FINAL REPORT

EVALUATION AND GENETIC SCREENING OF ILLINOIS POPULATIONS OF THE STATE THREATENED REDSPOTTED SUNFISH (*Lepomis miniatus*) TO DETERMINE FEASIBILITY FOR REINTRODUCTION EFFORTS

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Job 1. Population assessment and collection of tissue samples of redspotted sunfish.

OBJECTIVE

To collect updated information regarding the status of redspotted sunfish populations in Illinois and to gather a supply of tissue samples for DNA analyses.

INTRODUCTION

In Illinois, the redspotted sunfish (*Lepomis miniatus*) has apparently never been overly abundant. In the statewide surveys of Forbes and Richardson (1920), this sunfish was only collected 24 times. However, the species does appear to have been more widely distributed then. They collected this sunfish mostly from bottomland lakes along the Illinois River, but also from two localities on the Wabash River, one on the Mississippi River in Hancock County, and one from the Iroquois River.

By the time Smith (1979) wrote The Fishes of Illinois, the redspotted sunfish had already been extirpated from the Mississippi River location and the Iroquois River location. Its distribution had also been reduced along the Illinois River. However, the sunfish did expand its range into the Lower Sangamon River Basin and into Crane Creek. It was also collected from new localities in the Big Muddy River Basin in southwest Illinois and a new locality along the Wabash River in southeast Illinois.

The most current distribution map for the redspotted sunfish by Mike Retzer (current Illinois Natural History Survey collection data) shows a greatly reduced post-1979 range in Illinois. The INHS has no post-1979 collections from the Illinois River Basin, including the Sangamon River and Crane Creek. This map also shows no post-1979 collections from the Big Muddy River Basin in southwest Illinois. However, the map does show current collections from the Wabash River in southeast Illinois.

Prior to our surveys for this project, the statewide FAS database for the Illinois Department of Natural Resources listed only three collections of redspotted sunfish. These collections were from the Little Wabash River (1) in 1989, Cypress Ditch (6) in 1993, and Alcorn Creek (5) in 1994. All of these collection sites are in southern Illinois. Three (3) redspotted sunfish were also collected from Crane Creek during the 1996 survey of the Lower Sangamon River Basin by Doug Carney (IDNR). Four (4) redspotted sunfish were collected by Eastern Illinois University in Indian Creek during the 1996 survey of the Embarras River Basin. This was the only time redspotted sunfish had been collected from the Embarras River Basin.

The goal of Job 1 of this project was to conduct targeted surveys for redspotted sunfish at the recorded collection sites of recent history to determine population status and gather tissue samples for DNA analyses.

PROCEDURES

Collection sites of recent historical records for redspotted sunfish in Illinois were visited, and targeted surveys were conducted to identify presence/absence and population status for the species at the sites. These records were obtained from the fish collections at the Illinois Natural History Survey and Southern Illinois University, the Illinois Natural Heritage Database, and the Illinois Department of Natural Resources Streams Database. All sites with vouchered redspotted sunfish collections since 1970 were visited. Following the first year of surveys without locating any large stable populations of redspotted sunfish within Illinois waters, several locations in neighboring states were also surveyed to acquire tissue samples for the genetic testing.

Larger backwater lakes were surveyed for approximately one hour, targeting favorable habitat with 3-phase AC boat electrofishing gear. Smaller backwater lakes were surveyed to completion of 100% shoreline coverage with the boat electrofishing gear. Stream sites were surveyed for a minimum of 100 yards with DC backpack electrofishing gear.

Redspotted sunfish collected during the surveys were enumerated, measured for total length (TL), partial caudal fin-clipped for tissue sample, and often photographed for proof of identification. Tissue samples were preserved in alcohol for subsequent delivery to the genetics lab at the Illinois Natural History Survey.

RESULTS

Our survey efforts for redspotted sunfish can be classified into four separate areas of concentration: Central Illinois, Southeast Illinois, Southwest Illinois, and Out of State which can be subdivided into Missouri, Kentucky, and Indiana. Surveys conducted prior to the grant cycle and selected survey efforts conducted independent of the grant funding will be included in the results for the sake of a full coverage discussion.

Figure 1 shows an Illinois distribution map taken from The Fishes of Illinois written by P.W. Smith in 1979. As a note, Smith referred to this fish as the spotted sunfish (*Lepomis punctatus*). Warren (1992) produced the evidence which elevated the redspotted sunfish (*Lepomis miniatus*) to full species level. With Smith's distribution map as the foundation, the targeted sampling areas within Illinois have been identified and labeled to summarize our efforts.

Also labeled on the map are two historic sampling locations over 100 years old from the efforts of Forbes and Richardson around the turn of the last century (Forbes and Richardson 1920). Reviews of collection data from the Illinois Natural History Survey (INHS) and the Illinois Department of Natural Resources (IDNR), as well as conversations with biologists of these two institutions, excluded these sites as likely locations of extant populations. Therefore, these sites were not surveyed in this effort in an attempt to conserve time and expenses.

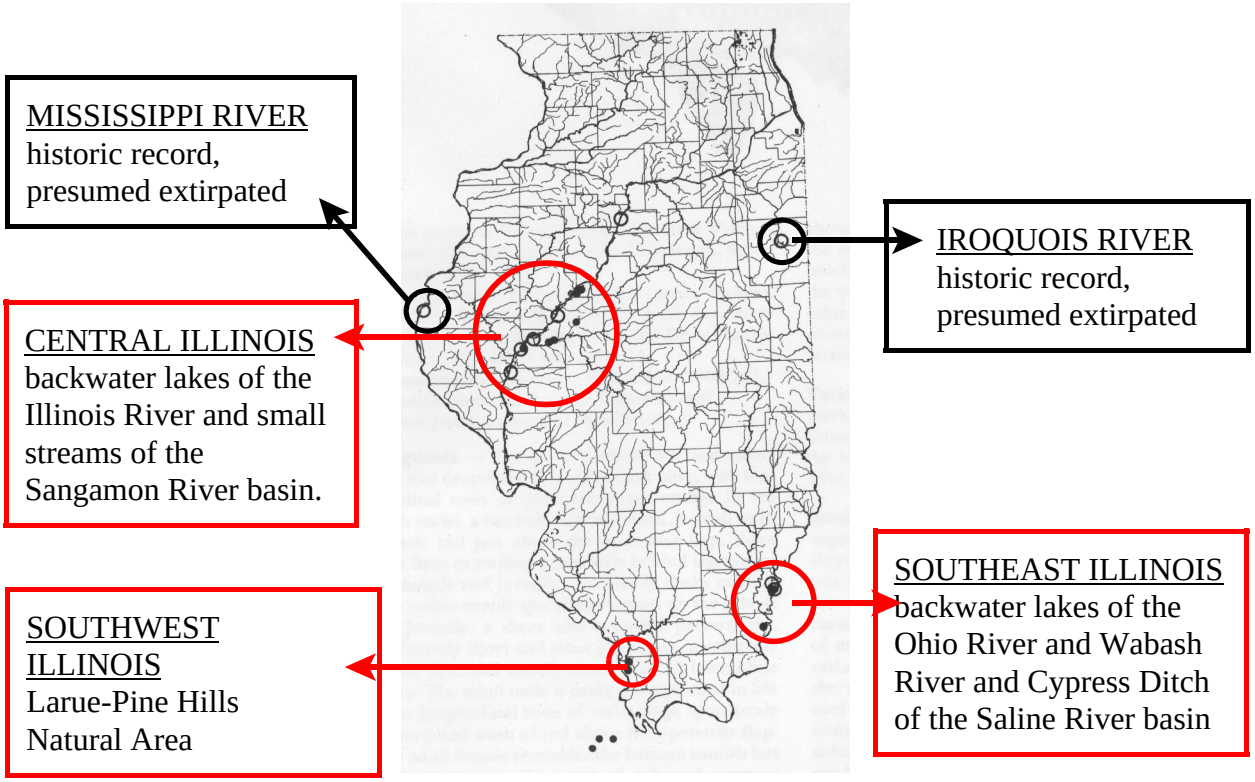

Figure 1. Redspotted sunfish Illinois distribution map from Smith (1979). Open circles represent pre-1908 collections and closed circles represent post-1950 collections. Black circles and text boxes show historic locations of individuals presumed extirpated due the failure of multiple recent attempts to locate redspotted sunfish in these localities. Red circles and text boxes show areas of concentrated sampling efforts to collect redspotted sunfish in execution of this grant.

CENTRAL ILLINOIS

The localities of Central Illinois represent the northern range of the known extant distribution of redspotted sunfish. This species was a former resident of backwater lakes along the Illinois River. It appears that the backwater lakes went through morphological changes that no longer appealed to redspotted sunfish, causing their demise in these former habitats. Literature and personal observations indicate that these fish are strongly associated with aquatic vegetation. The backwater lakes of the Illinois River no longer support large tracts of aquatic vegetation, most likely due to turbidity, sedimentation, water fluctuations, levees, and the presence of injurious species such as common carp and grass carp.

Our collections indicate that the Central Illinois populations have become greatly reduced to their current status as remnant populations inhabiting lowland ditches of the Sand Prairie Natural Division near the confluence of the Illinois River and Sangamon River. These ditches possess

relatively stable flows of clear water and support extensive aquatic vegetation in parts of their drainage system. However, evidence was collected that showed these small streams may be quite vulnerable to perturbations of herbicide applications and grass carp (*Ctenopharyngodon idella*) invasions that threaten the existence of the essential aquatic vegetation. Large stands of aquatic vegetation in stretches of Crane Creek were nearly completely eliminated for several years, but are now showing signs of slow recovery (Carney pers. comm.). It is difficult to determine the cause of this disturbance. The drainage is composed almost completely of agricultural landuse, lending itself to bouts with chemical run-off. Schools of grass carp were also observed during sampling efforts in the stream.

Following are accounts for specific locations of collections within the Central Illinois area:

Illinois River: Several vouchered specimens were collected from the Illinois River along Mason and Morgan Counties from 1894 to 1900. No redspotted sunfish have been collected from the Illinois River for over 100 years. The INHS does extensive sampling in these localities as part of their Long Term River Monitoring Program. Conversations with INHS staff indicated that no redspotted sunfish have been collected from these localities and no large tracts of aquatic vegetation persist in these areas, so their continued presence is highly doubtful. No locations on the Illinois River were surveyed during this effort.

Crane Creek, Sangamon River Basin (Mason County): Doug Carney (IDNR Streams Biologist) collected three (3) juvenile redspotted sunfish from Crane Creek, west of Easton, IL in Mason County (Station Code: EH-02) on August 26, 1996 during the Sangamon River Intensive Basin Survey. This site and several other locations along Crane Creek were sampled May 4-5 and September 1, 2004. Four (4) juveniles were collected during these efforts northwest of Easton at EH-04, about a mile north of EH-02, and one (1) juvenile at EH-03, about a mile south of EH-02. Aquatic vegetation was greatly reduced from past observations of the stream at this time (Carney, pers. comm.). Notably, significant numbers of the State-Threatened starhead topminnow (*Fundulus dispar*) and ironcolor shiner (*Notropis chalybaeus*) were observed at several locations along Crane Creek during these sampling efforts.

Central Ditch, Sangamon River Basin (Mason County): Central Ditch is a tributary in the upper reaches of the Crane Creek watershed. This small stream was believed to be a strong candidate for redspotted sunfish habitat as it contains plenty of aquatic vegetation and is immediately upstream of the Crane Creek site where juveniles were collected. This stream was sampled by Thomas on September 1, 2004 and May 26, 2005. No redspotted sunfish were collected. However, large numbers of State-Threatened starhead topminnows and ironcolor shiners were collected here.

Figure 2. Crane Creek (EH-04) collection site of four (4) juvenile redspotted sunfish.

Figure 3. Grass carp collected from Crane Creek.

Figure 4. Redspotted sunfish collected from Crane Creek on May 5, 2004.

Figure 5. Redspotted sunfish collected from Crane Creek on September 1, 2004.

Angelo Lake (Mason County): Angelo Lake is a small lake situated in-line with Pecan Creek of the Sangamon River Basin in Mason County. 100% of the shoreline was surveyed by Thomas, Carney, and Sauer on May 4, 2004. No redspotted sunfish were collected, and the lake contained no aquatic vegetation.

Figure 6. Central Ditch **Figure 7.** Angelo Lake **Figure 8.** Fish Lake

Fish Lake (Mason County): Fish Lake is a small lake situated in-line with Fish Creek of the Sangamon River Basin in Mason County. 100% of the shoreline was surveyed by Thomas and Carney on May 7, 2004. No redspotted sunfish were collected. The lake was silting in and contained no aquatic vegetation.

Wolf Lake (Mason County - T19N, R9W, Section 19): One (1) vouchered specimen was collected from Wolf Lake in 1976. Wolf Lake was surveyed by Thomas and Carney on May 7, 2004. 100% of the shoreline was surveyed by AC boat electrofishing. No redspotted sunfish were collected and the lake no longer contained aquatic vegetation. The lake was sampled again in 2007 by Tiemann and none were collected.

Fish Creek (Mason County): There are no historical records of redspotted sunfish from Fish Creek. The stream was sampled late in the grant cycle on a tip from a member of the North American Native Fishes Association (NANFA), who had stumbled across the population while on a collecting trip with the Illinois Chapter of NANFA. The site was surveyed by Thomas, Tiemann, and Retzer (INHS) on August 28, 2007. Twelve (12) redspotted sunfish were collected by DC backpack electrofishing in a sample length of 440 meters. This part of Fish Creek contained several varieties of aquatic vegetation representing suitable conditions for redspotted sunfish habitat. This site easily proved to be the highest concentration of redspotted sunfish that we found within the Central Illinois region.

Figure 9. Redspotted sunfish collected from Fish Creek on August 28, 2007.

Figure 10. Fish Creek

Figure 11. Aquatic Vegetation at Fish Creek

Spring Lake and Sunset Lake (Tazewell County): These two backwater lakes of the Illinois River had vouchered collections of redspotted sunfish from 1960 and 1967. The lakes were not surveyed on the advice of Wayne Herndon (IDNR District Biologist). Spring Lake was rehabilitated in the 1990's for common carp (*Cyprinus carpio*) removal and sportfish population enhancement. The lake is intensively sampled and no redspotted sunfish are currently present in the lake. Sunset Lake is a shallow lake that experiences winter fish kills on a regular basis, making the presence of redspotted sunfish unlikely (Herndon, pers. comm.).

SOUTHWEST ILLINOIS

Seven (7) specimens of redspotted sunfish had been vouchered in the fish collection of the INHS from southwest Illinois from 1971 to 1973. Records indicate that the specimens were collected from Pine Hills Swamp and Wolf Lake in Union County. Winters Pond in the Pine Hills Swamp area and Wolf Lake were both sampled for redspotted sunfish as indicated in the following accounts:

Winters Pond (La Rue-Pine Hills Ecological Area, Union County): Winters Pond was surveyed by Thomas, Carney, and Sauer on June 22, 2004 using AC boat electrofishing. Navigating through the pond proved to be extremely difficult due to high concentrations of aquatic vegetation of several varieties. Electrofishing efficiency also appeared to be poor, most likely due to high conductivity of the tea-colored water. No redspotted sunfish were collected. However, the State-Threatened starhead topminnow was collected during the effort.

Winters Pond was surveyed again on August 11-12, 2004 by Thomas and Garthaus, using four mini-fyke nets set overnight. Several juvenile sunfishes were collected in the nets. The majority of these were warmouth (*Chaenobryttus gulosus*) and State-Threatened bantam sunfish (*Lepomis symmetricus*). Eighteen (18) tissue samples for the genetic analyses were collected from small individuals in which the spot in the dorsal fin (indicative of bantam sunfish) was not readily apparent.

Figures 12-13. Views of Winters Pond at La Rue-Pine Hills Ecological Area in Union County in southwest Illinois. The pond contained extensive aquatic vegetation of several varieties. The pond was home to State-Threatened bantam sunfish and starhead topminnows.

Wolf Lake (Union County): Wolf Lake was surveyed by Thomas, Carney, and Sauer on June 22, 2004 using AC boat electrofishing. The lake contained large expanses of lotus that lined the banks, making sampling difficult. No redspotted sunfish were collected, but the State-Threatened starhead topminnow was again collected here. This lake is also the former home of the State-Extirpated bluehead shiner (*Pteronotropis hubbsi*).

Running Lake Ditch (Union County - T11S, R3W, Section 20): Southern Illinois University vouchered redspotted sunfish from Running Lake Ditch in 1979. Thomas and Tiemann sampled a swampy area of this ditch near La Rue-Pine Hills on October 26, 2006, using DC backpack electrofishing. No redspotted sunfish were found during this effort.

SOUTHEAST ILLINOIS

The redspotted sunfish records from southeast Illinois represented the most recent collections of any significant numbers of this species in the state. The majority of the records came from a targeted survey conducted by Burr and Warren (1987) of Southern Illinois University (SIU). The

authors sampled 21 sites within the backwater lakes, ponds, wetlands, and sloughs along the Ohio and Lower Wabash Rivers. Redspotted sunfish were found at nine of their collection sites, using 100-meter gill nets, boat electrofishing, and seines as collection methods. Our efforts were to visit these confirmed sites and conduct more intensive sampling to determine continued persistence and estimate population health of the species. A few other sites from this region also came from IDNR collection records, as noted below:

Big Lake (Gallatin County - T9S, R10E, Sections 21 & 28): Big Lake is a large floodplain lake along the Ohio River. Redspotted sunfish were recorded here from the 1987 SIU report. The lake was sampled by Thomas and Tiemann on August 24, 2005, using AC boat electrofishing concentrating mostly on the south end of the lake. No redspotted sunfish were collected and no aquatic vegetation was present in the lake. Our results may not indicate extirpation of the species here, but they certainly indicate that the sunfish does not occur here in significant numbers.

Figure 14. Big Lake, looking south

Figure 15. South end of Big Lake showing submerged woody debris, but no aquatic vegetation.

Fish Lake (Gallatin County - T9S, R10E, Section 22): No previous records of redspotted sunfish from Fish Lake exist. The lake was sampled by Frankland and Hirst on August 24, 2005, using AC boat electrofishing. They collected two (2) redspotted sunfish during this effort, representing a new species record for this body of water. However, the sunfish appears to be present in very small numbers.

Unnamed Wetland between Big Lake and Fish Lake (Gallatin County - T9S, R10E, Section 21): This wetland was sampled during the 1987 study, and no redspotted sunfish were collected. Thomas, Tiemann, Frankland, and Hirst sampled the wetland on August 24, 2005, using AC boat electrofishing. One (1) redspotted sunfish was collected. The wetland contained no aquatic

vegetation, but habitat was present in the form of numerous logs and debris. The collection represents a another new location record for the species, but it is again only present in low numbers.

Figure 16. Fish Lake

Figure 17. Unnamed Wetland between Big Lake and Fish Lake

Hulda Lake or AB Lake (Gallatin County - T8S, R10E, Sections 28 & 34): Burr collected a redspotted sunfish from Hulda Lake in 1975, but no individuals were collected during their 1987 surveys (Burr and Warren 1987). Thomas, Tiemann, Frankland, and Hirst sampled the lake on August 26, 2005, using AC boat electrofishing and DC backpack electrofishing. No redspotted sunfish were collected. The lake contained no aquatic vegetation.

Beaver Pond (Gallatin County - T7S, R10E, Section 27): Redspotted sunfish were recorded from this pond in the 1987 SIU report. Thomas, Tiemann, Frankland, and Hirst sampled the pond on August 26, 2005, using AC boat electrofishing. Five individuals were collected from the rip rap below the road bridge, none were collected throughout the rest of the pond. No aquatic vegetation was present in the pond. This site represented our largest catch of redspotted sunfish from the backwater habitats of southeast Illinois.

Figure 18. Beaver Pond, looking east

Figure 19. Beaver Pond, showing rip rap

Figures 20-21. Redspotted sunfish collected from Beaver Pond

Yellowbank Slough (Gallatin County - T8S, R10E, Section 7): The 1987 SIU report recorded redspotted sunfish from Yellowbank Slough. Thomas and Tiemann surveyed the slough on November 3, 2005, using DC backpack electrofishing. No redspotted sunfish were collected.

Cypress Ditch (Gallatin County): Cypress Ditch is a small tributary in the Saline River Basin. The IDNR Heritage database recorded a collection of redspotted sunfish from Cypress Ditch in 1993. Tiemann sampled this stream on June 23, 2004 and was also successful in finding the sunfish. Thomas and Tiemann surveyed this stream again on November 3, 2005, using DC backpack electrofishing. Eight (8) individuals were collected during this effort. The stream contains aquatic vegetation (mostly coontail) that is serving as favorable habitat for the sunfish. This represented the highest concentration of redspotted sunfish that we were able to locate in the southeast Illinois region.

Figure 22. Redspotted sunfish collected from Cypress Creek by Tiemann

Wabash River - Old Channel (White County): The IDNR Heritage database recorded a collection of redspotted sunfish from the old channel of the Wabash River about 3.5 miles northeast of Maunie in 1975. Thomas and Tiemann surveyed the old channel at this location on November 3, 2005, using AC boat electrofishing. No redspotted sunfish were collected. Abundant jumping silver carp (*Hypopthalmichthys molitrix*) were present in large numbers.

Brushy Slough (White County - T7S, R10E, Section15): The 1987 SIU report recorded redspotted sunfish from Brushy Slough. Thomas and Tiemann surveyed the slough on November 3, 2005, using DC backpack electrofishing. No redspotted sunfish were collected.

Beaver Dam Lake (Massac County -T16 & 17S, R6E, Sections 1 & 35): The 1987 SIU report recorded redspotted sunfish from Beaver Dam Lake. Thomas and Tiemann surveyed the lake on November 4, 2005, using AC boat electrofishing. No redspotted sunfish were collected.

Loon Lake (Massac County - T16S, R6E, Section 20): The 1987 SIU report recorded redspotted sunfish from Loon Lake. Thomas and Tiemann surveyed the lake on November 4, 2005, using DC backpack electrofishing. No redspotted sunfish were collected.

Long Reach and Cypress Pond (Pulaski County): The IDNR Heritage database recorded a collection of redspotted sunfish from Long Reach and Cypress Pond about 1.5 miles south of Perks in 1979. Thomas and Tiemann surveyed the pond on November 4, 2005, using AC boat electrofishing. No redspotted sunfish were collected.

Haney Creek (Hardin County - T12S, R10E, Section 10): No previous records of redspotted sunfish exist for Haney Creek. Jana Hirst (IDNR) collected a single individual during regular basin survey activities on July 5, 2005, using an AC electric seine at Station Code AR-01. Haney Creek is a small tributary stream to the Ohio River that had no aquatic vegetation. Habitat consisted of boulders and submerged logs and roots. This collection represents a new species location record for redspotted sunfish.

Indian Creek (Lawrence County - T3N, R12W, Section 13): Indian Creek is a tributary to the Embarras River south of Lawrence. Four (4) redspotted sunfish were collected by Eastern Illinois University in 1996 during contracted basin survey work. This site has been sampled by Thomas and IDNR personnel on August 6, 2001 and again by Hirst, Thomas, and IDNR personnel on August 17, 2006, using an AC electric seine. No redspotted sunfish have been collected here or elsewhere in the Embarras River Basin since the 1996 EIU find.

OUT-OF-STATE

INDIANA

Thomas and Tiemann traveled to southwest Indiana to sample known locations for redspotted sunfish in that state. Brant Fisher of the Indiana Department of Natural Resources hosted the sampling trip. Several locations were sampled in a single day. Several redspotted sunfish were collected and fin-clipped for DNA analyses. The redspotted sunfish population in this part of Indiana appeared to be stable and healthy. Also of note, several banded pygmy sunfish were collected during the sampling. This is a new species record for the state of Indiana.

River Deshee, Indiana (Knox County): River Deshee is a direct tributary to the Wabash River, south of Vincennes, Indiana. It is a channelized drainage ditch with a significant amount of aquatic vegetation and mostly soft bottom substrate. This river closely resembles the Crane Creek and Fish Creek locations in Central Illinois for habitat and fish assemblage. 18 redspotted sunfish were collected from two River Deshee locations on October 25, 2006. Sites were sampled using DC backpack electrofishing.

Figures 23-25. Three views of redspotted sunfish collection sites on River Deshee, Indiana.

Barren Ditch, Indiana (Pike County): Barren Ditch is a tributary to the Patoka River southwest of Ayrshire, Indiana. The site sampled possessed a forested riparian corridor with significant woody debris in the stream. Aquatic vegetation was not prevalent here. 11 redspotted sunfish were collected on October 25, 2006 using DC backpack electrofishing.

Figures 26-31. Redspotted sunfish collected from River Deshee and Barren Ditch, Indiana.

KENTUCKY

Bayou Creek, Kentucky (McCracken County): Bayou Creek is a direct tributary to the Ohio River in northwest Kentucky. Thomas and Tiemann sampled a site one mile east of Cimota City on October 26, 2006 using DC backpack electrofishing. No redspotted sunfish were collected. The stream had an all rock substrate and no aquatic vegetation. The site was not consistent with other locations where redspotted sunfish had been collected during the study. Shawnee Creek was also visited, but not sampled as the habitat did not look promising at this location either.

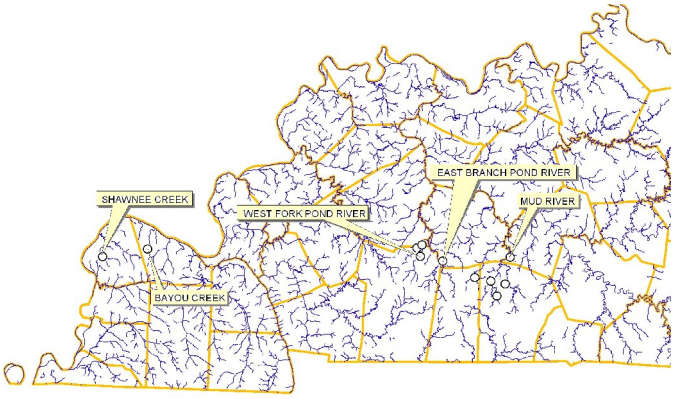

Figure 32. Historic redspotted sunfish collection sites in Kentucky. Bayou Creek and Shawnee Creek were the only sites visited in Kentucky.

MISSOURI

After mediocre survey results and failure to collect significant numbers of redspotted sunfish in Illinois, it was decided to sample locations in Missouri where the species was known to be abundant. Thomas and Epifanio traveled to Missouri to collect redspotted sunfish from Jacks Fork with Jeff Koppelman of the Missouri Department of Conservation. In addition to this trip, Tiemann collected redspotted sunfish from the Black River at a later date. Biologists from the Missouri Department of Conservation also collected additional redspotted sunfish tissue samples during their routine sampling efforts and supplied us with a number of samples from other locations throughout the area.

Jacks Fork, Missouri: Jacks Fork was sampled by Thomas, Epifanio, and Koppelman on May 9, 2006 using DC boat electrofishing. High water conditions prevailed at the time, which made sampling difficult. However, it quickly became evident that redspotted sunfish inhabited the well-vegetated slack water pockets that formed on the downstream sides of the larger point bars. Redspotted sunfish were predictably and consistently collected from these pockets throughout the sampling reach. 28 individuals were collected and fin-clipped for DNA analyses.

Figure 33. Jacks Fork, Missouri during high water sampling conditions.

Figures 34-35. Jacks Fork, Missouri showing slack water areas where redspotted sunfish were consistently collected.


Figures 36-37. Jacks Fork, Missouri - closer views of the slack water areas. Most of these pockets possessed abundant aquatic vegetation, consisting mostly of coontail and lillies.

Black River, Missouri: Several locations on the Black River were sampled by Thomas and Epifanio on May 10, 2006 using a seine net. However, water conditions were not conducive to efficient sampling due to persistent rains and no redspotted sunfish were collected. Tiemann returned to the Black River on September 17, 2006 and collected six (6) individuals about one mile south of Hendrickson, Missouri using a seine net among emergent water willow plants.

Additional samples were collected by Missouri Department of Conservation biologists and supplied for inclusion in the genetic analyses. These samples came from 11-Points Creek, Little Black River, Duck Creek, and Ten Mile Creek.

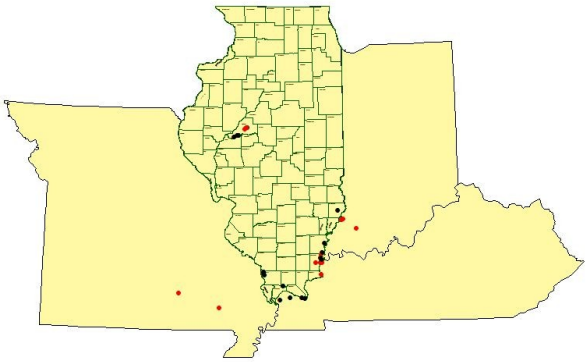

Figure 38. Redspotted Sunfish survey site summary. Red dots indicate locations where redspotted sunfish were collected, and black dots indicate sites sampled with no specimens collected.

Job 2. DNA analysis of redspotted sunfish populations – a genetic survey of *L. miniatus* in Illinois and neighboring states.

Background

Restoration and recovery of imperiled aquatic species populations may require reintroduction of extirpated populations within a species' former range as well as the creation of *ex situ* refuge populations in cases where suitable critical habitat has been lost or severely degraded. Historically, the relatively diffuse extant distribution of redspotted sunfish in Illinois suggests that remnant populations are sufficiently isolated and occur in separate watersheds warranting a more site-or population-specific approach to restoration, especially where population reintroduction is under consideration. As a basic tenet of conservation genetics, such reintroduced populations are expected to have a greater probability of success and a longer term viability where the re-founded population essentially mimics or is otherwise compatible with aboriginal patterns of genetic-level biodiversity (e.g., Brown et al. 2000, Brown et al. submitted). Therefore, during the planning stages for any reintroduction effort, it becomes vital to 1) choose an appropriate founding source and 2) ensure maximization of local genetic diversity to the greatest practicable extent.

In order to choose an appropriate founding source, a prudent and risk-averse precursor requires an examination of the species' phylogeographic variation within and across watersheds and basins. From this kind of assessment, a decision can be made as to which candidate source population(s) is/are most evolutionarily comparable (i.e., most similar genetically or phylo-geographically) to the extirpated population as possible. Following from this decision, then specific approaches can then be undertaken to maximize within population diversity to avoid bottleneck and potential inbreeding effects. As a practical matter when dealing with imperiled taxa, background data on historical patterns of spatial diversity (i.e., genetic population structure) are not available; thus, we generally try to examine patterns from extant populations to get an accurate view of how the candidate and extirpated populations fit into broader landscape patterns. The central question this job is attempting to answer is, "Are remnant Illinois watershed populations unique or divergent from those of neighboring states' watersheds?" Answering this question is at the heart of Objective 3 for this project – to conduct a mitochondrial DNA analysis of redspotted sunfish representing the existing populations in Illinois. Ultimately, the data and analyses gathered from this project will inform decisions regarding geographical (watershed) sources for potential reintroduction into suitable and historical habitats in Illinois.

Methods and Approach

Mitochondrial DNA analyses used for Job 2 included both a coarse-level screening of restriction fragment length polymorphism (RFLP) approach and a more fine-level screening of specific nucleotide sequences. Tissues (caudal fin clips) collected from Job 1 by IDNR and INHS

biologists were augmented by those from collaborators with the Missouri Department of Conservation. Including samples from neighboring states provided a broader spatial context for genetic variation (that is, phylogeography). Collection methods included backpack and boat-mounted electroshocking, as well as pulling of a shoreline bag seine.

Tissues were collected and stored in labeled vials containing 95% ethanol from populations during spring and summer 2005-07. Effort and population sample sizes are described in Job 1, reflecting size and accessibility of habitats, as well as population condition/status. Several voucher specimens were collected from locales with robust populations along with other sunfish species. These specimens were deposited in the INHS Ichthyologic collection. Field identification of Winter's Pond samples was provided by Dr. Brooks Burr, Southern Illinois University and determined to be bantam sunfish rather than redspotted sunfish.

DNA extraction – In the laboratory, we extracted total genomic DNA from all individuals. We followed a modified version of the PUREGENE® (GentraSystems, Inc., Minneapolis, MN, USA) protocol for extraction of fixed animal tissue. Briefly, approximately 0.005g (1 mm^2) of tissue was excised from each fin clip and briefly blotted with a paper towel to remove excess ethanol. The tissue sample was macerated with a grinding pestle in a vial containing 300 µL of cell lysis solution (10mM tris-HCl, 10mM EDTA, pH 8.0 and 2% SDS) and 1.5 µL of proteinase-K (20 mg/mL). The mixture was incubated at 65°C for three hours to emulsify the tissue. Then 1.5 µL of pancreatic ribonuclease-A (100 mg/mL) was added to the emulsification and incubated at 35°C for 30 min. After cooling the sample to room temperature, 100 µL of a protein precipitation solution (7.5 M ammonium acetate) was added and the solution gently agitated. The solution was incubated at 0°C for 5-10 min and centrifuged for 3 min at 13,000 x g. The supernatant was transferred to a new vial containing 300 µL of 100% iso-propanol and 0.5 µL of a DNA carrier solution (glycogen , 20 mg/ml). The solution was centrifuged for 7 min at 13,000 x *g*. The supernatant was discarded and the resulting pellet washed with 300 µL of 70% EtOH. The washed pellet was centrifuged for 1 min at 13,000 x *g*, the supernatant discarded, and the pellet dried at room temperature for 15 min. The extracted DNA was suspended in solution and rehydrated by dissolving the dried pellet in 25 µL of 0.1X TE Buffer (10mM Tris-HCL and 1mM Tetra-EDTA) at room temperature for 12 to 18 hours. All extracted DNA samples were stored at 4°C until further processing.

ND-3/4 amplification and isolation – A 1 µL sample of genomic DNA was subjected to a PCR amplification of the ND-3/4 domain of the mitochondrial DNA region. We chose the ND-3/4 domain because it is a large domain (~2.2Kb) which is helpful for finding variation using a PCR-RFLP method. Moreover, this domain has been shown to be highly variable and phylo-geographically informative in other Centrarchidae species. Briefly, PCR amplifications were performed in reaction mixtures with a total volume of 15 µL which included 8.95 µL of ddiH20; 1.50 µL of 10X Buffer-A (100 mM Tris-HCl, (pH 9.0), 500 mM KCl, 15 mM $MgCl_2$); 1.50 µL of 100 µM DNTP's (20 µL of each dATP, dTTP, dGTP, and dCTP); 0.40 µL of 10 mM

spermidine trihydrochloride; 0.75 µL of a 10 µM stock of each ND-3/4 primer (forward and reverse); and 0.15 µL of Fisher BioReagents *Taq*® DNA polymerase in buffer A (Fisher Scientific International). Amplifications were performed using a MJ Research PTC-100 thermal cycler (MJ Research, Waltham, MA, USA). PCR conditions were optimized by systematically increasing or decreasing the annealing temperature until PCR products had adequate levels of the target amplicons and minimal non-specific annealing. Specific primer sequences and PCR conditions are available by contacting <epifanio@inhs.uiuc.edu>. In some cases a second round of PCR was performed to elevate concentration or total volumes of amplicon for RFLP and sequence analyses.

PCR products were then screened for variation by digesting with a suite of Restriction Endonucleases including *Alu I, Apa I, Bam HI, Eco RI, Eco RV, Hind III, KpnI, Pst I, Sau I, Xba I* to permit a rapid RFLP analysis. This broad screening failed to produce a predicted data set of variation useful for population analysis, therefore, we sent n ~ 89 samples of cleaned-up (QiaQuik Kit by Qiagen) ND-3/4 amplicons to the University of Illinois Core Sequencing Laboratory (http://www.biotech.uiuc.edu/centers/Keck/Core/) to perform sequence analysis on the samples. This analysis provided sequences of sufficient quality on the ~ 1000 bp (1 Kb) for 3' end of the sequence for n ~ 40 of the samples and partial (<1000 bp) for the remaining samples. Raw sequences were aligned and edited with SeaView and Sequencher software to produce a set of edited sequences for data analyses.

In addition to the direct analysis of sequence variation, we used the edited sequences to search for variable restriction sites from a broad suite of restriction endonucleases that would be useful for rapid PCR-RFLP analyses. Several including *Dra II*, previously unscreened, display promise for this kind of rapid application in efforts for the next phase of the project.

Data analyses – Initial data analyses was conducted with Felsenstein's Phylip 3.67 Package (available at http://evolution.genetics.washington.edu/phylip.html). We specifically examined the overall genetic divergence among all individuals by constructing a Neighbor-Joining tree to look for spatially-defined cluster patterns of genetically related individuals. From a topology drawn, we look for divergences in two ways – one, for groupings with branching points and two, for branch lengths. Sequences that are divergent (i.e., individuals with large differences in sequences) are expected to not be in the same cluster group and have longer branch lengths. Thus, if deep divergences are associated with population subdivision that is organized by spatial patterns, we would expect clusters of individuals drawn from locations with branch points increasing with spatial distance. Conversely, if divergence is not due associated with physical distance, then a more random pattern would be expected.

Archiving of tissues – All tissues (fin clips) will be maintained and curated at the Illinois Natural History Survey for any future analyses that might be required for future monitoring or

for new techniques that might shed additional light on distribution or variation within the species. Specifically, all remaining tissues will be stored in 95% ethanol at -20ºC.

Results

As stated above, insufficient restriction site polymorphism was observed to take this approach for data analysis. Direct sequence data, however, revealed a significant amount of variation, especially at the front and back ends of sequences (see Table 1 below for nucleotide sequences for 914 aligned bases; the unedited sequences are included in Appendices I). Sufficiently complete sequence information was obtained from 59 individuals across the study regions to permit distance/divergence analysis. Upon inspection, it is clear that individuals from Fish Creek in Illinois display considerably more sequence variation than other individuals from other sources. This indicates that the population has not experienced a severe bottleneck and is a diverse local candidate source for constructing a captive bred population for reintroduction and refuge populations (Objective 4 in this project).

Beyond simple inspection, we computed the pairwise distances among all individuals and displayed these as a Neighbor-Joining topology (PHYLIP 3.67). While population-level distances are generally preferred over individual distances, the final sample sizes for some of the populations, were sufficiently small that the risk of a Type II bias was significant - as is often the case with rare or imperiled species.

In Figure 1, the Neighbor-Joining topology of all individuals reveals a low level of deep divergence and clustering in spite of the amount of sequence variation. Also, we anticipated there to be a high level of clustering of individuals by sampling location at least at a broad scale. In fact, this does not appear to be the case. Several of the individuals from the Fish Creek (Sangamon River watershed, Illinois – identified in the dashed circle) have long branches suggesting a high level of variation within this population. While it might be suggested that these individuals were misidentified in the field, we note that the field trip for this collection included an identification expert (Dr. Michael Retzer, Curator of Fishes, Illinois Natural History Survey), thus minimizing the chances that such a suggestion merits further consideration.

To explore the relationships among the individuals more deeply, we examined the individuals from each state individually (Figures 2-4) because they ultimately occupy spatially discreet watersheds (i.e., the Wabash in Indiana, the Black River in Missouri, and the Sangamon/Saline/Ohio rivers in Illinois). In Figure 2, the topology for Illinois individuals again reveals no discrete clustering of the different collection locations but does indicate long branch lengths for several of the Fish Creek individuals. In Figures 3 and 4 (Indiana and Missouri, respectively), the topologies reveal little clustering and deep divergence by collection location.

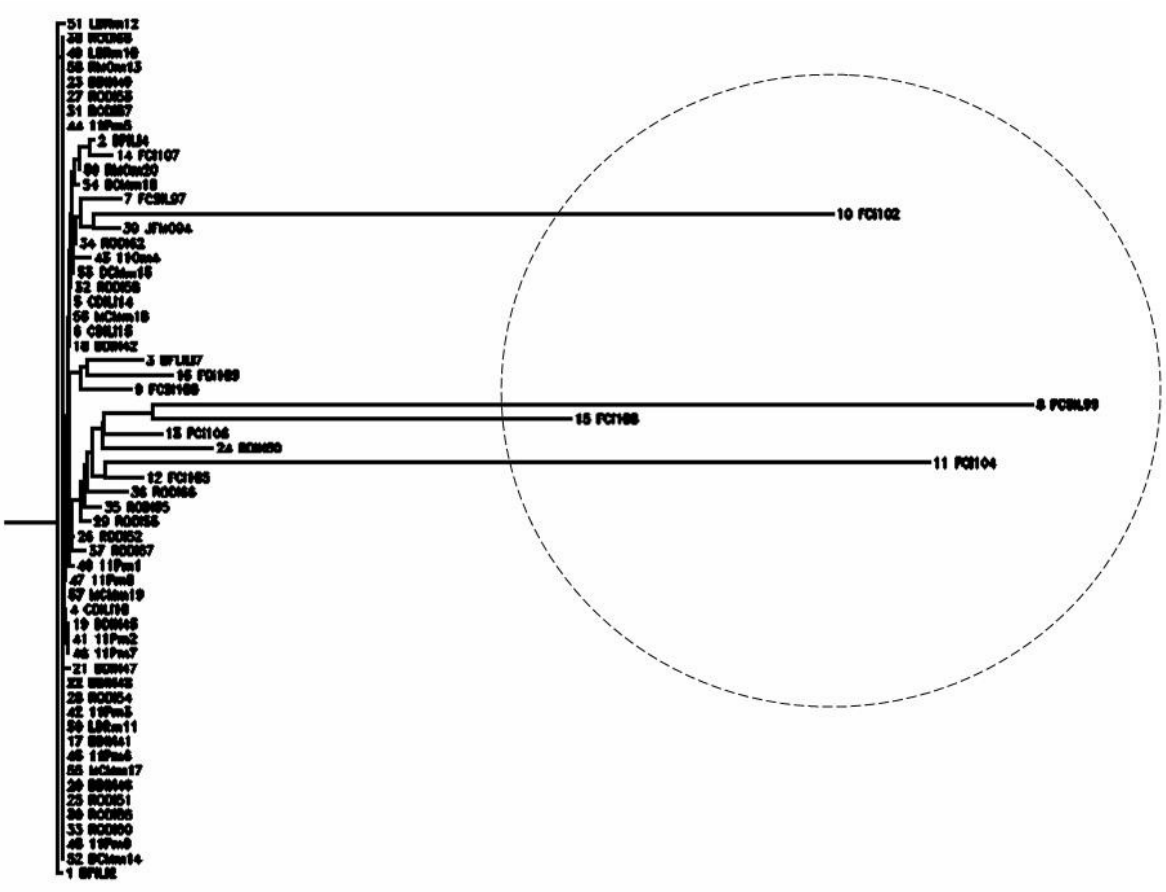

Figure 1. The Neighbor-Joining topology of all individuals

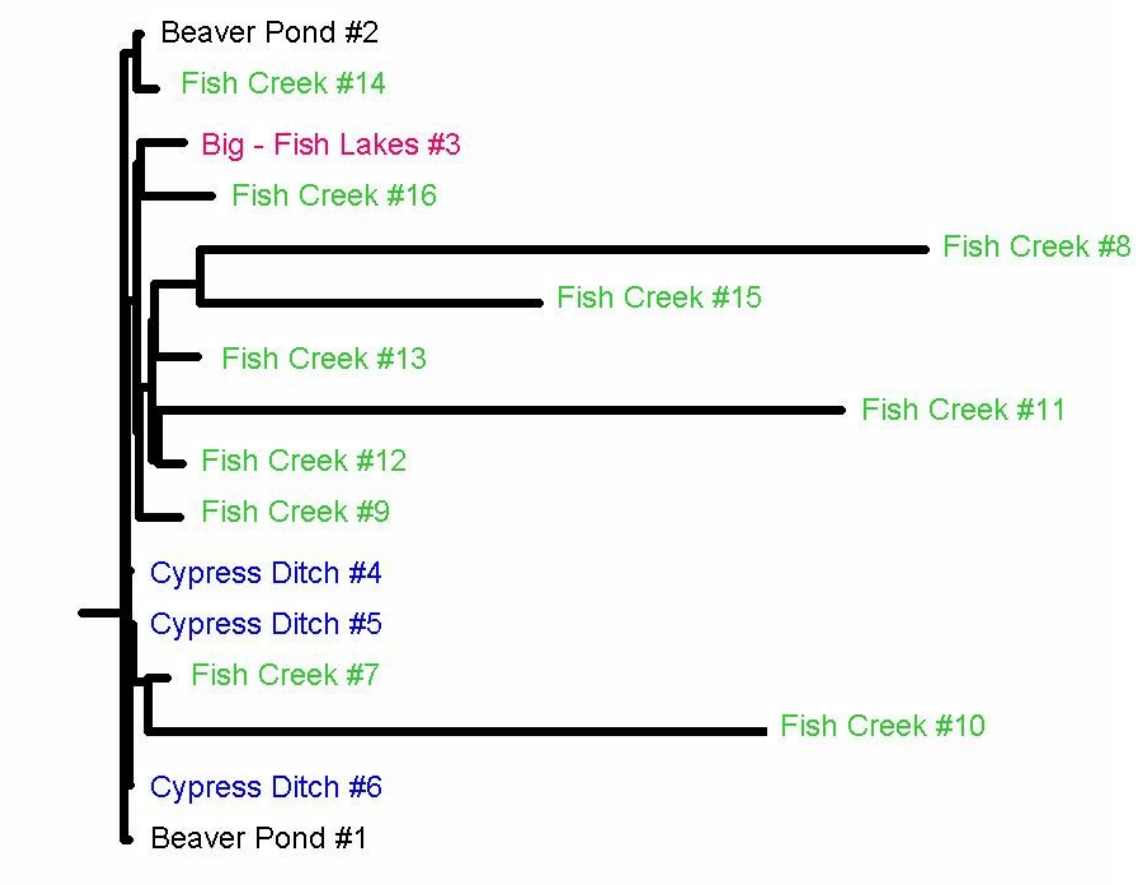

Figure 2. Topology of Illinois individuals

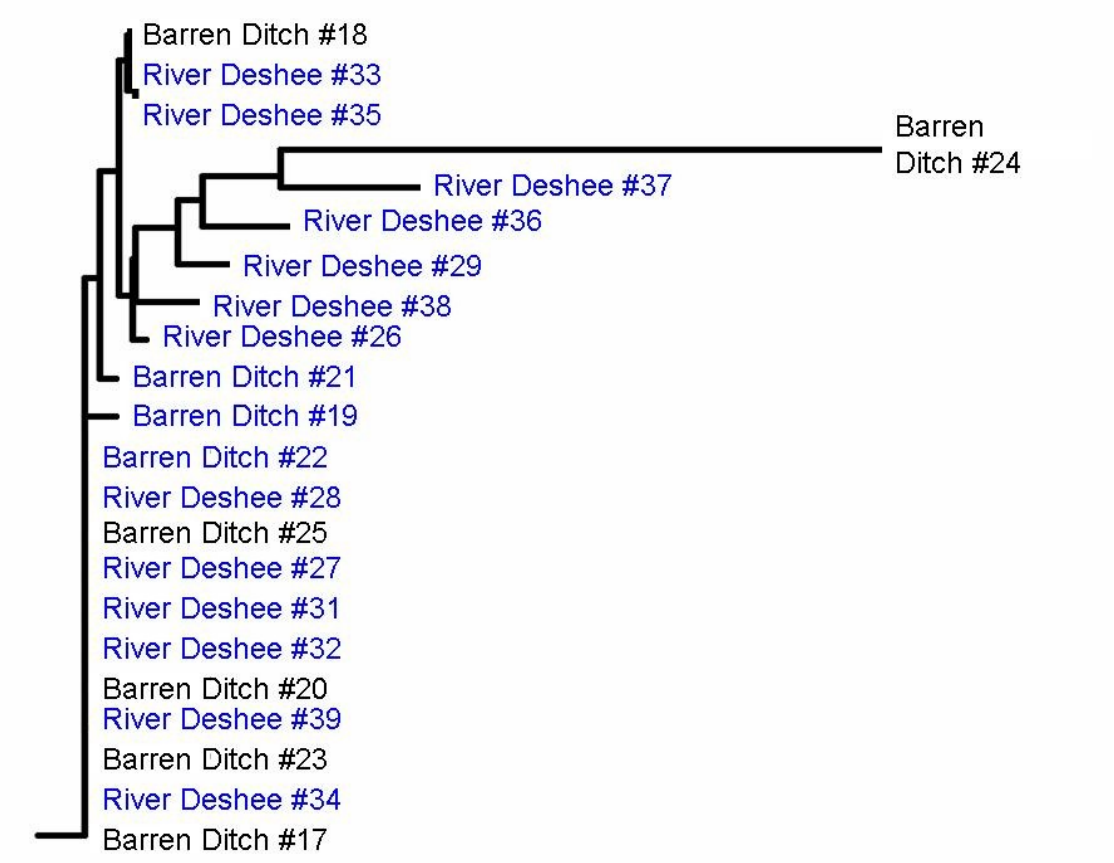

Figure 3. Topology of Indiana individuals

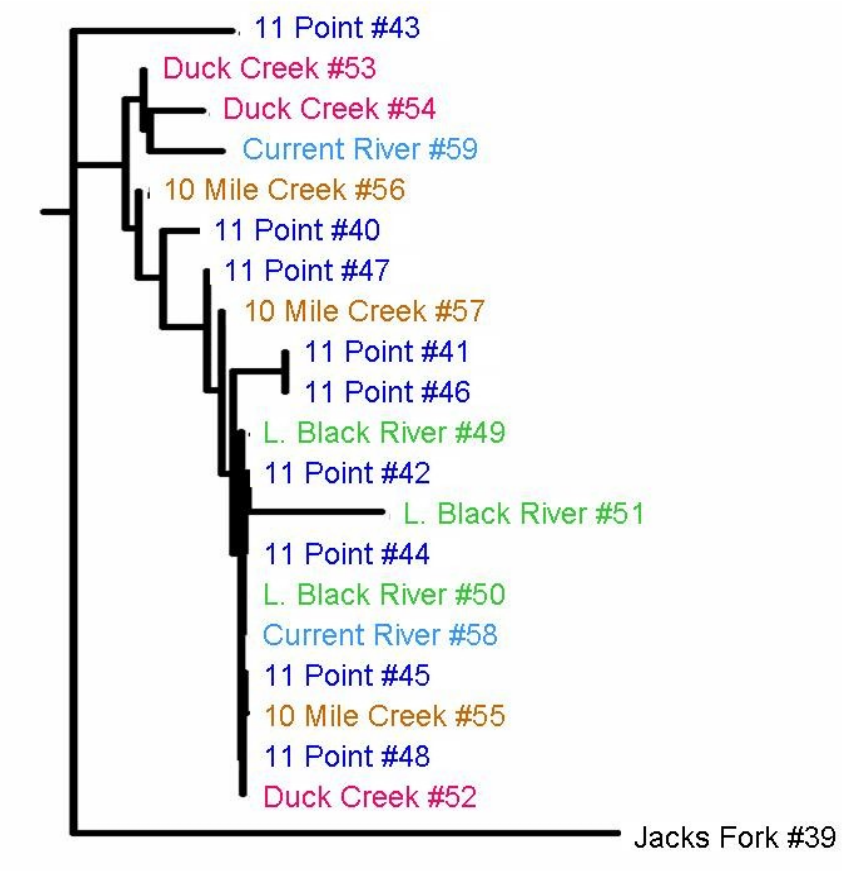

Figure 4. Topology of Missouri individuals

Job 3. Determine feasibility of broodstock collection for reintroduction efforts of redspotted sunfish in Illinois.

Conclusions and Recommendations

The general absence of any deeply rooted clustering among individuals along geographical lines indicates that the phylogeographic divergence among populations is not very deep. While this somewhat ambiguous pattern might suggest there will be little concern over choice of source population, this conclusion should be tempered by the observation of high sequence variation from the Fish Creek population. As such, to avoid introducing any unintended outbreeding (mixing of lineages) effects (e.g., Philipp et al. 2002), it remains prudent in Illinois to choose and use two different source populations for constructing recovery brood stock – one from Fish Creek (and other nearby sources should these be uncovered) for use in the vicinity of the Sangamon River basin in central Illinois and another from Cypress Ditch (and other nearby sources should these be uncovered) for use in southern Illinois.

With this in mind and given the overall demographic health (see Job 1 for this project) for each of these populations, we will wish to proceed cautiously in constructing a captive population that also 1) maximizes *within-population* genetic variation of each of the brood sources while in captivity, and 2) minimizes the risk to the source populations from any unintended "brood mining" effects.

Commonly in conservation circles, a target of 50 parents is suggested as a rule to avoid severe demographic bottlenecks and loss of genetic variation due to random drift. While this goal may ultimately be difficult to achieve in any single brood year if we are to avoid brood mining effects, this goal may ultimately be achieved across years within the species typical generation cycle (likely 5-8 years). Thus, we recommend a brood program that rotates in new individuals each year for the duration of the captive breeding program.

Moreover, we recommend an active program to monitor *within-population* genetic variation for both the captive population, the newly founded populations, and the source population(s) to assess how well the program is doing at avoiding bottlenecks. Such monitoring is necessary if we are to take an *adaptive management* approach that permits modification of actions based on measured responses of treatment to reference groups (see Bisson et al. 2007).

Finally, we recommend some descriptive observation of basic reproductive biology for this species in association with the captive breeding program to establish whether males preferentially construct nests in densely-packed colonies (such as bluegill), loose aggregates (such as pumpkinseed sunfish), or isolates (such as green sunfish). Moreover, we can also learn about effective sex ratios of spawners in nests that may guide stocking recommendations for recovery or refuge populations.

Potential Reintroduction Sites

At this time, priority reintroduction efforts should likely concentrate on the Emiquon Nature Preserve. This is a former backwater lake near Havana, Illinois now isolated from the Illinois River. The lakes and ditches on this property were rehabilitated in 2007 with restocking efforts of native fishes already begun. The Preserve should provide excellent habitat for redspotted sunfish in the form of clear water with abundant aquatic vegetation. Emiquon lies in close proximity to the Fish Creek population, making that an ideal founding source.

Hennepin-Hopper Lakes and Spunky Bottoms have also been identified as possible reintroduction sites. Both of these locations are currently experiencing significant problems involving common carp. These locations should be closely scrutinized and monitored for progress towards correcting deleterious conditions caused by the activities of an overabundant carp population. An inability to support significant stands of aquatic vegetation would certainly deter reintroduction success for the redspotted sunfish.

Mansion Pond at Allerton Park in the Sangamon River basin has been identified as a good location for an ex situ refuge population for redspotted sunfish. Other rare fish species of the Sangamon River basin are already being propagated in the pond with excellent results. Redspotted sunfish would mix well with the current population of the pond.

Other areas in Central Illinois will require further investigation for habitat suitability and concurrence with management objectives at these sites.

BUDGET SUMMARY

Requested Federal Funds:	2005	2006	Total
Contractual (INHS genetic lab work)	$6000	$6000	$12000
Paid reimbursement to INHS (09/19/2007)			$12000
Required State Match:			$4000
John Epifanio, INHS (08/01/05-07/31/07)			$5506
Trent Thomas, IDNR Streams Biologist	$7277.84	$2397.38	$9675.22
Overmatch:			11181.22

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Page, L. M. and B. M. Burr. 1991. A field guide to freshwater fishes of North America north of Mexico. The Peterson Field Guide Series. Houghton Mifflin Co., Boston. 432 pp.

Pflieger, W. L. 1975. The fishes of Missouri. Missouri Department of Conservation. 343 pp.

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Table 1. Redspotted sunfish ND-3/4 amplicon sequence data comparing edited & aligned sequences (916 nucleotide sites) from 59 individuals collected in Illinois, Missouri, and Indiana. A dot indicates an aligned basepair similarity with the first individual (Beaver Pond #1). A dash indicates no basepair present and substitutions are indicated with a different nucleotide letter (G, C, T, or A).

Individual Sample Name	Aligned Sequences base pairs (3' to 5') from 0 → 60
ILLINOIS	
Beaver Pond #1	-----AAGAT -ATGG----- -ACC-TCA-T TACA-GCCG- TCATT-GGCA TCGCCGCAG-
Beaver Pond #2	....A.... ....
Big Fish Lake #3	....--G.... ....
Cypress Ditch #4	....-..... ....
Cypress Ditch #5	....A.... ....
Cypress Ditch #6	....-.A.... ....
Fish Creek #7	....T.A.... ....
Fish Creek #8	........C. A..A.GTCTG NT..T..C.. ...G.....G ....-- ......TCTA
Fish Creek #9	....A.... ....G....G ....
Fish Creek #10	....CT.N.... ....T.GG.. .....A..AG ......A... .....T...A
Fish Creek #11	.......T.. ...A.GCCTG N.......C. ....TC...A ....AG.... ......TC..
Fish Creek #12	....CT.T.... ....A.
Fish Creek #13	....C-.T.... ....A.
Fish Creek #14	....-.N.... ....G ....
Fish Creek #15	........G. .----..... .G..CC.... ....G....C ....TC-.
Fish Creek #16	..... AC...A.... ....C..... ...T.....G ....A.
INDIANA	
Barren Ditch #17	....-.A.... ....
Barren Ditch #18	..... A....A.... ....G.... .....
Barren Ditch #19	....AA.... ....
Barren Ditch #20	....-.A.... ....
Barren Ditch #21	..... A..-.A.... ....
Barren Ditch #22	....-.A.... ....G.... .....
Barren Ditch #23	....-.A.... ....
Barren Ditch #24	....-.A.... ....C.. ....A.
Barren Ditch #25	....-.A.... ....
River Deshee #26	....-.A.... ....
River Deshee #27	....-.A.... ....
River Deshee #28	....-.A.... ....G.... .....
River Deshee #29	....--G.... ....
River Deshee #30	....-.A.... ....
River Deshee #31	....-.A.... ....
River Deshee #32	....-.A.... ....

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River Deshee #33        .......... ...-.A.... .......... .......... .......... ..........
River Deshee #34        .......... ...-.A.... .......... .......... .......... ..........
River Deshee #35        .......... ...--G.... .......... .........T .......... ..........
River Deshee #36        .......... ...-.G.... .......... .......... .......... ..........
River Deshee #37        .......... ...-.A.... .......... .......... .......... ..........
River Deshee #38        .......... ...-.A.... .......... .......... .......... ..........

MISSOURI
Jacks Fork #39          .......... ...-.A.... .......... .......... .......... ..........
11 Point #40            .......... ....AA.... .......... .......... .......... ..........
11 Point #41            .......... ....AA.... .......... .......... .......... ..........
11 Point #42            .......... ...-.A.... .......... .......... .......... ..........
11 Point #43            .......... ...-.A.... .......... .......... .......... ..........
11 Point #44            .......... ...-.A.... .......... .......... .......... ..........
11 Point #45            .......... .....A.... .......... ....G..... .......... ..........
11 Point #46            .......... ....AA.... .......... .......... .......... ..........
11 Point #47            .......... .....A.... .......... .......... .....G.... ..........
11 Point #48            .......... ...-.A.... .......... .......... .......... ..........
Little Black #49        .......... .....A.... .......... .......... .......... ..........
Little Black #50        .......... ...-.A.... .......... .......... .......... ..........
Little Black #51        .......... ...-.A.... .......... .......... .......... ..........
Duck Creek #52          .......... ...-.A.... .......... .......... .......... ..........
Duck Creek #53          .......... .....A.... .......... .......... .......... ..........
Duck Creek #54          .......... .....A.... .......... .......... .......... ..........
10 Mile Creek #55       .......... ...-.A.... .......... .......... .......... ..........
10 Mile Creek #56       .......... .....A.... ....T..... .......... .....G.... ..........
10 Mile Creek #57       .......... ...-.A.... .......... .......... .......... ..........
Current River #58       .......... .....A.... .......... ....G..... .......... ..........
Current River #59       .......... .....A.... .......... .......... .......... ..........
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Individual Sample Name	Aligned Sequences base pairs (3' to 5') from 61 ➔ 120
Beaver Pond #1	--CA-TTATG -CATTA-TTC TTG-CAATTG TATC-TTTCT GG-CTCCCCC A--AATTA-C
Beaver Pond #2	....
Big Fish Lake #3	....
Cypress Ditch #4	....
Cypress Ditch #5	....
Cypress Ditch #6	....
Fish Creek #7	....--... ....
Fish Creek #8	....C.CC. AA..GGG.G. ..ATC..... ..CG..... .AG.....
Fish Creek #9	....C.. .....GG... ....
Fish Creek #10	GA.TT..... .T.CCC.--- ----..GGGC. ....T. .A.....T. ......A...
Fish Creek #11	..AGA..... A.....CCCT C.TTG.GG.. A...G....A ..C..... .....
Fish Creek #12	....C.. .....---... ....
Fish Creek #13	....CC.. ....--.... ....
Fish Creek #14	....--. ....--.... ....
Fish Creek #15	....T..... .....TCC.. .GAGGGG.GT ....A..... ....C.
Fish Creek #16	....---C.. ....
Barren Ditch #17	....
Barren Ditch #18	....
Barren Ditch #19	....
Barren Ditch #20	....
Barren Ditch #21	....C.. ....
Barren Ditch #22	....
Barren Ditch #23	....
Barren Ditch #24	....T....A ....-C. C..---... ....C.
Barren Ditch #25	....
River Deshee #26	....C.. ....-..... ....
River Deshee #27	....
River Deshee #28	....
River Deshee #29	....CC.. ....-..... ....
River Deshee #30	....
River Deshee #31	....
River Deshee #32	....
River Deshee #33	....
River Deshee #34	....
River Deshee #35	....C.. ....GCG... ....
River Deshee #36	....CC. ...A..G... ....
River Deshee #37	....CC.. ....--.... ....
River Deshee #38	....
Jacks Fork #39	....CC.. .G..--G... ....

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Individual Sample Name	Aligned Sequences base pairs (3' to 5') from 121 ➔ 180
Beaver Pond #1	CCCTG-ACCA C-GAAAAGC- TCTCCCCCTA -TGAATGCGG -CTTCGACCC ATTAGGAAGC
Beaver Pond #2	....
Big Fish Lake #3	....
Cypress Ditch #4	....
Cypress Ditch #5	....
Cypress Ditch #6	....
Fish Creek #7	....
Fish Creek #8	......C... ....T A..... ....
Fish Creek #9	....
Fish Creek #10	...A..... .TA.....T ..... .C..... ....
Fish Creek #11	....T..... .....CT A..... C..... .....
Fish Creek #12	....
Fish Creek #13	....
Fish Creek #14	....
Fish Creek #15	..G..T.... .... T..... ....
Fish Creek #16	....A ....
Barren Ditch #17	....
Barren Ditch #18	....
Barren Ditch #19	....
Barren Ditch #20	....
Barren Ditch #21	....
Barren Ditch #22	....
Barren Ditch #23	....
Barren Ditch #24	....
Barren Ditch #25	....
River Deshee #26	....
River Deshee #27	....
River Deshee #28	....
River Deshee #29	....
River Deshee #30	....
River Deshee #31	....
River Deshee #32	....
River Deshee #33	....
River Deshee #34	....
River Deshee #35	....
River Deshee #36	....
River Deshee #37	....
River Deshee #38	....

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Jacks Fork #39 ....
11 Point #40 ....
11 Point #41 ....
11 Point #42 ....
11 Point #43 ....
11 Point #44 ....
11 Point #45 ....
11 Point #46 ....
11 Point #47 ....
11 Point #48 ....
Little Black #49 ....
Little Black #50 ....
Little Black #51 ....
Duck Creek #52 ....
Duck Creek #53 ....
Duck Creek #54 ....
10 Mile Creek #55 ....
10 Mile Creek #56 ....
10 Mile Creek #57 ....
Current River #58 ....
Current River #59 ....

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Individual Sample Name	Aligned Sequences base pairs (3' to 5') from 181 ➔ 240
Beaver Pond #1	GCCCGACTGC CC-TTCTCAC TTCGATTTTT CCT-TGTTGC AATTCTCTTT CTCC-TCTTT
Beaver Pond #2	....
Big Fish Lake #3	....
Cypress Ditch #4	....
Cypress Ditch #5	....
Cypress Ditch #6	....
Fish Creek #7	....
Fish Creek #8	....A.....
Fish Creek #9	....
Fish Creek #10	......T... ..... .C..... ....C..C.. ...CT.A..C .....
Fish Creek #11	....A... ....AG..... ....--.
Fish Creek #12	....
Fish Creek #13	....
Fish Creek #14	....
Fish Creek #15	....A..... ....--.. ....
Fish Creek #16	....
Barren Ditch #17	....
Barren Ditch #18	....
Barren Ditch #19	....
Barren Ditch #20	....
Barren Ditch #21	....
Barren Ditch #22	....
Barren Ditch #23	....
Barren Ditch #24	....
Barren Ditch #25	....
River Deshee #26	....
River Deshee #27	....
River Deshee #28	....
River Deshee #29	....
River Deshee #30	....
River Deshee #31	....
River Deshee #32	....
River Deshee #33	....
River Deshee #34	....
River Deshee #35	....
River Deshee #36	....
River Deshee #37	....
River Deshee #38	....

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Individual Sample Name	Aligned Sequences base pairs (3' to 5') from 241 ➔ 300
Beaver Pond #1	GACCTAGAAA TTGCCCTC-T TACTCCCGCT TCCCTGAGGA GATCAACTAA CCTCCCCCCT
Beaver Pond #2	....
Big Fish Lake #3	....G. ....
Cypress Ditch #4	....
Cypress Ditch #5	....
Cypress Ditch #6	....
Fish Creek #7	....
Fish Creek #8	....
Fish Creek #9	....
Fish Creek #10	....T.. ....A.. C......... ......T... .A......AC
Fish Creek #11	....
Fish Creek #12	....
Fish Creek #13	....
Fish Creek #14	....
Fish Creek #15	....T. ....
Fish Creek #16	....
Barren Ditch #17	....
Barren Ditch #18	....
Barren Ditch #19	....
Barren Ditch #20	....
Barren Ditch #21	....
Barren Ditch #22	....
Barren Ditch #23	....
Barren Ditch #24	....
Barren Ditch #25	....
River Deshee #26	....
River Deshee #27	....
River Deshee #28	....
River Deshee #29	....
River Deshee #30	....
River Deshee #31	....
River Deshee #32	....
River Deshee #33	....
River Deshee #34	....
River Deshee #35	....
River Deshee #36	....
River Deshee #37	....
River Deshee #38	....

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Individual Sample Name	Aligned Sequences base pairs (3' to 5') from 301 ➔ 360
Beaver Pond #1	CATGACCTTC TTATGGGCCT CTGCCGTACT AGCCCTCCTT ACATTAGGCC TAATTTATGA
Beaver Pond #2	....
Big Fish Lake #3	....
Cypress Ditch #4	....
Cypress Ditch #5	....
Cypress Ditch #6	....
Fish Creek #7	....
Fish Creek #8	....A ....T .....
Fish Creek #9	....
Fish Creek #10	..CA..A... .....A..T. ....T.. .....C ...C..... ....C..C..
Fish Creek #11	....--... .....
Fish Creek #12	....
Fish Creek #13	....
Fish Creek #14	....
Fish Creek #15	....
Fish Creek #16	....
Barren Ditch #17	....
Barren Ditch #18	....
Barren Ditch #19	....
Barren Ditch #20	....
Barren Ditch #21	....
Barren Ditch #22	....
Barren Ditch #23	....
Barren Ditch #24	....
Barren Ditch #25	....
River Deshee #26	....
River Deshee #27	....
River Deshee #28	....
River Deshee #29	....
River Deshee #30	....
River Deshee #31	....
River Deshee #32	....
River Deshee #33	....
River Deshee #34	....
River Deshee #35	....
River Deshee #36	....
River Deshee #37	....
River Deshee #38	....

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Individual Sample Name	Aligned Sequences base pairs (3' to 5') from 361 ➔ 420
Beaver Pond #1	ATGAACTCAA GGGGGCC-TT GAATGAGCTG AATAGACAGT TAGTTTAAGC AAAACAC-TT
Beaver Pond #2	....
Big Fish Lake #3	....
Cypress Ditch #4	....
Cypress Ditch #5	....
Cypress Ditch #6	....
Fish Creek #7	....
Fish Creek #8	.A....G..T ....T.. ......T.A. ..... C....C.... ....T..
Fish Creek #9	....
Fish Creek #10	.....TC... ....G.... ....AT .....C....
Fish Creek #11	.C......T. ....C ....T..C.. ......T... .....
Fish Creek #12	....
Fish Creek #13	....
Fish Creek #14	....
Fish Creek #15	.........T ....
Fish Creek #16	....
Barren Ditch #17	....
Barren Ditch #18	....
Barren Ditch #19	....
Barren Ditch #20	....
Barren Ditch #21	....
Barren Ditch #22	....
Barren Ditch #23	....
Barren Ditch #24	....
Barren Ditch #25	....
River Deshee #26	....
River Deshee #27	....
River Deshee #28	....
River Deshee #29	....
River Deshee #30	....
River Deshee #31	....
River Deshee #32	....
River Deshee #33	....
River Deshee #34	....
River Deshee #35	....
River Deshee #36	....
River Deshee #37	....
River Deshee #38	....

APPENDIX I

APPENDIX I

Individual Sample Name	Aligned Sequences base pairs (3' to 5') from 421 ➔ 480
Beaver Pond #1	GAT-TTCGGC -TCAAGAACT TGT-GGTTAA AGTCCATAAC TATCTA-ATG ACTCCCAC-T
Beaver Pond #2	....
Big Fish Lake #3	....
Cypress Ditch #4	....
Cypress Ditch #5	....
Cypress Ditch #6	....
Fish Creek #7	....
Fish Creek #8	...A..... G...T..... .....C.... ...-------A .....TG... GACT.....-
Fish Creek #9	....
Fish Creek #10	....--. ....C... ..C..... C
Fish Creek #11	TG-..G.... ....G....G. .AG..... .....TG.. .....G
Fish Creek #12	....
Fish Creek #13	....
Fish Creek #14	....
Fish Creek #15	....T..... ..A...--.. ....G.
Fish Creek #16	....
Barren Ditch #17	....
Barren Ditch #18	....
Barren Ditch #19	....
Barren Ditch #20	....
Barren Ditch #21	....
Barren Ditch #22	....
Barren Ditch #23	....
Barren Ditch #24	....
Barren Ditch #25	....
River Deshee #26	....
River Deshee #27	....
River Deshee #28	....
River Deshee #29	....
River Deshee #30	....
River Deshee #31	....
River Deshee #32	....
River Deshee #33	....
River Deshee #34	....
River Deshee #35	....
River Deshee #36	....
River Deshee #37	....
River Deshee #38	....

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APPENDIX I

Individual Sample Name	Aligned Sequences base pairs (3' to 5') from 481 ➔ 540
Beaver Pond #1	CACTTTGCTT TCTCTTCAAC TTTCCTACT- -GGGGCT--A ACGGGCCTGG CCTTTC-ACC
Beaver Pond #2	....
Big Fish Lake #3	....
Cypress Ditch #4	....
Cypress Ditch #5	....
Cypress Ditch #6	....
Fish Creek #7	....
Fish Creek #8	--.C...GC. ....------ -........C AA.....TA. .....TTG.T A.....T...
Fish Creek #9	....
Fish Creek #10	....A..... C..T..... .A..A....C ....C.....
Fish Creek #11	.G.......-- .....AT... ..A..... ....
Fish Creek #12	....
Fish Creek #13	....
Fish Creek #14	....
Fish Creek #15	.....G.... ....T.. ....
Fish Creek #16	....
Barren Ditch #17	....
Barren Ditch #18	....
Barren Ditch #19	....
Barren Ditch #20	....
Barren Ditch #21	....
Barren Ditch #22	....
Barren Ditch #23	....
Barren Ditch #24	....
Barren Ditch #25	....
River Deshee #26	....
River Deshee #27	....
River Deshee #28	....
River Deshee #29	....
River Deshee #30	....
River Deshee #31	....
River Deshee #32	....
River Deshee #33	....
River Deshee #34	....
River Deshee #35	....
River Deshee #36	....
River Deshee #37	....
River Deshee #38	....

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APPENDIX I

Individual Sample Name	Aligned Sequences base pairs (3' to 5') from 541 ➔ 600
Beaver Pond #1	GAAC-ACACC TTCT-TTCCG CCCTACT-CT GCTTAGAGGG GATAATAT-T GTCTTTATTT
Beaver Pond #2	....
Big Fish Lake #3	....
Cypress Ditch #4	....
Cypress Ditch #5	....
Cypress Ditch #6	....
Fish Creek #7	....
Fish Creek #8	...-....TT .C..C..T.. ..T....CTG .T...A.... ....G.C-..C.
Fish Creek #9	....
Fish Creek #10	....C..... ....A.. A......C.. A...C.....
Fish Creek #11	T...T...T. ..TG..... ....A.. ....A..A..A. .....
Fish Creek #12	....
Fish Creek #13	....
Fish Creek #14	....C .....
Fish Creek #15	...-.--... .CT..... ....A.... ....C.. .C.....
Fish Creek #16	....
Barren Ditch #17	....
Barren Ditch #18	....
Barren Ditch #19	....
Barren Ditch #20	....
Barren Ditch #21	....
Barren Ditch #22	....
Barren Ditch #23	....
Barren Ditch #24	....
Barren Ditch #25	....
River Deshee #26	....
River Deshee #27	....
River Deshee #28	....
River Deshee #29	....
River Deshee #30	....
River Deshee #31	....
River Deshee #32	....
River Deshee #33	....
River Deshee #34	....
River Deshee #35	....
River Deshee #36	....
River Deshee #37	....A.A .....
River Deshee #38	....

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Jacks Fork #39 ....
11 Point #40 ....
11 Point #41 ....
11 Point #42 ....
11 Point #43 ....
11 Point #44 ....
11 Point #45 ....
11 Point #46 ....
11 Point #47 ....
11 Point #48 ....
Little Black #49 ....
Little Black #50 ....
Little Black #51 ....
Duck Creek #52 ....
Duck Creek #53 ....
Duck Creek #54 ....
10 Mile Creek #55 ....
10 Mile Creek #56 ....
10 Mile Creek #57 ....
Current River #58 ....
Current River #59 ....

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Individual Sample Name	Aligned Sequences base pairs (3' to 5') from601 ➔ 660
Beaver Pond #1	ATTGCCCTCT CACTATGAAC CCT-CCAACT AGACTCAACT AAC-TTCTCA GCC---GCCC
Beaver Pond #2	....
Big Fish Lake #3	....
Cypress Ditch #4	....
Cypress Ditch #5	....
Cypress Ditch #6	....
Fish Creek #7	....
Fish Creek #8	....TA..-... ...CA...T. .T.G..TG.. T..T..... TT.CTGT...
Fish Creek #9	....
Fish Creek #10	..C..G.... ..T..... A.....T. ...T.....C .....G .....
Fish Creek #11	.....A...A .G-..... ....-T.... ...G..... T.A.C.G..C AG.....G.T
Fish Creek #12	....
Fish Creek #13	....
Fish Creek #14	....A..... ....
Fish Creek #15	..GC..TC.. ...G..... ...C..... ...G..... .....T. .....
Fish Creek #16	....- -.....
Barren Ditch #17	....
Barren Ditch #18	....
Barren Ditch #19	....
Barren Ditch #20	....
Barren Ditch #21	....
Barren Ditch #22	....
Barren Ditch #23	....
Barren Ditch #24	....T..... .....
Barren Ditch #25	....
River Deshee #26	....
River Deshee #27	....
River Deshee #28	....
River Deshee #29	....
River Deshee #30	....
River Deshee #31	....
River Deshee #32	....
River Deshee #33	....
River Deshee #34	....
River Deshee #35	....
River Deshee #36	....
River Deshee #37	....
River Deshee #38	....

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Jacks Fork #39 ....
11 Point #40 ....
11 Point #41 ....
11 Point #42 ....
11 Point #43 ....
11 Point #44 ....
11 Point #45 ....
11 Point #46 ....
11 Point #47 ....
11 Point #48 ....
Little Black #49 ....
Little Black #50 ....
Little Black #51 ....
Duck Creek #52 ....
Duck Creek #53 ....
Duck Creek #54 ....
10 Mile Creek #55 ....
10 Mile Creek #56 ....
10 Mile Creek #57 ....
Current River #58 ....
Current River #59 ....

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Individual Sample Name	Aligned Sequences base pairs (3' to 5') from 661 ➔ 720
Beaver Pond #1	CTATACTCC- TACT-GGCA- TTCTCAGCC- T--GC-GAAG C-AAGCGCC- GGAC-TTGCC
Beaver Pond #2	....
Big Fish Lake #3	....
Cypress Ditch #4	....
Cypress Ditch #5	....
Cypress Ditch #6	....
Fish Creek #7	....
Fish Creek #8	T..-----. .T.....T .CT....G.T .....GA TG..TT.G.. ...T.GGAT.
Fish Creek #9	....
Fish Creek #10	.C..... .-...A...T .CT..... ....T..A. ..G...C...
Fish Creek #11	....G....A.. ......A... .....ATG.A A..C..C.TG ....T.G..G
Fish Creek #12	....
Fish Creek #13	....G..... ....
Fish Creek #14	....G.... ....
Fish Creek #15	....T .....G .GT...A..- -.T..... ....T.....
Fish Creek #16	....
Barren Ditch #17	....
Barren Ditch #18	....
Barren Ditch #19	....
Barren Ditch #20	....
Barren Ditch #21	....
Barren Ditch #22	....
Barren Ditch #23	....
Barren Ditch #24	.....T .... CGG..... .T.....G .....
Barren Ditch #25	....
River Deshee #26	....
River Deshee #27	....
River Deshee #28	....
River Deshee #29	....
River Deshee #30	....
River Deshee #31	....
River Deshee #32	....
River Deshee #33	....
River Deshee #34	....
River Deshee #35	....
River Deshee #36	....
River Deshee #37	....
River Deshee #38	....

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Jacks Fork #39 ....
11 Point #40 ....
11 Point #41 ....
11 Point #42 ....
11 Point #43 ....
11 Point #44 ....
11 Point #45 ....
11 Point #46 ....
11 Point #47 ....
11 Point #48 ....
Little Black #49 ....
Little Black #50 ....
Little Black #51 ....
Duck Creek #52 ....
Duck Creek #53 ....
Duck Creek #54 ....
10 Mile Creek #55 ....
10 Mile Creek #56 ....
10 Mile Creek #57 ....
Current River #58 ....
Current River #59 ....

APPENDIX I

Individual Sample Name	Aligned Sequences base pairs (3' to 5') from 721 ➔ 780
Beaver Pond #1	C-TAC-TTGT -AGCAACCGC CC-GAACT-- -CACG-GC-T CCG-ATCG-- CTT-ACAAAA
Beaver Pond #2	....
Big Fish Lake #3	.T..... .... GC.T.....
Cypress Ditch #4	....
Cypress Ditch #5	....
Cypress Ditch #6	....
Fish Creek #7	.....T.... ....ACA....
Fish Creek #8	.T.T.T...G ....CG ..C.G.TATT CG.G.G...A ...C....GC T..T.G.GG.
Fish Creek #9	.....- .... T.....
Fish Creek #10	....T..... T.G..... .....G.. .....C.... ..-.....
Fish Creek #11	.T..T..G.. .....TA..- --...GTCGT CT.A...G.. ...A..T... T...--.C..
Fish Creek #12	....-.....
Fish Creek #13	.T..... ....
Fish Creek #14	....G...T... ....
Fish Creek #15	.....T.... G..T..A... T-......TA A......AT. .....AAT.. A.A.....G.
Fish Creek #16	....T..T. ....
Barren Ditch #17	....
Barren Ditch #18	....
Barren Ditch #19	....
Barren Ditch #20	....
Barren Ditch #21	....
Barren Ditch #22	....
Barren Ditch #23	....
Barren Ditch #24	.....- ..... .G....--.. .... A.....
Barren Ditch #25	....
River Deshee #26	....
River Deshee #27	....
River Deshee #28	....
River Deshee #29	....
River Deshee #30	....
River Deshee #31	....
River Deshee #32	....
River Deshee #33	....
River Deshee #34	....
River Deshee #35	....T. ....
River Deshee #36	....
River Deshee #37	....
River Deshee #38	....

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Jacks Fork #39 ....
11 Point #40 ....
11 Point #41 ....
11 Point #42 ....
11 Point #43 ....A.....
11 Point #44 ....
11 Point #45 ....
11 Point #46 ....
11 Point #47 ....
11 Point #48 ....
Little Black #49 ....
Little Black #50 ....
Little Black #51 ....
Duck Creek #52 ....
Duck Creek #53 ....
Duck Creek #54 ....
10 Mile Creek #55 ....
10 Mile Creek #56 ....
10 Mile Creek #57 ....
Current River #58 ....
Current River #59 ....

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Individual Sample Name	Aligned Sequences base pairs (3' to 5') from 781 ➔ 840
Beaver Pond #1	CCTAAA--CC TCCT-AC-AA T-GCT-AAAA ATTTT--AAT CCCCC---AC CCT-GATAC-
Beaver Pond #2	....A.... ..... -..... ...GA.....
Big Fish Lake #3	.......C.. ....A.. .....A.... .....TA.TC -..... ...GA.....
Cypress Ditch #4	....A.... ..... -..... .....
Cypress Ditch #5	.... -..... .....
Cypress Ditch #6	....A.... ..... -..... .....T
Fish Creek #7	....GA... -..... ...G..A...
Fish Creek #8	A...T..... C---..... .GTG.GTGTG .....--- -GT.....G. .T..-----.
Fish Creek #9	....TA... T..... .....
Fish Creek #10	...G..... ....A.. .....T.... TA.C...C.. T..... .T....GCT.
Fish Creek #11	..C.....A. .TT-..... ...T.TGTT. .A.G..... ....T...-- ---.AG..G.
Fish Creek #12	....A.... ..... -..... ....-G..AA
Fish Creek #13	.... .G...A.... T..... -....CACC. .TNGA....T
Fish Creek #14	....A..A.. .....A.... ......A... -..... ...GA.....
Fish Creek #15	....G..... .T....GAG. .G...T...G .G.....--- -T..GCCC.. ..C.TG..A.
Fish Creek #16	.....C...T C..... .....A.... ....TT.
Barren Ditch #17	.... -..... .....
Barren Ditch #18	.... -..... .....
Barren Ditch #19	.... -..... .....
Barren Ditch #20	.... -..... .....
Barren Ditch #21	.... -..... .....
Barren Ditch #22	.... -..... .....
Barren Ditch #23	.... -..... .....
Barren Ditch #24	.....C...T C.....TC.. .G...T.... ....G..... T....CAAC. .T.T.....
Barren Ditch #25	.... -..... .....
River Deshee #26	.... -..... .....
River Deshee #27	.... -..... .....
River Deshee #28	.... -..... .....
River Deshee #29	.... -..... .....
River Deshee #30	.... -..... .....
River Deshee #31	.... -..... .....
River Deshee #32	.... -..... .....
River Deshee #33	.... -..... .....
River Deshee #34	.... -..... .....
River Deshee #35	....AT.... ..... -..... ...T.....
River Deshee #36	....T.... ..... -.T..... .....G
River Deshee #37	......AC.. ....A.... ..... -..... .....
River Deshee #38	.... -..... ...G.....

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Jacks Fork #39          ......... ......... N....A.... T......... -......... .........
11 Point #40            ......... ......... ......... ......... -......... .........
11 Point #41            ......... ......... ......... ......... -......... .........
11 Point #42            ......... ......... ......... ......... -......... .........
11 Point #43            ......... ......... ......... ......... -......... .........
11 Point #44            ......... ......... ......... ......... -......... .........
11 Point #45            ......... ......... ......... ......... -......... .........
11 Point #46            ......... ......... ......... ......... -......... .........
11 Point #47            ......... ......... ......... ......... -......... .........
11 Point #48            ......... ......... ......... ......... -......... .........
Little Black #49        ......... ......... ......... ......... -......... .........
Little Black #50        ......... ......... ......... ......... -......... .........
Little Black #51        ......... ......... ......... ......... -......... .........
Duck Creek #52          ......... ......... ......... ......... -......... .........
Duck Creek #53          ......... ......... ......... ......... -......... .........
Duck Creek #54          ......... ......... ......... ......... -......... .........
10 Mile Creek #55       ......... ......... ......... ......... -......... .........
10 Mile Creek #56       ......... ......... ......... ......... -......... ...G......
10 Mile Creek #57       ......... ......... ......... ......... -......... .........
Current River #58       ......... ......... ......... ......... -......... .........
Current River #59       ......... ......... ......... ......... -......... .........
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Individual Sample Name	Aligned Sequences base pairs (3' to 5') from841 ➔ 900
Beaver Pond #1	TAAT--CCCT -ACC--ATCT -G--AGCAA- CCCCCCG--C CAAATG-AC- TGGG-----A
Beaver Pond #2	.... GA..... .....-..C. A..G--.... .....
Big Fish Lake #3	...ATC.... .....TC T..... A.....C. A.....A..T G...A.....
Cypress Ditch #4	....--.G. ....T.....
Cypress Ditch #5	....-..C. -.....A... ..T.....
Cypress Ditch #6	....G..C. -.....G... ..T.A.....
Fish Creek #7	...ATT.... .....T- ....G..C. A.....G... ...A.....T
Fish Creek #8	----.-... .---..G... T....AT... .TTT.....- ----.AA..T ....GCC...
Fish Creek #9	.....G.... .....ATC T..... .....-..T. .....T G.T.T.....
Fish Creek #10	.GT...-.. .G..AA.... T....CT..G .....G..CT A.....A AAT.A....C
Fish Creek #11	AG..... .-.....CA. T..-----. TA...GTTCT AT.....N. ..T.NTACC.
Fish Creek #12	.....C..T. A.....TC T..A..... .....-..C. A.....A..T G.T.....
Fish Creek #13	A...TC.... T.....CATC T.GA..... .....-.CCA A..T..G..T ..T.G.....
Fish Creek #14	..... A..... GA.....C .....GC. A.....G..T ...TG.....
Fish Creek #15	A.G....TA. .GAA...C.. ....---... .....G..GA A.GT....TT ..T.A.....
Fish Creek #16	A...CC.... .....TC T.GA..... A..... G..... ..T.A....C
Barren Ditch #17	....--.G. ....T.....
Barren Ditch #18	....-..C. -..... ..T.....
Barren Ditch #19	....--.G. ....T.....
Barren Ditch #20	....--.G. ....T.....
Barren Ditch #21	....--.G. ....T.....
Barren Ditch #22	....--.G. ....T.....
Barren Ditch #23	....--.G. ....T.....
Barren Ditch #24	..T..... .....ATC G.....T... .....C. A..T...--. ..T.T.....
Barren Ditch #25	....--.G. ....T.....
River Deshee #26	....G..C. -..... ..T.A.....
River Deshee #27	....--.G. ....T.....
River Deshee #28	....--.G. ....T.....
River Deshee #29	....-..C. -..... ..TTG.....
River Deshee #30	....--.G. ....T.....
River Deshee #31	....--.G. ....T.....
River Deshee #32	....-..C. -..... ..T.....
River Deshee #33	....--.G. ....T.....
River Deshee #34	....G-.C. A..... ..T.....
River Deshee #35	....C. -.....T ..T.A.....
River Deshee #36	.....C..T. .....T. GA..... .....C. A.....A..T ..T.A.....
River Deshee #37	....C. ....T.A.....
River Deshee #38	....--.G. ....T.....

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Jacks Fork #39          ...ATC..T. .......... .....A-... .....-..C. A......... -.T.A.....
11 Point #40            .......... .......... .......... .....-..C. -......... ..T.......
11 Point #41            .......... .......... .......... .....--.G. .......... ..T.......
11 Point #42            .......... .......... .......... .....--.G. .......... ..T.......
11 Point #43            ...A.T.... .......... G......... .....-..C. A..TG.A... ..T.A.....
11 Point #44            .......... .......... .......... .....--.G. .......... ..T.......
11 Point #45            .......... .......... .......... .....--.G. .......... ..T.A.....
11 Point #46            .......... .......... .......... .....--.G. .......... ..T.......
11 Point #47            .......... .......... .......... .....G.... .......... ..T.......
11 Point #48            .......... .......... .......... .....--.G. .......... ..T.......
Little Black #49        .......... .......... .......... .....--.G. .......... ..T.......
Little Black #50        .......... .......... .......... .....--.G. .......... ..T.......
Little Black #51        .........C T......... .......... .....--.G. ......A... ....G.....
Duck Creek #52          .......... .......... .......... .....--.G. .......... ..T.......
Duck Creek #53          .......... .......... .......... .....-..C. A......... ..T.......
Duck Creek #54          ....AC.... .......... .......... .....G..C. A......... ....A.....
10 Mile Creek #55       .......... .......... .......... .....-C.G. ......A... ..T.A.....
10 Mile Creek #56       .......... .......... .......... .....-..C. -......... ..T.A.....
10 Mile Creek #57       .......... .......... .......... .....GN... .......... ..T.A.....
Current River #58       .......... .......... .......... .....--.G. .......... ..T.......
Current River #59       .....C.... .......... .......... .......NC. A......... ....G.....
```

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Individual Sample Name	Aligned Sequences base pairs (3' to 5') from 901 ➔ 916
Beaver Pond #1	CCTA-C-GGT --CTTA
Beaver Pond #2	C
Big Fish Lake #3	....CGG... C
Cypress Ditch #4	C
Cypress Ditch #5	C
Cypress Ditch #6	....C.G... C
Fish Creek #7	......C... C
Fish Creek #8	...-..G..- ..N..C
Fish Creek #9	A.A-.TG.N. C
Fish Creek #10	.AC.A.C--- C
Fish Creek #11	.T...TG.A. TNANAC
Fish Creek #12	.TC...G... NCT..C
Fish Creek #13	......G..N CCT..C
Fish Creek #14	......G... TN..NC
Fish Creek #15	.ACGATNT.G TAT...
Fish Creek #16	....CGG.AC CC
Barren Ditch #17	......G... C
Barren Ditch #18	......G... C
Barren Ditch #19	C
Barren Ditch #20	C
Barren Ditch #21	......G... C
Barren Ditch #22	C
Barren Ditch #23	C
Barren Ditch #24	...GA.G... .....
Barren Ditch #25	C
River Deshee #26	C
River Deshee #27	C
River Deshee #28	C
River Deshee #29	...TA..... C
River Deshee #30	C
River Deshee #31	C
River Deshee #32	......G... C
River Deshee #33	C
River Deshee #34	C
River Deshee #35	C
River Deshee #36	...TA.G... .....
River Deshee #37	C

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River Deshee #38         .......... .....C
Jacks Fork #39           ....CGG... ...C.C
11 Point #40             .......... .....C
11 Point #41             .......... .....C
11 Point #42             .......... .....C
11 Point #43             ......G... .....C
11 Point #44             .......... .....C
11 Point #45             .......... .....C
11 Point #46             .......... .....C
11 Point #47             .......... .....C
11 Point #48             .......... .....C
Little Black #49         .......... .....C
Little Black #50         .......... .....C
Little Black #51         ......G... .....C
Duck Creek #52           .......... .....C
Duck Creek #53           .......... .....C
Duck Creek #54           ......G... .....C
10 Mile Creek #55        .......... .....C
10 Mile Creek #56        .......... .....C
10 Mile Creek #57        .......... .....C
Current River #58        .......... .....C
Current River #59        ......G... .....C
```

APPENDIX II

Table 2. Pairwise divergence comparisons (matrix) among individuals. Values along diagonal equal zero with those above being identical as those below the diagonal.

Beaver Pond #1 0.0000 0.0064 0.0168 0.0025 0.0025 0.0038 0.0142 0.2253 0.0181 0.1864
0.2088 0.0209 0.0237 0.0103 0.1208 0.0277 0.0025 0.0025 0.0038 0.0025 0.0038 0.0025 0.0025
0.0348 0.0025 0.0051 0.0025 0.0025 0.0064 0.0025 0.0025 0.0025 0.0025 0.0051 0.0090 0.0128
0.0064 0.0025 0.0156 0.0038 0.0038 0.0025 0.0077 0.0025 0.0025 0.0038 0.0038 0.0025 0.0025
0.0025 0.0025 0.0025 0.0038 0.0038 0.0038 0.0025 0.0038 0.0025 0.0025

Beaver Pond #2 0.0064 0.0000 0.0181 0.0076 0.0051 0.0051 0.0142 0.2297 0.0235 0.1883
0.2105 0.0235 0.0236 0.0064 0.1247 0.0318 0.0076 0.0051 0.0089 0.0076 0.0089 0.0076 0.0076
0.0390 0.0076 0.0064 0.0076 0.0076 0.0102 0.0076 0.0076 0.0051 0.0076 0.0051 0.0155 0.0167
0.0102 0.0076 0.0168 0.0063 0.0089 0.0076 0.0064 0.0076 0.0076 0.0089 0.0064 0.0076 0.0076
0.0076 0.0076 0.0076 0.0051 0.0038 0.0089 0.0051 0.0064 0.0076 0.0038

Big & Fish L. #3 0.0168 0.0181 0.0000 0.0182 0.0168 0.0193 0.0261 0.2372 0.0262 0.2059
0.2164 0.0234 0.0316 0.0234 0.1387 0.0330 0.0195 0.0168 0.0195 0.0195 0.0208 0.0195 0.0195
0.0521 0.0195 0.0195 0.0195 0.0195 0.0222 0.0195 0.0195 0.0168 0.0195 0.0181 0.0248 0.0287
0.0221 0.0195 0.0220 0.0168 0.0195 0.0195 0.0220 0.0195 0.0195 0.0195 0.0195 0.0195 0.0195
0.0195 0.0207 0.0195 0.0168 0.0180 0.0208 0.0168 0.0195 0.0195 0.0167

Cypress Ditch #4 0.0025 0.0076 0.0182 0.0000 0.0013 0.0013 0.0143 0.2283 0.0155 0.1829
0.2045 0.0195 0.0223 0.0116 0.1208 0.0250 0.0000 0.0013 0.0000 0.0000 0.0013 0.0000 0.0000
0.0364 0.0000 0.0025 0.0000 0.0000 0.0051 0.0000 0.0000 0.0013 0.0000 0.0025 0.0090 0.0154
0.0051 0.0000 0.0143 0.0013 0.0000 0.0000 0.0064 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000
0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

Cypress Ditch #5 0.0025 0.0051 0.0168 0.0013 0.0000 0.0013 0.0129 0.2271 0.0154 0.1762
0.2012 0.0181 0.0222 0.0103 0.1190 0.0236 0.0013 0.0000 0.0025 0.0013 0.0025 0.0013 0.0013
0.0320 0.0013 0.0013 0.0013 0.0013 0.0051 0.0013 0.0013 0.0000 0.0013 0.0000 0.0076 0.0127
0.0038 0.0013 0.0116 0.0013 0.0025 0.0013 0.0038 0.0013 0.0013 0.0025 0.0000 0.0013 0.0013
0.0013 0.0038 0.0013 0.0000 0.0013 0.0025 0.0000 0.0000 0.0013 0.0013

Cypress Ditch #6 0.0038 0.0051 0.0193 0.0013 0.0013 0.0000 0.0116 0.2330 0.0168 0.1795
0.2009 0.0194 0.0221 0.0115 0.1222 0.0249 0.0013 0.0000 0.0025 0.0013 0.0025 0.0013 0.0013
0.0376 0.0013 0.0013 0.0013 0.0013 0.0076 0.0013 0.0013 0.0000 0.0013 0.0000 0.0102 0.0192
0.0051 0.0013 0.0115 0.0013 0.0025 0.0013 0.0050 0.0013 0.0013 0.0025 0.0000 0.0013 0.0013
0.0013 0.0063 0.0013 0.0000 0.0013 0.0038 0.0000 0.0000 0.0013 0.0038

Fish Creek #7 0.0142 0.0142 0.0261 0.0143 0.0129 0.0116 0.0000 0.2399 0.0264 0.1822
0.2144 0.0262 0.0305 0.0182 0.1310 0.0375 0.0142 0.0129 0.0156 0.0129 0.0156 0.0129 0.0129
0.0436 0.0129 0.0116 0.0129 0.0129 0.0143 0.0129 0.0129 0.0116 0.0129 0.0103 0.0182 0.0248
0.0169 0.0129 0.0209 0.0129 0.0156 0.0129 0.0142 0.0129 0.0142 0.0156 0.0129 0.0129 0.0142
0.0129 0.0155 0.0129 0.0116 0.0141 0.0155 0.0116 0.0116 0.0142 0.0142

APPENDIX II

Fish Creek #8 0.2253 0.2297 0.2372 0.2283 0.2271 0.2330 0.2399 0.0000 0.2349 0.4243 0.4140 0.2370 0.2323 0.2223 0.3039 0.2509 0.2257 0.2266 0.2301 0.2261 0.2231 0.2261 0.2261 0.2391 0.2261 0.2253 0.2261 0.2261 0.2217 0.2261 0.2261 0.2253 0.2261 0.2284 0.2255 0.2287 0.2280 0.2261 0.2265 0.2297 0.2301 0.2261 0.2340 0.2261 0.2297 0.2301 0.2298 0.2261 0.2279 0.2261 0.2244 0.2261 0.2275 0.2284 0.2298 0.2289 0.2302 0.2279 0.2236

Fish Creek #9 0.0181 0.0235 0.0262 0.0155 0.0154 0.0168 0.0264 0.2349 0.0000 0.1939 0.2132 0.0222 0.0291 0.0263 0.1238 0.0333 0.0155 0.0154 0.0168 0.0155 0.0142 0.0155 0.0155 0.0362 0.0155 0.0155 0.0155 0.0155 0.0182 0.0155 0.0155 0.0154 0.0155 0.0168 0.0195 0.0247 0.0169 0.0155 0.0223 0.0168 0.0168 0.0155 0.0248 0.0155 0.0168 0.0168 0.0141 0.0155 0.0155 0.0155 0.0194 0.0155 0.0168 0.0207 0.0181 0.0168 0.0155 0.0154 0.0207

Fish Creek #10 0.1864 0.1883 0.2059 0.1829 0.1762 0.1795 0.1822 0.4243 0.1939 0.0000 0.4049 0.1844 0.1952 0.1997 0.2808 0.2066 0.1807 0.1782 0.1825 0.1787 0.1807 0.1787 0.1787 0.2132 0.1787 0.1741 0.1787 0.1787 0.1806 0.1787 0.1787 0.1762 0.1787 0.1741 0.1827 0.1910 0.1811 0.1787 0.1791 0.1781 0.1825 0.1787 0.1842 0.1787 0.1804 0.1825 0.1782 0.1787 0.1807 0.1787 0.1864 0.1787 0.1760 0.1795 0.1804 0.1757 0.1762 0.1807 0.1836

Fish Creek #11 0.2088 0.2105 0.2164 0.2045 0.2012 0.2009 0.2144 0.4140 0.2132 0.4049 0.0000 0.2020 0.2103 0.2089 0.3064 0.2204 0.2016 0.2005 0.2058 0.2020 0.1995 0.2016 0.2020 0.2275 0.2020 0.1949 0.2020 0.2016 0.1982 0.2020 0.2020 0.1991 0.2020 0.1966 0.2000 0.2057 0.2067 0.2020 0.2033 0.2033 0.2058 0.2020 0.2085 0.2020 0.2059 0.2058 0.2030 0.2020 0.2037 0.2020 0.2041 0.2020 0.2008 0.2026 0.2037 0.2008 0.1995 0.2059 0.2051

Fish Creek #12 0.0209 0.0235 0.0234 0.0195 0.0181 0.0194 0.0262 0.2370 0.0222 0.1844 0.2020 0.0000 0.0221 0.0248 0.1123 0.0345 0.0195 0.0181 0.0222 0.0195 0.0181 0.0195 0.0195 0.0434 0.0195 0.0155 0.0195 0.0195 0.0182 0.0195 0.0195 0.0168 0.0195 0.0168 0.0181 0.0233 0.0195 0.0195 0.0208 0.0194 0.0222 0.0195 0.0234 0.0195 0.0208 0.0222 0.0195 0.0195 0.0208 0.0195 0.0235 0.0195 0.0181 0.0194 0.0208 0.0181 0.0182 0.0208 0.0194

Fish Creek #13 0.0237 0.0236 0.0316 0.0223 0.0222 0.0221 0.0305 0.2323 0.0291 0.1952 0.2103 0.0221 0.0000 0.0303 0.1217 0.0373 0.0236 0.0209 0.0250 0.0236 0.0223 0.0236 0.0236 0.0404 0.0236 0.0209 0.0236 0.0236 0.0223 0.0236 0.0236 0.0209 0.0236 0.0209 0.0263 0.0343 0.0249 0.0236 0.0289 0.0222 0.0250 0.0236 0.0275 0.0236 0.0250 0.0250 0.0223 0.0236 0.0236 0.0236 0.0276 0.0236 0.0209 0.0248 0.0276 0.0222 0.0236 0.0236 0.0222

Fish Creek #14 0.0103 0.0064 0.0234 0.0116 0.0103 0.0115 0.0182 0.2223 0.0263 0.1997 0.2089 0.0248 0.0303 0.0000 0.1296 0.0360 0.0116 0.0090 0.0129 0.0116 0.0116 0.0116 0.0116 0.0448 0.0116 0.0116 0.0116 0.0116 0.0090 0.0116 0.0116 0.0090 0.0116 0.0103 0.0168 0.0193 0.0129 0.0116 0.0209 0.0103 0.0129 0.0116 0.0155 0.0116 0.0129 0.0129 0.0116 0.0116 0.0116 0.0116 0.0142 0.0116 0.0090 0.0102 0.0155 0.0102 0.0129 0.0116 0.0076

Fish Creek #15 0.1208 0.1247 0.1387 0.1208 0.1190 0.1222 0.1310 0.3039 0.1238 0.2808 0.3064 0.1123 0.1217 0.1296 0.0000 0.1416 0.1190 0.1190 0.1190 0.1190 0.1173 0.1190 0.1190 0.1394 0.1190 0.1152 0.1190 0.1190 0.1189 0.1190 0.1190 0.1190 0.1190 0.1189 0.1184 0.1232

APPENDIX II

0.1211 0.1190 0.1195 0.1190 0.1190 0.1190 0.1206 0.1190 0.1187 0.1190 0.1189 0.1190 0.1190 0.1190 0.1243 0.1190 0.1189 0.1204 0.1206 0.1204 0.1189 0.1189 0.1241

Fish Creek #16 0.0277 0.0318 0.0330 0.0250 0.0236 0.0249 0.0375 0.2509 0.0333 0.2066 0.2204 0.0345 0.0373 0.0360 0.1416 0.0000 0.0250 0.0235 0.0263 0.0250 0.0263 0.0250 0.0250 0.0493 0.0250 0.0263 0.0250 0.0250 0.0319 0.0250 0.0250 0.0236 0.0250 0.0263 0.0304 0.0386 0.0290 0.0250 0.0344 0.0249 0.0263 0.0250 0.0318 0.0250 0.0250 0.0263 0.0263 0.0250 0.0250 0.0250 0.0291 0.0250 0.0249 0.0289 0.0263 0.0249 0.0263 0.0250 0.0275

Barren Ditch #17 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0142 0.2257 0.0155 0.1807 0.2016 0.0195 0.0236 0.0116 0.1190 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000 0.0348 0.0000 0.0025 0.0000 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0025 0.0089 0.0154 0.0051 0.0000 0.0142 0.0025 0.0013 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0000 0.0000 0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

Barren Ditch #18 0.0025 0.0051 0.0168 0.0013 0.0000 0.0000 0.0129 0.2266 0.0154 0.1782 0.2005 0.0181 0.0209 0.0090 0.1190 0.0235 0.0013 0.0000 0.0025 0.0013 0.0025 0.0013 0.0013 0.0320 0.0013 0.0013 0.0013 0.0013 0.0051 0.0013 0.0013 0.0000 0.0013 0.0000 0.0076 0.0127 0.0038 0.0013 0.0116 0.0013 0.0025 0.0013 0.0038 0.0013 0.0013 0.0025 0.0000 0.0013 0.0013 0.0013 0.0038 0.0013 0.0000 0.0013 0.0025 0.0000 0.0000 0.0013 0.0013

Barren Ditch #19 0.0038 0.0089 0.0195 0.0000 0.0025 0.0025 0.0156 0.2301 0.0168 0.1825 0.2058 0.0222 0.0250 0.0129 0.1190 0.0263 0.0013 0.0025 0.0000 0.0013 0.0025 0.0013 0.0013 0.0363 0.0013 0.0038 0.0013 0.0013 0.0064 0.0013 0.0013 0.0025 0.0013 0.0038 0.0089 0.0167 0.0064 0.0013 0.0155 0.0013 0.0000 0.0013 0.0077 0.0013 0.0013 0.0000 0.0013 0.0013 0.0013 0.0013 0.0038 0.0013 0.0038 0.0051 0.0013 0.0025 0.0013 0.0013 0.0051 20

Barren Ditch #20 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0129 0.2261 0.0155 0.1787 0.2020 0.0195 0.0236 0.0116 0.1190 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000 0.0349 0.0000 0.0025 0.0000 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0025 0.0089 0.0154 0.0051 0.0000 0.0142 0.0025 0.0013 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0000 0.0000 0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

Barren Ditch #21 0.0038 0.0089 0.0208 0.0013 0.0025 0.0025 0.0156 0.2231 0.0142 0.1807 0.1995 0.0181 0.0223 0.0116 0.1173 0.0263 0.0013 0.0025 0.0025 0.0013 0.0000 0.0013 0.0013 0.0348 0.0013 0.0013 0.0013 0.0013 0.0051 0.0013 0.0013 0.0025 0.0013 0.0038 0.0077 0.0141 0.0038 0.0013 0.0129 0.0038 0.0025 0.0013 0.0076 0.0013 0.0013 0.0025 0.0013 0.0013 0.0013 0.0013 0.0038 0.0013 0.0038 0.0051 0.0013 0.0025 0.0013 0.0013 0.0051

Barren Ditch #22 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0129 0.2261 0.0155 0.1787 0.2016 0.0195 0.0236 0.0116 0.1190 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000 0.0349 0.0000 0.0025 0.0000 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0025 0.0089 0.0154 0.0051 0.0000 0.0142 0.0025 0.0013 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0000 0.0000 0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

APPENDIX II

Barren Ditch #23 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0129 0.2261 0.0155 0.1787 0.2020 0.0195 0.0236 0.0116 0.1190 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000 0.0349 0.0000 0.0025 0.0000 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0025 0.0089 0.0154 0.0051 0.0000 0.0142 0.0025 0.0013 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0000 0.0000 0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

Barren Ditch #24 0.0348 0.0390 0.0521 0.0364 0.0320 0.0376 0.0436 0.2391 0.0362 0.2132 0.2275 0.0434 0.0404 0.0448 0.1394 0.0493 0.0348 0.0320 0.0363 0.0349 0.0348 0.0349 0.0349 0.0000 0.0349 0.0348 0.0349 0.0349 0.0363 0.0349 0.0349 0.0320 0.0349 0.0334 0.0361 0.0345 0.0391 0.0363 0.0450 0.0334 0.0363 0.0349 0.0347 0.0349 0.0363 0.0363 0.0348 0.0349 0.0349 0.0349 0.0391 0.0349 0.0320 0.0361 0.0377 0.0348 0.0363 0.0349 0.0347

Barren Ditch #25 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0129 0.2261 0.0155 0.1787 0.2020 0.0195 0.0236 0.0116 0.1190 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000 0.0349 0.0000 0.0025 0.0000 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0025 0.0089 0.0154 0.0051 0.0000 0.0142 0.0025 0.0013 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0000 0.0000 0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

River Deshee #26 0.0051 0.0064 0.0195 0.0025 0.0013 0.0013 0.0116 0.2253 0.0155 0.1741 0.1949 0.0155 0.0209 0.0116 0.1152 0.0263 0.0025 0.0013 0.0038 0.0025 0.0013 0.0025 0.0025 0.0348 0.0025 0.0000 0.0025 0.0025 0.0051 0.0025 0.0025 0.0013 0.0025 0.0013 0.0064 0.0128 0.0038 0.0025 0.0103 0.0025 0.0038 0.0025 0.0051 0.0025 0.0025 0.0038 0.0013 0.0025 0.0025 0.0025 0.0064 0.0025 0.0013 0.0025 0.0038 0.0013 0.0013 0.0025 0.0051

River Deshee #27 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0129 0.2261 0.0155 0.1787 0.2020 0.0195 0.0236 0.0116 0.1190 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000 0.0349 0.0000 0.0025 0.0000 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0025 0.0089 0.0154 0.0051 0.0000 0.0142 0.0025 0.0013 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0000 0.0000 0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

River Deshee #28 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0129 0.2261 0.0155 0.1787 0.2016 0.0195 0.0236 0.0116 0.1190 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000 0.0349 0.0000 0.0025 0.0000 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0025 0.0089 0.0154 0.0051 0.0000 0.0142 0.0025 0.0013 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0000 0.0000 0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

River Deshee #29 0.0064 0.0102 0.0222 0.0051 0.0051 0.0076 0.0143 0.2217 0.0182 0.1806 0.1982 0.0182 0.0223 0.0090 0.1189 0.0319 0.0064 0.0051 0.0064 0.0064 0.0051 0.0064 0.0064 0.0363 0.0064 0.0051 0.0064 0.0064 0.0000 0.0064 0.0064 0.0051 0.0064 0.0051 0.0077 0.0115 0.0077 0.0064 0.0169 0.0051 0.0064 0.0064 0.0102 0.0064 0.0076 0.0064 0.0051 0.0064 0.0064 0.0064 0.0089 0.0064 0.0051 0.0076 0.0089 0.0064 0.0064 0.0064 0.0064

River Deshee #30 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0129 0.2261 0.0155 0.1787 0.2020 0.0195 0.0236 0.0116 0.1190 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000 0.0349 0.0000 0.0025 0.0000 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0025 0.0089 0.0154

APPENDIX II

0.0051 0.0000 0.0142 0.0025 0.0013 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0000 0.0000
0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

River Deshee #31 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0129 0.2261 0.0155 0.1787
0.2020 0.0195 0.0236 0.0116 0.1190 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000
0.0349 0.0000 0.0025 0.0000 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0025 0.0089 0.0154
0.0051 0.0000 0.0142 0.0025 0.0013 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0000 0.0000
0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

River Deshee #32 0.0025 0.0051 0.0168 0.0013 0.0000 0.0000 0.0116 0.2253 0.0154 0.1762
0.1991 0.0168 0.0209 0.0090 0.1190 0.0236 0.0013 0.0000 0.0025 0.0013 0.0025 0.0013 0.0013
0.0320 0.0013 0.0013 0.0013 0.0013 0.0051 0.0013 0.0013 0.0000 0.0013 0.0000 0.0076 0.0127
0.0038 0.0013 0.0116 0.0013 0.0025 0.0013 0.0038 0.0013 0.0013 0.0025 0.0000 0.0013 0.0013
0.0013 0.0038 0.0013 0.0000 0.0013 0.0025 0.0000 0.0000 0.0013 0.0013

River Deshee #33 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0129 0.2261 0.0155 0.1787
0.2020 0.0195 0.0236 0.0116 0.1190 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000
0.0349 0.0000 0.0025 0.0000 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0025 0.0089 0.0154
0.0051 0.0000 0.0142 0.0025 0.0013 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0000 0.0000
0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

River Deshee #34 0.0051 0.0051 0.0181 0.0025 0.0000 0.0000 0.0103 0.2284 0.0168 0.1741
0.1966 0.0168 0.0209 0.0103 0.1189 0.0263 0.0025 0.0000 0.0038 0.0025 0.0038 0.0025 0.0025
0.0334 0.0025 0.0013 0.0025 0.0025 0.0051 0.0025 0.0025 0.0000 0.0025 0.0000 0.0089 0.0141
0.0064 0.0025 0.0116 0.0013 0.0038 0.0025 0.0038 0.0025 0.0025 0.0038 0.0013 0.0025 0.0025
0.0025 0.0051 0.0025 0.0000 0.0013 0.0025 0.0000 0.0013 0.0025 0.0025

River Deshee #35 0.0090 0.0155 0.0248 0.0090 0.0076 0.0102 0.0182 0.2255 0.0195 0.1827
0.2000 0.0181 0.0263 0.0168 0.1184 0.0304 0.0089 0.0076 0.0089 0.0089 0.0077 0.0089 0.0089
0.0361 0.0089 0.0064 0.0089 0.0089 0.0077 0.0089 0.0089 0.0076 0.0089 0.0089 0.0000 0.0128
0.0076 0.0102 0.0129 0.0076 0.0089 0.0089 0.0115 0.0089 0.0089 0.0089 0.0089 0.0089 0.0089
0.0089 0.0128 0.0089 0.0076 0.0102 0.0102 0.0089 0.0089 0.0089 0.0102

River Deshee #36 0.0128 0.0167 0.0287 0.0154 0.0127 0.0192 0.0248 0.2287 0.0247 0.1910
0.2057 0.0233 0.0343 0.0193 0.1232 0.0386 0.0154 0.0127 0.0167 0.0154 0.0141 0.0154 0.0154
0.0345 0.0154 0.0128 0.0154 0.0154 0.0115 0.0154 0.0154 0.0127 0.0154 0.0141 0.0128 0.0000
0.0167 0.0154 0.0194 0.0141 0.0167 0.0154 0.0179 0.0154 0.0154 0.0167 0.0154 0.0154 0.0154
0.0154 0.0193 0.0154 0.0127 0.0153 0.0167 0.0127 0.0154 0.0154 0.0153

River Deshee #37 0.0064 0.0102 0.0221 0.0051 0.0038 0.0051 0.0169 0.2280 0.0169 0.1811
0.2067 0.0195 0.0249 0.0129 0.1211 0.0290 0.0051 0.0038 0.0064 0.0051 0.0038 0.0051 0.0051
0.0391 0.0051 0.0038 0.0051 0.0051 0.0077 0.0051 0.0051 0.0038 0.0051 0.0064 0.0076 0.0167
0.0000 0.0051 0.0142 0.0051 0.0064 0.0051 0.0090 0.0051 0.0051 0.0064 0.0051 0.0051 0.0051
0.0051 0.0089 0.0051 0.0051 0.0076 0.0064 0.0038 0.0051 0.0051 0.0076

APPENDIX II

River Deshee #38 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0129 0.2261 0.0155 0.1787 0.2020 0.0195 0.0236 0.0116 0.1190 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000 0.0363 0.0000 0.0025 0.0000 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0025 0.0102 0.0154 0.0051 0.0000 0.0142 0.0025 0.0013 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0000 0.0000 0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

Jacks Fork #39 0.0156 0.0168 0.0220 0.0143 0.0116 0.0115 0.0209 0.2265 0.0223 0.1791 0.2033 0.0208 0.0289 0.0209 0.1195 0.0344 0.0142 0.0116 0.0155 0.0142 0.0129 0.0142 0.0142 0.0450 0.0142 0.0103 0.0142 0.0142 0.0169 0.0142 0.0142 0.0116 0.0142 0.0116 0.0129 0.0194 0.0142 0.0142 0.0000 0.0129 0.0155 0.0142 0.0141 0.0142 0.0142 0.0155 0.0129 0.0142 0.0142 0.0142 0.0182 0.0142 0.0116 0.0141 0.0155 0.0116 0.0129 0.0142 0.0141

11 Point #40 0.0038 0.0063 0.0168 0.0013 0.0013 0.0013 0.0129 0.2297 0.0168 0.1781 0.2033 0.0194 0.0222 0.0103 0.1190 0.0249 0.0025 0.0013 0.0013 0.0025 0.0038 0.0025 0.0025 0.0334 0.0025 0.0025 0.0025 0.0025 0.0051 0.0025 0.0025 0.0013 0.0025 0.0013 0.0076 0.0141 0.0051 0.0025 0.0129 0.0000 0.0013 0.0025 0.0051 0.0025 0.0025 0.0013 0.0013 0.0025 0.0025 0.0025 0.0051 0.0025 0.0013 0.0025 0.0038 0.0013 0.0013 0.0025 0.0025

11 Point #41 0.0038 0.0089 0.0195 0.0000 0.0025 0.0025 0.0156 0.2301 0.0168 0.1825 0.2058 0.0222 0.0250 0.0129 0.1190 0.0263 0.0013 0.0025 0.0000 0.0013 0.0025 0.0013 0.0013 0.0363 0.0013 0.0038 0.0013 0.0013 0.0064 0.0013 0.0013 0.0025 0.0013 0.0038 0.0089 0.0167 0.0064 0.0013 0.0155 0.0013 0.0000 0.0013 0.0077 0.0013 0.0013 0.0000 0.0013 0.0013 0.0013 0.0013 0.0038 0.0013 0.0038 0.0051 0.0013 0.0025 0.0013 0.0013 0.0051

11 Point #42 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0129 0.2261 0.0155 0.1787 0.2020 0.0195 0.0236 0.0116 0.1190 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000 0.0349 0.0000 0.0025 0.0000 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0025 0.0089 0.0154 0.0051 0.0000 0.0142 0.0025 0.0013 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0000 0.0000 0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

11 Point #43 0.0077 0.0064 0.0220 0.0064 0.0038 0.0050 0.0142 0.2340 0.0248 0.1842 0.2085 0.0234 0.0275 0.0155 0.1206 0.0318 0.0064 0.0038 0.0077 0.0064 0.0076 0.0064 0.0064 0.0347 0.0064 0.0051 0.0064 0.0064 0.0102 0.0064 0.0064 0.0038 0.0064 0.0038 0.0115 0.0179 0.0090 0.0064 0.0141 0.0051 0.0077 0.0064 0.0000 0.0064 0.0064 0.0077 0.0051 0.0064 0.0064 0.0064 0.0102 0.0064 0.0038 0.0063 0.0076 0.0038 0.0051 0.0064 0.0076

11 Point #44 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0129 0.2261 0.0155 0.1787 0.2020 0.0195 0.0236 0.0116 0.1190 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000 0.0349 0.0000 0.0025 0.0000 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0025 0.0089 0.0154 0.0051 0.0000 0.0142 0.0025 0.0013 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0000 0.0000 0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

11 Point #45 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0142 0.2297 0.0168 0.1804 0.2059 0.0208 0.0250 0.0129 0.1187 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000 0.0363 0.0000 0.0025 0.0000 0.0000 0.0076 0.0000 0.0000 0.0013 0.0000 0.0025 0.0089 0.0154

APPENDIX II

0.0051 0.0000 0.0142 0.0025 0.0013 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0000 0.0000
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11 Point #46 0.0038 0.0089 0.0195 0.0000 0.0025 0.0025 0.0156 0.2301 0.0168 0.1825
0.2058 0.0222 0.0250 0.0129 0.1190 0.0263 0.0013 0.0025 0.0000 0.0013 0.0025 0.0013 0.0013
0.0363 0.0013 0.0038 0.0013 0.0013 0.0064 0.0013 0.0013 0.0025 0.0013 0.0038 0.0089 0.0167
0.0064 0.0013 0.0155 0.0013 0.0000 0.0013 0.0077 0.0013 0.0013 0.0000 0.0013 0.0013 0.0013
0.0013 0.0038 0.0013 0.0038 0.0051 0.0013 0.0025 0.0013 0.0013 0.0051

11 Point #47 0.0038 0.0064 0.0195 0.0000 0.0000 0.0000 0.0129 0.2298 0.0141 0.1782
0.2030 0.0195 0.0223 0.0116 0.1189 0.0263 0.0000 0.0000 0.0013 0.0000 0.0013 0.0000 0.0000
0.0348 0.0000 0.0013 0.0000 0.0000 0.0051 0.0000 0.0000 0.0000 0.0000 0.0013 0.0089 0.0154
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11 Point #48 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0129 0.2261 0.0155 0.1787
0.2020 0.0195 0.0236 0.0116 0.1190 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000
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0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

Little Black #49 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0142 0.2279 0.0155 0.1807
0.2037 0.0208 0.0236 0.0116 0.1190 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000
0.0349 0.0000 0.0025 0.0000 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0025 0.0089 0.0154
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0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

Little Black #50 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0129 0.2261 0.0155 0.1787
0.2020 0.0195 0.0236 0.0116 0.1190 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000
0.0349 0.0000 0.0025 0.0000 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0025 0.0089 0.0154
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0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

Little Black #51 0.0025 0.0076 0.0207 0.0025 0.0038 0.0063 0.0155 0.2244 0.0194 0.1864
0.2041 0.0235 0.0276 0.0142 0.1243 0.0291 0.0025 0.0038 0.0038 0.0025 0.0038 0.0025 0.0025
0.0391 0.0025 0.0064 0.0025 0.0025 0.0089 0.0025 0.0025 0.0038 0.0025 0.0051 0.0128 0.0193
0.0089 0.0025 0.0182 0.0051 0.0038 0.0025 0.0102 0.0025 0.0038 0.0038 0.0025 0.0025 0.0025
0.0025 0.0000 0.0025 0.0051 0.0050 0.0038 0.0051 0.0038 0.0025 0.0038

Duck Creek #52 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0129 0.2261 0.0155 0.1787
0.2020 0.0195 0.0236 0.0116 0.1190 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000
0.0349 0.0000 0.0025 0.0000 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0025 0.0089 0.0154
0.0051 0.0000 0.0142 0.0025 0.0013 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0000 0.0000
0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

Duck Creek #53 0.0038 0.0051 0.0168 0.0025 0.0000 0.0000 0.0116 0.2275 0.0168 0.1760 0.2008 0.0181 0.0209 0.0090 0.1189 0.0249 0.0025 0.0000 0.0038 0.0025 0.0038 0.0025 0.0025 0.0320 0.0025 0.0013 0.0025 0.0025 0.0051 0.0025 0.0025 0.0000 0.0025 0.0000 0.0076 0.0127 0.0051 0.0025 0.0116 0.0013 0.0038 0.0025 0.0038 0.0025 0.0025 0.0038 0.0013 0.0025 0.0025 0.0025 0.0051 0.0025 0.0000 0.0013 0.0038 0.0000 0.0013 0.0025 0.0013

Duck Creek #54 0.0038 0.0038 0.0180 0.0038 0.0013 0.0013 0.0141 0.2284 0.0207 0.1795 0.2026 0.0194 0.0248 0.0102 0.1204 0.0289 0.0038 0.0013 0.0051 0.0038 0.0051 0.0038 0.0038 0.0361 0.0038 0.0025 0.0038 0.0038 0.0076 0.0038 0.0038 0.0013 0.0038 0.0013 0.0102 0.0153 0.0076 0.0038 0.0141 0.0025 0.0051 0.0038 0.0063 0.0038 0.0038 0.0051 0.0025 0.0038 0.0038 0.0038 0.0050 0.0038 0.0013 0.0000 0.0051 0.0013 0.0025 0.0038 0.0025

10 Mile Cr. #55 0.0038 0.0089 0.0208 0.0000 0.0025 0.0038 0.0155 0.2298 0.0181 0.1804 0.2037 0.0208 0.0276 0.0155 0.1206 0.0263 0.0000 0.0025 0.0013 0.0000 0.0013 0.0000 0.0000 0.0377 0.0000 0.0038 0.0000 0.0000 0.0089 0.0000 0.0000 0.0025 0.0000 0.0025 0.0102 0.0167 0.0064 0.0000 0.0155 0.0038 0.0013 0.0000 0.0076 0.0000 0.0000 0.0013 0.0013 0.0000 0.0000 0.0000 0.0038 0.0000 0.0038 0.0051 0.0000 0.0025 0.0000 0.0000 0.0063

10 Mile Cr. #56 0.0025 0.0051 0.0168 0.0013 0.0000 0.0000 0.0116 0.2289 0.0168 0.1757 0.2008 0.0181 0.0222 0.0102 0.1204 0.0249 0.0013 0.0000 0.0025 0.0013 0.0025 0.0013 0.0013 0.0348 0.0013 0.0013 0.0013 0.0013 0.0064 0.0013 0.0013 0.0000 0.0013 0.0000 0.0089 0.0127 0.0038 0.0013 0.0116 0.0013 0.0025 0.0013 0.0038 0.0013 0.0013 0.0025 0.0000 0.0013 0.0013 0.0013 0.0051 0.0013 0.0000 0.0013 0.0025 0.0000 0.0000 0.0013 0.0025

10 Mile Cr. #57 0.0038 0.0064 0.0195 0.0000 0.0000 0.0000 0.0116 0.2302 0.0155 0.1762 0.1995 0.0182 0.0236 0.0129 0.1189 0.0263 0.0000 0.0000 0.0013 0.0000 0.0013 0.0000 0.0000 0.0363 0.0000 0.0013 0.0000 0.0000 0.0064 0.0000 0.0000 0.0000 0.0000 0.0013 0.0089 0.0154 0.0051 0.0000 0.0129 0.0013 0.0013 0.0000 0.0051 0.0000 0.0000 0.0013 0.0000 0.0000 0.0000 0.0000 0.0038 0.0000 0.0013 0.0025 0.0000 0.0000 0.0000 0.0000 0.0051

Current R. #58 0.0025 0.0076 0.0195 0.0000 0.0013 0.0013 0.0142 0.2279 0.0154 0.1807 0.2059 0.0208 0.0236 0.0116 0.1189 0.0250 0.0000 0.0013 0.0013 0.0000 0.0013 0.0000 0.0000 0.0349 0.0000 0.0025 0.0000 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0025 0.0089 0.0154 0.0051 0.0000 0.0142 0.0025 0.0013 0.0000 0.0064 0.0000 0.0000 0.0013 0.0000 0.0000 0.0000 0.0000 0.0025 0.0000 0.0025 0.0038 0.0000 0.0013 0.0000 0.0000 0.0038

Current R. #59 0.0025 0.0038 0.0167 0.0038 0.0013 0.0038 0.0142 0.2236 0.0207 0.1836 0.2051 0.0194 0.0222 0.0076 0.1241 0.0275 0.0038 0.0013 0.0051 0.0038 0.0051 0.0038 0.0038 0.0347 0.0038 0.0051 0.0038 0.0038 0.0064 0.0038 0.0038 0.0013 0.0038 0.0025 0.0102 0.0153 0.0076 0.0038 0.0141 0.0025 0.0051 0.0038 0.0076 0.0038 0.0051 0.0051 0.0038 0.0038 0.0038 0.0038 0.0038 0.0038 0.0013 0.0025 0.0063 0.0025 0.0051 0.0038 0.0000

APPENDIX III

Population studies reveal critically low numbers of this "new" species in Illinois.

The Redspotted Sunfish

Photos By Trent Thomas

Most people have never heard of the redspotted sunfish (*Lepomis miniatus*)—and for good reason. Until 1992 when researchers at Southern Illinois University determined this fish to be a separate species, it was known as the spotted sunfish (*Lepomis punctatus*). The redspotted sunfish is found as far south as Florida and Texas, and Illinois is at the northern edge of its distribution.

Although never abundant in Illinois, when the first collections of Illinois fish were made 100 years ago, the redspotted sunfish was more widespread than it is today. Historically, the redspotted sunfish was found along the Illinois and Ohio rivers, and in the Mississippi and the Iroquois rivers. Currently, this species appears to be much less abundant along the Illinois and Ohio rivers, and has disappeared from the other two rivers.

The habitat of this sunfish is larger river backwater lakes with aquatic plants—habitats that have been lost through draining, filling or construction of extensive levee systems. While many of the backwater lakes still exist, siltation and introduced species (grass carp) have diminished the aquatic vegetation, leading to the demise of the redspotted sunfish except in small, slow-moving, vegetated streams—habitats that are quite vulnerable to disturbance.

With the help of a federal grant, the Department of Natural Resources' Division of Fisheries has begun a statewide survey to determine the population status of the redspotted sunfish. Small fin-clips are being taken from fish collected in Illinois and neighboring states for use in genetic studies. Results from these surveys and studies will guide efforts to reintroduce this species into restored habitats throughout their historic Illinois range.

Redspotted sunfish habitat

Preliminary results of the population surveys show the redspotted sunfish is quite rare in Illinois. An adult population is yet to be found along the Illinois River (a few juvenile fish were found in a single, small stream in the lower Sangamon River basin), which was once the heart of its existence in the state.

The news is better along the Ohio River as the occasional redspotted sunfish is found in a few backwater lakes despite the lack of necessary aquatic vegetation and presence of exotic silver carp. Several adults have been collected from a remnant population in a small stream in the lower Saline River basin.

Plans for the Illinois population of this sunfish include continuation of population surveys and genetic analyses to determine if genetic variation exists among the isolated populations, allowing for identification of the most appropriate source of fish for stocking into restored habitats. Adult redspotted sunfish will be collected and spawned in rearing ponds, producing the number of fish needed for stocking efforts.

—**Trent Thomas**, Region 3 Streams Biologist, DNR Division of Fisheries