hedrick 2001), while not of immediate concern, raises alarms regarding the long-term persistence of these species (Hedrick 2001; Hedrick and Fredrickson 2010; Reed 2010).

Although low genetic diversity and potential elevated genetic load are of concern, predicting the actual risk is difficult and implementing steps to minimize risk are often contentious. Difficulty and contention arise because one way to increase genetic diversity (other than via the mutational process) and lower the genetic load in a population involves the introduction of unrelated individuals from another population or closely related species (often termed genetic rescue, Hedrick 2001, Hedrick and Fredrickson 2010). Genetic rescue is not the ultimate solution for recovery of endangered species; rather, it provides for a temporary increase in population size with the intent of lowering the probability of extinction and providing time to correct the actual problem(s) associated with endangerment (Hedrick and Fredrickson 2010). Instead, maintaining or increasing the census size and effective population size should minimize risks associated with the loss of genetic diversity and genetic load (Lande 1994; Hedrick and Fredrickson 2010). We, therefore, advocate the need for defining and protecting critical habitat for these species and monitoring of both the census and effective populations size for this population. Monitoring temporal fluctuations in population genetic metrics or other population data generated using molecular markers is an integral tool for the conservation of threatened or endangered species because it can provide for 1) an understanding of the present and historical levels genetic diversity in a population or species (e.g., prior to release of hatchery individuals), 2) an assessment of the alteration of these characteristics (i.e., perhaps due anthropogenic factors), and 3) an evaluation of the biological consequences of management and conservation initiatives (Schwartz et al. 2007; Laikre et al. 2010).

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Table 1. Sample size (*N*), locality, and sampling information of fishes used to estimate indices genetic diversity.

Species	Drainage	<i>N</i>	Year sampled
<i>Noturus baileyi</i>	Abrams Creek	16	2009
		7	2010
	Citico Creek	48	2008
		13	2010
		3	2010
<i>Noturus flavipinnis</i>	Abrams Creek	4	2009
		25	2010
	Citico Creek	17	2008
		4	2009
		12	2010
<i>Etheostoma sitikuense</i>	Abrams Creek	31	2010
	Citico Creek	25	2010

Table 2. Molecular microsatellite markers used to estimate genetic diversity for *N. flavipinnis*. The abbreviation (bp) represents base pairs.

Locus	Primer sequence(5'-3')	Repeat Motif	Size Range (bp)	Citation
<i>Nfl</i> A3	F: CCATTTTGGTCAACTACCTG R: GAGGGTTAGCATCACAGAAGT	AAC	160	Moyer et al (2011)
<i>Nfl</i> A9	F: TGCCTCCAGTGCTGTAGT R: CGAGCGTATTTCAATCTTTC	AAC	225	Moyer et al (2011)
<i>Nfl</i> A12	F: GACCTGATTGAGTCAGAATGAC R: AAATTCCACTGCACACTTAGAG	AAC	241	Moyer et al (2011)
<i>Nfl</i> C1a	F: AAAGCAAAAGAGCCGTA AAAAG R: TGACCCTGAAAAGGAGTAAGC	CATC	174	Moyer et al (2011)
<i>Nfl</i> C7	F: TGACCCTGAAAAGGAGTAAGC R: GGTGTGAGGAAACCAGAGAAC	CATC	177	Moyer et al (2011)
<i>Nfl</i> C119	F: ATGCCCTCTTGTGTTCTGG R: GAGTGGGTGTGTGTGTGATG	CATC	200	Moyer et al (2011)
<i>Nfl</i> C122	F: CCGTGACACTGAAAGGAAG R: CTGTGATGGTCTATGG	CATC	141-149	Moyer et al (2011)
<i>Nfl</i> C126	F: AGCAGTTCTGTCAAGTGCCTTAG R: ATTCCACATTCCACAATCTACG	CATC	183	Moyer et al (2011)
<i>Nfl</i> C138	F: GGATTGCCTTGTAAGTCCAAC R: AACCCTAAGTGCTGATGCTG	CATC	207	Moyer et al (2011)
<i>Nfl</i> C143	F: AATGGAGCAATGGGTGAAAC R: TGATGGGCGTGTCTAAAGTG	CATC	262-266	Moyer et al (2011)
<i>Nfl</i> C145	F: TGACCCTGAAAAGGAGTAAGC R: AAGCAGTCGTTCCCTCACTAG	CATC	236-246	Moyer et al (2011)
<i>Nfl</i> D105	F: CCAGAGCATTAAAGAAGAGTAGG R: GGAGTTGATCCAATTTGTTG	TAGA	259-263	Moyer et al (2011)
<i>Nfl</i> D109	F: AGTGCGACAGACAAAGTTTG R: CCTGGGGGATCAATATAGTATC	TAGA	127	Moyer et al (2011)
<i>Nfl</i> D123	F: GCTTTTTGTCCATTTATCTCTG R: GCAACCCTGATTGGATTC	TAGA	270-274	Moyer et al (2011)
<i>Nfl</i> D129	F: TGCAGTTCCAGCTCTTAAAC R: TCCTTGGGGGTAAATGTAA	TAGA	237	Moyer et al (2011)
<i>Nfl</i> D137	F: AGCGCACAAAAATGTACG R: CGGGCTCTAAATACTGTGG	TAGA	235	Moyer et al (2011)
<i>Nfl</i> D138	F: GTAGAAATGCGACACAGACAC R: GACCCTGAAAAGGAGTAAGC	TAGA	250-274	Moyer et al (2011)
<i>Nfl</i> D139	F: ACTGAATGGCAGGCTTAGA R: ACAAGGGCAAGAGGTGAC	TAGA	227-244	Moyer et al (2011)
<i>Nfl</i> D140	F: GTTTGGTCTGTGAGGGTAATC R: TTTATTTTGGTGCGATGTG	TAGA	282	Moyer et al (2011)
<i>Nfl</i> D145	F: ATGGATGGATGGATGGATC R: TCACGTTTACAGAGTGGAACAG	TAGA	255-259	Moyer et al (2011)
<i>Nfl</i> D146	F: TGTGTTTTGTGCGACTACTGTG R: CTTATCAGGGGCTTCTGTCTGT	TAGA	226-234	Moyer et al (2011)

Table 3. Molecular microsatellite markers used to estimate genetic diversity for *N. baileyi*. The abbreviation (bp) represents base pairs.

Locus	Primer sequence(5'-3')	Repeat Motif	Size Range (bp)	Citation
<i>Nfi</i> A12	F: GACCTGATTGAGTCAGAATGAC R: AAATTCCACTGCACACTTAGAG	AAC	226-246	Moyer et al (2011)
<i>Nfi</i> D109	F: AGTGCGACAGACAAAGTTTG R: CCTGGGGGATCAATATAGTATC	TAGA	112-144	Moyer et al (2011)
<i>Nfi</i> D129	F: TGCAGTTCCAGCTCTTAAAC R: TCCTTGGGGGTAAATGTAA	TAGA	254	Moyer et al (2011)
<i>Nfi</i> A10	F: TTGTCGCTGTGGTGATACC R: TTTCCTTATTGCCCTCGTG	AAC	160-168	Moyer and Williams unpublished data
<i>Nfi</i> C120	F: GCATCTTCGACATATTTGACCT R: CCCTGGCTCTTAATGTATCATG	CATC	205	Moyer and Williams unpublished data
<i>Nfi</i> C135	F: GGCTGTCTTTACCTGTTCAG R: TCGTCCATAGTGTGTGATTG	CATC	254-270	Moyer and Williams unpublished data
<i>Nfi</i> C142	F: GTGCCCTGTGATGGACTG R: TGCTGGTTGTGCTAAGACG	CATC	273-293	Moyer and Williams unpublished data
<i>Nfi</i> D2	F: ACGGTCTTTCTCAGTGATTG R: ATTACCACAGATTTTCCTCAGA	TAGA	105-145	Moyer and Williams unpublished data
<i>Nfi</i> D9	F: CATTAAAGCATGGACGAGTTTA R: GGTTTCCCTACGATGTAGAGC	TAGA	206-214	Moyer and Williams unpublished data
<i>Nfi</i> D120	F: CACCAATTAGCCATTTAGCAG R: CAAGATATGGGTGGGTGTATG	TAGA	145-157	Moyer and Williams unpublished data

Table 4. Molecular microsatellite markers used to estimate genetic diversity for *E. sitikuense*. The abbreviation (bp) represents base pairs.

Locus	Primer Sequence (5'-3')	Repeat Motif	Size Range (bp)	Citation
<i>Ebl</i> 1	F: CCCTTTCGTAACCCTTTTTCA R: GGGACCAGATGCTGTGAGAT	(CA) ₁₂	237-258	Beneteau et al. (2007)
<i>Ebl</i> 2	F: TGGTGCGACTGAACAAGAAC R: TACCACAACCACCTGCATTC	(AC) ₂₈	150	Beneteau et al. (2007)
<i>Ebl</i> 4	F: TGTGACTGATATTTTGCTGCTG R: TGCATATCAAGATTCCCATTG	(TATC) ₇ GT(TCTA) ₇	156-164	Beneteau et al. (2007)
<i>Ebl</i> 6	F: TATCATCCCATCGTCTGTCTG R: TGGCCCAACAACAAGCTG	(GT) ₂₂	244-280	Beneteau et al. (2007)
<i>Ebl</i> 8	F: ACAGGTATTAGGGCATTAGCA R: CGTTCAAGTGGCATCAGAGA	(CA) ₇ CG(CA) ₃ CG(CA) ₅	138-154	Beneteau et al. (2007)
<i>Eca</i> 11EPA	F: CGGGCCAGGTTGGTTTAAAGT R: GCAGAAGCACAGGAAAGCACCCCTCAA	(GATA) ₁₆	180-198	Tonnis (2006)
<i>Eca</i> 13EPA	F: CAGAAGCCCAAGAATGGTA R: TGTGTAAGTATATTTTGCTGCTG	(TAGA) ₁₇	182-190	Tonnis (2006)
<i>Esc</i> 26B	F: CAATGCGCCACATTGAGAAGG R: GCACAACATATGTCGTTAAGCTCC	(TAGA) ₂₇	192-232	Gabel et al. (2008)
<i>Esc</i> 187	F: ATCGGCCAGCCCTACTCTG R: GGTGATCAGTCTGGACCACAGC	(GTCT) ₁₃	182-200	Gabel et al. (2008)

Table 5. Comparison of population genetic parameters for sampled *N. flavipinnis* in Citico and Abrams creeks, TN. Abbreviations *N*, *Ar*, *Ho*, *He* and *F* represent the number of samples genotyped, allelic richness, observed heterozygosity, expected heterozygosity and fixation index. Note that average values between localities were not significantly different from one another.

Locality	Locus	<i>N</i>	<i>Ar</i>	<i>Ho</i>	<i>He</i>	<i>F</i>
Citico Creek	<i>Nfl A9</i>	30	1.000	0.000	0.000	
	<i>Nfl C122</i>	30	1.000	0.000	0.000	
	<i>Nfl C119</i>	30	1.000	0.000	0.000	
	<i>Nfl C143</i>	30	2.793	0.310	0.392	0.208
	<i>Nfl C1a</i>	30	1.000	0.000	0.000	
	<i>Nfl C7</i>	30	1.000	0.000	0.000	
	<i>Nf D105</i>	30	1.960	0.069	0.067	-0.036
	<i>Nfl D123</i>	30	2.000	0.154	0.204	0.246
	<i>Nfl D129</i>	30	2.000	0.000	0.000	
	<i>Nfl D139</i>	30	2.000	0.567	0.495	-0.145
	<i>Nfl D145</i>	30	2.000	0.310	0.383	0.191
	<i>Nfl C126</i>	30	1.000	0.000	0.000	
	<i>Nfl D138</i>	30	1.000	0.000	0.000	
	<i>NflC145</i>	30	2.000	0.667	0.444	-0.500
	<i>Nfl A12</i>	30	1.000	0.000	0.000	
	<i>Nfl D140</i>	30	1.000	0.000	0.000	
	<i>Nfl D146</i>	30	2.897	0.067	0.127	0.474
	<i>Nfl C138</i>	30	2.000	0.179	0.219	0.184
	<i>Nfl D137</i>	30	1.000	0.000	0.000	
	<i>Nfl A3</i>	30	1.000	0.000	0.000	
	<i>Nfl D109</i>	30	1.000	0.000	0.000	
	Average		1.507	0.110	0.110	0.077
Abrams Creek	<i>Nfl A9</i>	29	1.000	0.000	0.000	
	<i>Nfl C122</i>	29	1.793	0.034	0.034	-0.018
	<i>Nfl C119</i>	29	1.000	0.000	0.000	
	<i>Nfl C143</i>	29	2.000	0.172	0.158	-0.094
	<i>Nfl C1a</i>	29	1.000	0.000	0.000	
	<i>Nfl C7</i>	29	1.000	0.000	0.000	
	<i>Nf D105</i>	29	2.000	0.143	0.191	0.253
	<i>Nfl D123</i>	29	2.000	0.208	0.187	-0.116
	<i>Nfl D129</i>	29	1.000	0.000	0.000	
	<i>Nfl D139</i>	29	2.000	0.308	0.393	0.218
	<i>Nfl D145</i>	29	2.000	0.429	0.436	0.018
	<i>Nfl C126</i>	29	1.000	0.000	0.000	
	<i>Nfl D138</i>	29	1.000	0.000	0.000	
	<i>NflC145</i>	29	2.000	0.130	0.122	-0.070
	<i>Nfl A12</i>	29	1.000	0.000	0.000	
	<i>Nfl D140</i>	29	1.000	0.000	0.000	
	<i>Nfl D146</i>	29	1.980	0.074	0.071	-0.038
	<i>Nfl C138</i>	29	2.821	0.429	0.418	-0.026
	<i>Nfl D137</i>	29	1.000	0.000	0.000	
	<i>Nfl A3</i>	29	1.000	0.000	0.000	
	<i>Nfl D109</i>	29	1.000	0.000	0.000	
	Average		1.506	0.106	0.108	0.045

Table 6. Comparison of population genetic parameters for sampled *N. baileyi* in Citico and Abrams creeks, TN. Abbreviations *N*, *Ar*, *Ho*, *He* and *F* represent the number of samples genotyped, allelic richness, observed heterozygosity, expected heterozygosity and fixation index. Note that average values between localities were not significantly different from one another.

Locality	Locus	<i>N</i>	<i>Ar</i>	<i>Ho</i>	<i>He</i>	<i>F</i>
Citico Creek	<i>Nfl D109</i>	64	8.527	0.844	0.820	-0.029
	<i>Nfl A10</i>	64	1.571	0.033	0.032	-0.017
	<i>Nfl A12</i>	64	2.859	0.050	0.142	0.647
	<i>Nfl C142</i>	64	1.333	0.016	0.016	-0.008
	<i>Nfl D129</i>	64	1.000	0.000	0.000	
	<i>Nfl D120</i>	64	2.999	0.367	0.508	0.278
	<i>Nfl C135</i>	64	1.890	0.016	0.047	0.660
	<i>Nfl D2</i>	64	2.284	0.071	0.102	0.301
	<i>Nfl D9</i>	64	2.388	0.222	0.281	0.208
	<i>Nfl C120</i>	64	1.000	0.000	0.000	
	Average		2.585	0.161	0.194	0.255
Abrams Creek	<i>Nfl D109</i>	23	6.907	0.682	0.704	0.031
	<i>Nfl A10</i>	23	1.000	0.000	0.000	
	<i>Nfl A12</i>	23	2.000	0.045	0.127	0.642
	<i>Nfl C142</i>	23	2.000	0.136	0.127	-0.073
	<i>Nfl D129</i>	23	1.000	0.000	0.000	
	<i>Nfl D120</i>	23	2.000	0.478	0.405	-0.179
	<i>Nfl C135</i>	23	1.000	0.000	0.000	
	<i>Nfl D2</i>	23	1.913	0.043	0.043	-0.022
	<i>Nfl D9</i>	23	2.000	0.391	0.364	-0.075
	<i>Nfl C120</i>	23	1.000	0.000	0.000	
	Average		2.082	0.177	0.176	0.053

Table 7. Comparison of population genetic parameters for sampled *E. sitikuense* in Citico and Abrams creeks, TN. Abbreviations *N*, *Ar*, *Ho*, *He* and *F* represent the number of samples genotyped, allelic richness, observed heterozygosity, expected heterozygosity and fixation index. Note that average values between localities were not significantly different from one another.

Locality	Locus	<i>N</i>	<i>Ar</i>	<i>Ho</i>	<i>He</i>	<i>F</i>
Citico Creek	<i>Ebl 1</i>	25	1.000	0.000	0.000	
	<i>Ebl 6</i>	25	2.000	0.217	0.194	-0.122
	<i>Ebl 2</i>	25	1.000	0.000	0.000	
	<i>Eca 11EPA</i>	25	2.000	0.083	0.080	-0.043
	<i>Eca 13EPA</i>	25	3.000	0.095	0.172	0.447
	<i>Esc 187</i>	25	2.000	0.083	0.080	-0.043
	<i>Ebl 8</i>	25	1.000	0.040	0.113	0.645
	<i>Ebl 4</i>	25	3.000	0.857	0.635	-0.350
	<i>Esc 26B</i>	25	9.000	0.840	0.782	-0.074
	Average	25	2.667	0.246	0.228	0.066
Abrams Creek	<i>Ebl 1</i>	31	1.800	0.033	0.033	-0.017
	<i>Ebl 6</i>	31	1.000	0.000	0.000	
	<i>Ebl 2</i>	31	1.000	0.000	0.000	
	<i>Eca 11EPA</i>	31	2.855	0.143	0.135	-0.062
	<i>Eca 13EPA</i>	31	2.000	0.357	0.299	-0.194
	<i>Esc 187</i>	31	1.994	0.133	0.124	-0.071
	<i>Ebl 8</i>	31	2.942	0.000	0.062	1.000
	<i>Ebl 4</i>	31	3.000	0.667	0.651	-0.024
	<i>Esc 26B</i>	31	6.940	0.710	0.716	0.009
	Average		2.615	0.227	0.225	0.092

Table 8. Prior uniform distributions, posterior probabilities, and summary statistics for coalescent models used to compare competing evolutionary scenarios. Each scenario (A-D) was comprised of four parameters: ancestral effective population size (N_{ea}), contemporary effective population size (N_e), effective population size during the bottleneck (N_{eb}) and time of bottleneck (T , in generations). Each parameter was sampled from a uniform distribution with lower and upper bounds indicated in brackets (refer to Material and Methods for details about each uniform distribution). Also reported is the posterior probability (Posterior) for each evolutionary scenario along with summary statistics (average number of alleles, *No. alleles*; expected heterozygosity, *He*; allele size variance across loci, *Var.*; and *M*-index, *MG*) used to assess the goodness-of-fit between each model parameter posterior combination and the observed dataset. Test quantities (x), which corresponded to the summary statistics, were interpreted as the probability ($X_{\text{simulated}} < X_{\text{observed}}$); therefore, values greater than 0.95 and less than 0.05 were considered significant, and denoted with an asterisk.

		Scenario			
		A	B	C	D
<i>N. baileyi</i>	N_{ea}	[450-2180]	[450-2180]	[450-2180]	[450-2180]
	N_{eb}	[2-50]	[2-50]	[2-50]	[2-50]
	T	5000	5000	18	55
	N_e	[29-691]	[29-691]	[29-691]	[29-691]
	No. alleles	0.778	0.749	0.905	0.907
	<i>He</i>	0.295	0.301	0.270	0.413
	<i>Var.</i>	0.661	0.692	0.428	0.574
	<i>MG</i>	0.042*	0.032*	0.146	0.130
	Posterior ABCD	0.245	0.291	0.118	0.346
	Posterior CD			0.255	0.745
<i>N. flavipinnis</i>	N_{ea}	[450-2180]	[450-2180]	[450-2180]	[450-2180]
	N_{eb}	[2-50]	[2-50]	[2-50]	[2-50]
	T	3333	3333	12	36
	N_e	[15-750]	[15-750]	[15-750]	[15-750]
	No. alleles	0.007*	0.008*	0.063	0.072
	<i>He</i>	0.008*	0.008*	0.020*	0.060
	<i>Var.</i>	0.460	0.510	0.324	0.347
	<i>MG</i>	0.375	0.354	0.648	0.477
	Posterior ABCD	0.2935	0.333	0.134	0.239
	Posterior CD			0.357	0.643
<i>E. sitikuense</i>	N_{ea}	[450-2180]	[450-2180]	[450-2180]	[450-2180]
	N_{eb}	[2-50]	[2-50]	[2-50]	[2-50]
	T	5000	5000	18	55
	N_e	[19-291]	[19-291]	[19-291]	[19-291]
	No. alleles	0.813	0.783	0.806	0.865
	<i>He</i>	0.479	0.431	0.164	0.231
	<i>Var.</i>	0.950*	0.952*	0.712	0.730
	<i>MG</i>	0.015*	0.008*	0.120	0.110
	Posterior ABCD	0.078	0.086	0.330	0.506
	Posterior CD			0.397	0.603

Figure 1. Map of the middle Little Tennessee River system.

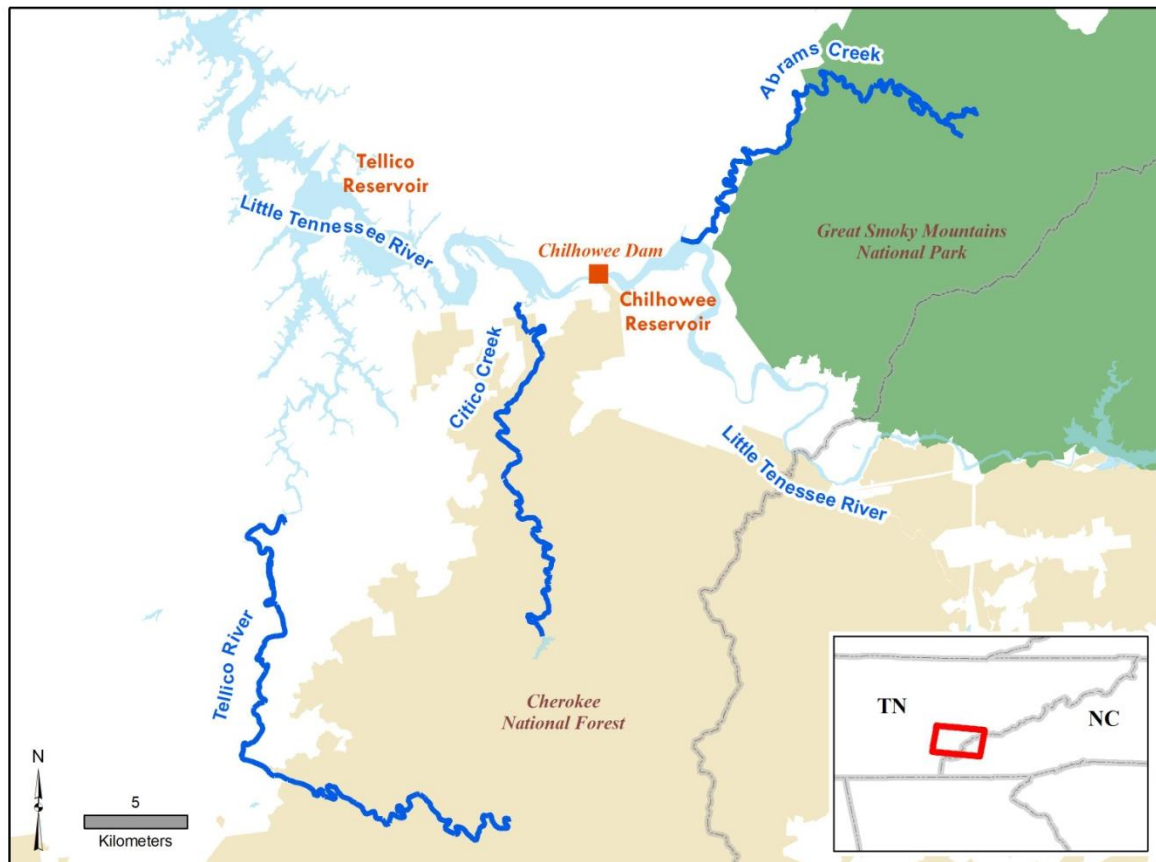
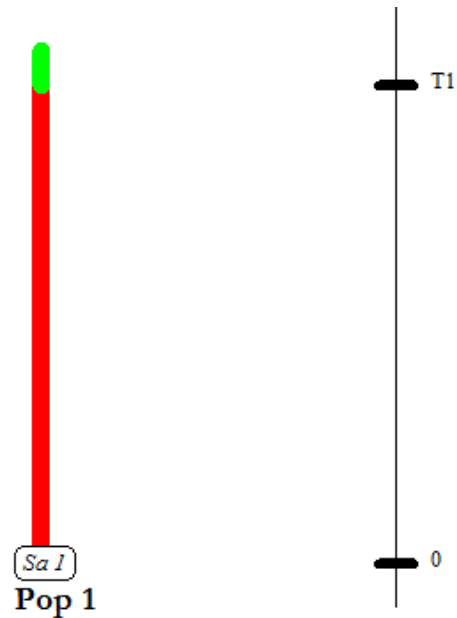


Figure 2. Depiction of evolutionary scenarios used for DIYABC simulations. A) Scenario A depicting a gradual loss of genetic diversity over time. In this scenario we identified T1 as the initial generation time for the gradual loss. For this scenario T1 was set to 5000 or 3333 generations depending on the species. Parameters N_{ea} and N_e were the ancestral and contemporary effective population size. B) Scenarios B-D depicting a bottleneck in genetic diversity. In these scenarios, T1 was the initial start of the bottleneck (see Table 8) and from T1 to T2, we modeled the effective population size during the bottleneck (N_{eb}) as a uniform distribution of [2-50]. T2 was modeled as a uniform distribution having a lower bound of 1 and an upper bound of T1-1. Finally, from T2 to 0, N_e was modeled as a uniform distribution with an upper and lower bound representing the 95% confidence intervals estimated by the program LDNe.

A)

 N_{ea}
 N_e



B)

 N_{ea}
 N_{eb}
 N_e

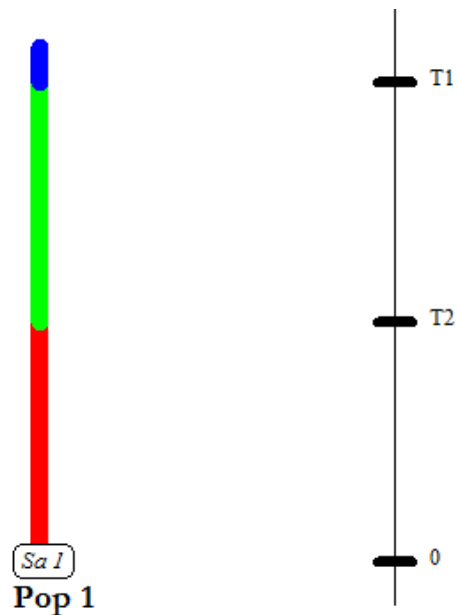
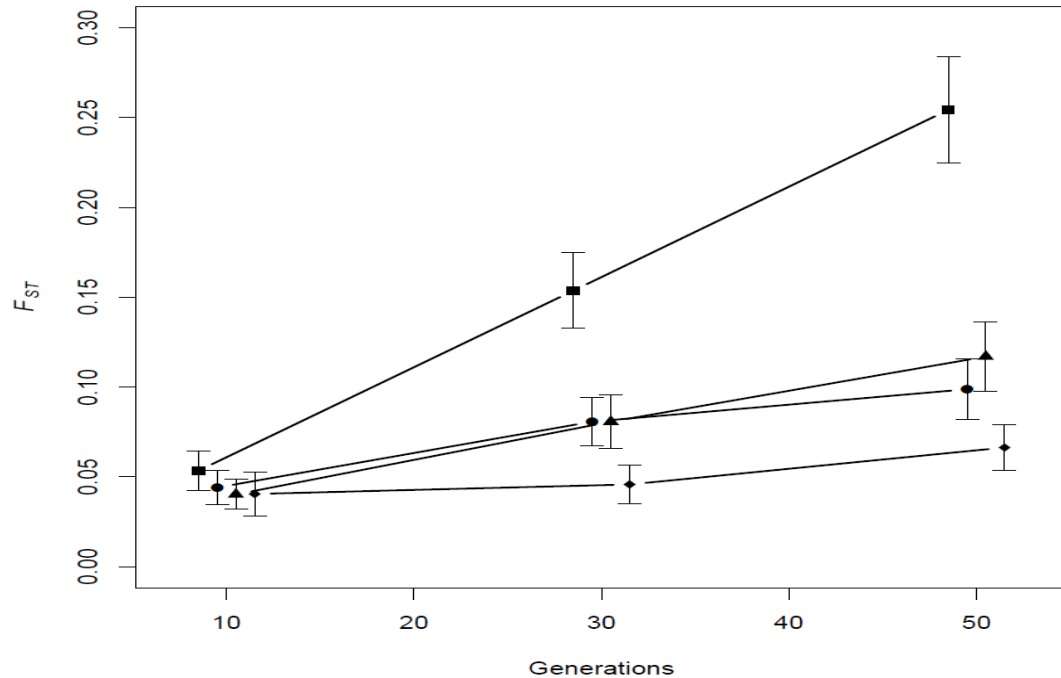


Figure 3. Simulation results for the amount of migration necessary to minimize population differentiation between Citico and Abrams creek populations of *N. flavipinnis*, *N. baileyi*, and *E. sitikuense* over a time span of 50 generations. All simulations assumed a one-way migration model from Citico Creek to Abrams Creek and are based on 10 microsatellite markers. Bars around each point estimate of F_{ST} represent 95% confidence intervals. A) Simulation results using four differing migration rates of 0.00 (■), 0.01 (●), 0.02 (▲) or 0.05 (◆) assuming a constant effective population size of 75. B) Simulation results for a continuous exponential growth model assuming a 0.05 migration rate at four differing growth rates of 0.00 (×), 0.009 (▲), 0.09 (●), 0.18 (■).

A)



B)

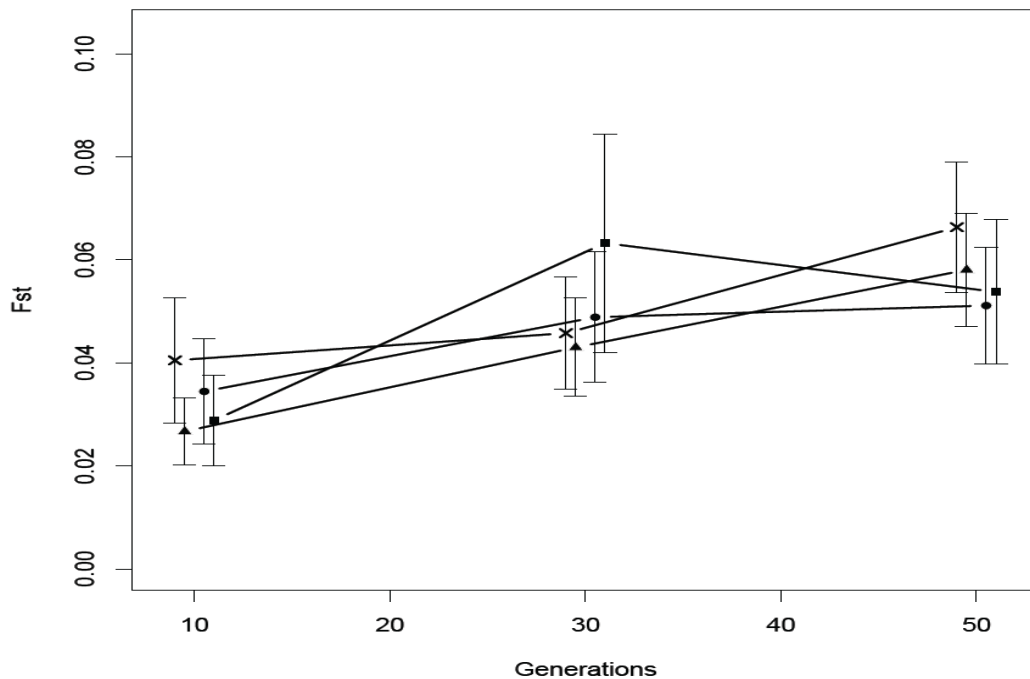
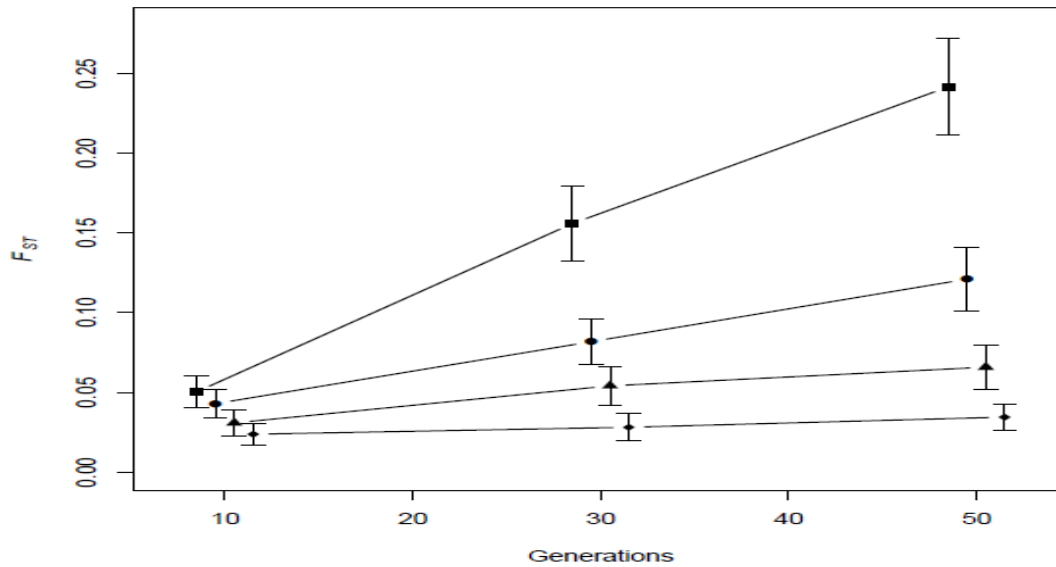
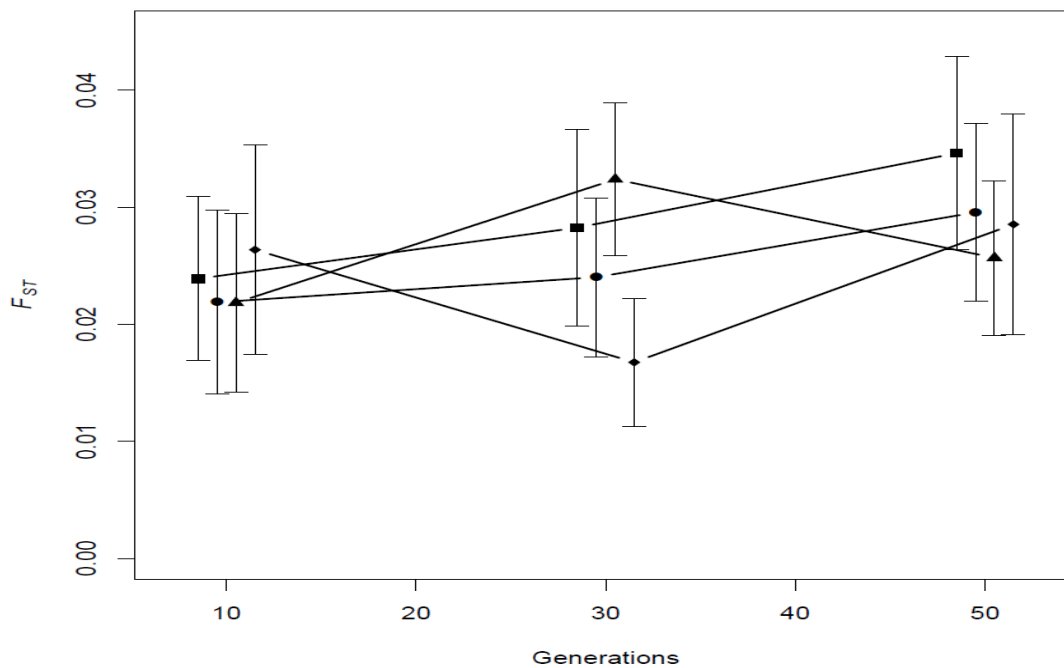


Figure 4. Simulation results for the amount of migration necessary to minimize population differentiation between Citico and Abrams creek populations of *N. flavipinnis*, *N. baileyi*, and *E. sitikuense* over a time span of 50 generations. All simulations assumed a two-way migration model and are based on 10 microsatellite markers. Bars around each point estimate of F_{ST} represent 95% confidence intervals. A) Simulation results using four differing migration rates of 0.00 (■), 0.01 (●), 0.02 (▲) or 0.05 (◆) assuming a constant effective population size of 75. B) Simulation results for a continuous exponential growth model assuming a 0.05 migration rate at four differing growth rates of 0.00 (■), 0.009 (●), 0.09 (▲), 0.18 (◆).

A)



B)



Appendix A.

Noturus flavipinnis microsatellite allele frequencies by population.

Locus	Allele/n	Citico Creek	Abrams Creek
<i>Nfl</i> A9	209	0.000	0.000
	225	1.000	1.000
	229	0.000	0.000
	237	0.000	0.000
	253	0.000	0.000
<i>Nfl</i> C122	141	1.000	0.983
	145	0.000	0.000
	149	0.000	0.017
<i>Nfl</i> C119	196	0.000	0.000
	200	1.000	1.000
	210	0.000	0.000
<i>Nfl</i> C143	246	0.000	0.000
	258	0.017	0.000
	262	0.741	0.914
	266	0.241	0.086
<i>Nfl</i> C1a	174	1.000	1.000
<i>Nfl</i> C7	177	1.000	1.000
<i>Nf</i> D105	259	0.966	0.893
	263	0.034	0.107
<i>Nfl</i> D123	262	0.000	0.000
	270	0.885	0.896
	274	0.115	0.104
	282	0.000	0.000
<i>Nfl</i> D129	233	0.000	0.000
	237	0.875	1.000
	244	0.000	0.000
	258	0.125	0.000
<i>Nfl</i> D139	227	0.450	0.731
	244	0.550	0.269
<i>Nfl</i> D145	255	0.259	0.321
	259	0.741	0.679
	263	0.000	0.000
	271	0.000	0.000
	275	0.000	0.000
<i>Nfl</i> C126			

Locus	Allele/n	Citico Creek	Abrams Creek
<i>Nfl</i> D138	167	0.000	0.000
	183	1.000	1.000
	187	0.000	0.000
	191	0.000	0.000
<i>Nfl</i> C145	207	1.000	1.000
<i>Nfl</i> A12	236	0.667	0.935
	246	0.333	0.065
	250	0.000	0.000
<i>Nfl</i> D140	241	1.000	1.000
<i>Nfl</i> D146	234	0.000	0.000
	242	0.000	0.000
	246	0.000	0.000
	254	0.000	0.000
	262	0.000	0.000
	274	0.000	0.000
	282	1.000	1.000
	218	0.000	0.000
<i>Nfl</i> C138	226	0.000	0.037
	234	0.933	0.963
	246	0.033	0.000
	254	0.033	0.000
	250	0.875	0.714
<i>Nfl</i> D137	270	0.000	0.018
	274	0.125	0.268
	314	0.000	0.000
	235	1.000	1.000
<i>Nfl</i> A3	160	1.000	1.000
<i>Nfl</i> D109	95	0.000	0.000
	107	0.000	0.000
	111	0.000	0.000
	119	0.000	0.000
	127	1.000	1.000
	131	0.000	0.000

Appendix B

Noturus baileyi microsatellite allele frequencies by population

Locus	Allele/n	Citico Creek	Abrams Creek
<i>Nfl</i> D109			
	112	0.055	0.045
	116	0.141	0.023
	120	0.094	0.455
	124	0.094	0.045
	128	0.336	0.227
	132	0.078	0.000
	136	0.047	0.000
	140	0.133	0.182
	144	0.023	0.023
<i>Nfl</i> A10			
	160	0.984	1.000
	168	0.016	0.000
<i>Nfl</i> A12			
	226	0.008	0.000
	234	0.050	0.068
	242	0.017	0.000
	246	0.925	0.932
<i>Nfl</i> C142			
	273	0.008	0.068
	293	0.992	0.932
<i>Nfl</i> D129			
	254	1.000	1.000
<i>Nfl</i> D120			
	145	0.158	0.000
	153	0.658	0.717
	157	0.183	0.283
<i>Nfl</i> C135			
	254	0.976	1.000
	258	0.016	0.000
	270	0.008	0.000
<i>Nfl</i> D2			
	105	0.000	0.022
	125	0.946	0.978
	133	0.000	0.000
	137	0.045	0.000
	145	0.009	0.000
<i>Nfl</i> D9			
	206	0.009	0.000
	210	0.833	0.761
	214	0.157	0.239
<i>Nfl</i> C120			
	205	1.000	1.000

Appendix C

Etheostoma sitikuense microsatellite allele frequencies by population

Locus	Allele/n	Citico Creek	Abrams Creek
<i>Ebl</i> 1			
	234	0.000	0.017
	258	1.000	0.983
<i>Ebl</i> 6			
	244	0.109	0.000
	280	0.891	1.000
<i>Ebl</i> 2			
	150	1.000	1.000
<i>Eca</i> 11EPA			
	180	0.000	0.018
	182	0.042	0.054
	198	0.958	0.929
<i>Eca</i> 13EPA			
	182	0.905	0.821
	186	0.000	0.018
	190	0.095	0.161
<i>Esc</i> 187			
	182	0.042	0.067
	200	0.958	0.933
<i>Ebl</i> 8			
	138	0.000	0.032
	142	0.060	0.000
	154	0.940	0.968
<i>Ebl</i> 4			
	156	0.190	0.300
	160	0.429	0.433
	164	0.381	0.267
<i>Esc</i> 26B			
	192	0.400	0.484
	196	0.060	0.065
	200	0.040	0.016
	204	0.060	0.000
	208	0.120	0.065
	212	0.040	0.081
	216	0.060	0.065
	228	0.060	0.161
	232	0.160	0.065

The Department of the Interior protects and manages the nation's natural resources and cultural heritage; provides scientific and other information about those resources; and honors its special responsibilities to American Indians, Alaska Natives, and affiliated Island Communities.

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National Park Service
U.S. Department of the Interior



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