GENETIC POPULATION STRUCTURE AND LIFE HISTORY ASPECTS OF THE FEDERALLY THREATENED CHEROKEE DARTER, *ETHEOSTOMA SCOTTI*

By

Casey Michael Storey

Under the Direction of Mary C. Freeman

Abstract

The Cherokee darter (*Etheostoma scotti*) is a Federally Threatened percid fish endemic to the Etowah River system, Georgia. Research was conducted on aspects of life history and genetic structure of this fish to aid in the protection and management of the species and its habitat. Through snorkel observations spawning behavior, spawning season, and preferred spawn habitat conditions were determined through two field seasons. Simultaneously, an analysis of population structure was made utilizing mtDNA with neighbor joining, maximum parsimony, and Bayesian analyses. Field observations found the habitat parameters required or preferred by Cherokee darters for spawning varied, with spawning occurring between the middle of March and early June. Genetic results show three distinct groups within the greater population of Cherokee darters in the Etowah River basin. These groups correspond with geographic breaks within the range of the species and fall out in the order of upper, middle and lower in terms of the Etowah drainage. These ESUs are supported, in part, by color variation in nuptial males between the ESUs.

Index Words: Cherokee darter; Control region; ESU; Snorkel; Etowah River; MtDNA; *Etheostoma*; Bayesian analysis; *Ulocentra*

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CHAPTER 1

INTRODUCTION

An overview of life history traits and genetics of the snubnose darters

The darter genus *Etheostoma* is one of the largest genera of freshwater fishes in North America. Within it the subgenus *Ulocentra*, the snubnose darters, has experienced numerous species descriptions over recent years. Currently comprised of twenty nominal species this subgenus has gained 12 members since 1982. The reason for this recent taxonomic explosion lies with a central characteristic of the members of the subgenus. Namely the members of the subgenus are characterized as possessing nearly identical cryptic coloration during a large portion of the year. However, when males of the group come into nuptial condition, beginning sometime in mid to late autumn, a profusion of species-specific colorations are exhibited. These colors are the basis by which the members of the subgenus have been described and the color “dormancy” is responsible for the delay in the recognition and description of the most recent additions to the group.

Although there is great diversity in male colorations between species, there are few if any true morphological synapomorphies that unite all members of the subgenus although phylogenetic structure within the group and its sister subgenus *Etheostoma* are shown to have phylogenetic support with mtDNA sequence data (Porter et al., 2002.).

One recently described member of the subgenus *Ulocentra* and a basal member of the *E. duryi* species group (Bailey and Etnier 1988) is the Cherokee darter, *Etheostoma* *scotti* (Bauer et al., 1995). The Cherokee darter is the only Federally protected (threatened) member of the *Ulocentra* subgenus and is endemic to the Etowah River system, Georgia, USA. This recently described fish was previously synonymous with the Coosa darter, *Etheostoma coosae* found throughout much of the Coosa River system of which the Etowah is part. The Cherokee darter was recognized and listed as a Federally threatened species (U.S. Federal Register, 1994) prior to the publication of its official description. Such listing was suggested by Bauer et al. (1995), based on the imminent imperilment of the species due to rapid land development in the Etowah River basin and the apparent loss of some populations within the basin.

The description of the Cherokee darter (Bauer et al., 1995) was based primarily on male color pattern differences in lateral bars and the spinous dorsal fin of nuptial males. In *E. coosae* the posterior lateral bars are wide and spawning males have a blue distal margin throughout the spinous dorsal fin as well as an area of red pigmentation between each that comprises a medial red band with a clear band above and below this area (Bauer et al., 1995). In *E. scotti* the posterior lateral bars are narrow and the males have a diffuse red pigmentation throughout the majority of the spinous dorsal fin with a partial black basal band in two-thirds of the first dorsal fin as depicted in the color plate from Bauer et al. (1995).

Collections conducted by Brady A. Porter in 1993 at Butler Creek, Cobb Co, GA as well as collections made by David M. Walters, Byron J. Freeman, Brady A. Porter, John R. Knight, Casey M. Storey, and others from the University of Georgia in tributaries to Pumpkinvine Creek and Raccoon Creek, as well as Raccoon Creek proper, Stamp Creek, Allatoona Creek, and Proctor Creek in years 2000-2002 have noted color differences at these localities. During these collections the presence of at least a partial blue distal margin in nuptial males, much like that observed in the Coosa darter was commonplace and set these fish apart from Cherokee darters collected in tributaries upstream of Butler Creek. Within the description of *E. scotti* (Bauer et al., 1995) the authors mention a narrow turquoise or blue distal band sometimes appearing on the first spinous dorsal fin of nuptial male Cherokee darters. This noted variation along with field observations from within the population led to the suggestion of greater diversity within the species than previously noted, possible previous misidentification, and also led to questions about the evolution of the sister species, *E. coosae* and *E. scotti*.

The spawning behavior of various members of the subgenus *Ulocentra* has been noted as the primary possible synapamorphy uniting the group (Bailey and Etnier 1988, Porter et al., 2002). This spawning behavior as noted for various members of *Ulocentra*, including *E. coosae* (O'Neil 1981), *E. baileyi*, *E. etnieri* (Porterfield 1998), *E. flavum* (Keevin et al. 1989), *E. scotti* (Bauer et al. 1995), *E. simoterum* (Page and Mayden 1981) and others is consistent with females typically depositing single eggs to rock substrate in a vertical position. The substrate selected has been noted to be of cobble and larger size class. Spawning temperatures and seasons vary among studied species within the subgenus, but have been noted to occur during April and May and with temperatures ranging from 10-20 degrees Celsius (Porterfield, 1998). Much of the temporal and temperature variation of different species within the subgenus may be related to the broad geographic and latitudinal range of the species within *Ulocentra*. Ranging from the Green and Barren River system in Kentucky (*E. rafinesque*) to the coastal plain of

Alabama (*E. colorosum*) the members of this subgenus are not uniform in the climate, water quality, or reproductive season that they experience.

In laboratory observations of spawning behavior, female Cherokee darters usually attached a single egg to a cobble, small boulder, or gravel substrate while in an inverted or vertical position (Bauer et al., 1995). Although this behavior closely resembles that of other members of the group, aquaria spawning (Porterfield, 1998) may not accurately reflect the behavior of individuals in the wild.

The primary concern in regard to the continued existence or persistence of the Cherokee darter is the impacts of development within its range. Suburban development around the northern perimeter of Atlanta as well as development around Dawsonville, Canton, Cartersville, and most areas in between all threaten the continued existence of the species and its habitat through increased sediment and pollutants as well as the alteration of flows, inundation of streams through damming, and channelization (Bauer et al., 1995, Burkhead 1993).

Objectives of Study

The rapid rate of urban, suburban, and rural development within the range of the Cherokee darter remains a major threat to the future of this species. However, the extent of this impact and the specific associated factors that are detrimental to the species are not known. Impoundment and inundation are obvious detriments to stream dwelling fishes, but the extent to which development plays a role in their extirpation or reduction in numbers is not known. Also, from a management perspective the habitat critical for the protection of populations and those populations requiring prioritization for protection must be understood. Such knowledge is needed for the goals of down listing (under the ESA) and protection to be achieved.

For these reasons this study has been undertaken with the intent of exploring factors essential for the effective management of the species. First, the goal is to explore potential genetic variation and population structure within the species as indicated by field observations in recent years. Results of this study would suggest potential priorities for conservation and preservation of habitat as well as populations in need of immediate action in terms of management attention. In addition, the habitat preferences of spawning Cherokee darters need to be related to the susceptibility of preferred habitat to impact from development. Specifically, the flow, spawning substrate size, water temperature, and depth required or preferred for spawning are needed to determine the factors that could have potential to impact the species.

Within the range of the Cherokee darter, Allatoona Reservoir and several other impoundments within the Etowah basin have inundated habitat and fragmented populations. This substantial fragmentation coupled with the impacts from development within the watershed makes addressing genetic variability a priority. To answer the numerous questions associated with this we have undertaken a genetic study.

Phylogenetic analyses of the mitochondrial DNA (mtDNA) sequence data were used to uncover potential population diversity, population structure, and phylogeography. An attempt has also been made to correlate variation in male color patterns with genetic results to understand the evolutionary history of the *E. coosae*-*E. scotti* clade and the genetic structure of the fragmented populations of the Federally listed Cherokee darter. In addition, two seasons of field observations via snorkeling were conducted at five localities across the range of the species in an attempt to determine the microhabitat preferences and requirements for the spawning of *Etheostoma scotti*. Spawning coordinates are overlain with total station survey data at two of the five localities to demonstrate habitat type selection within these study reaches. Finally, water temperature data were utilized to evaluate seasonal temperature factors as the potential driver(s) behind the Cherokee darter spawning season.

CHAPTER 2

GENETIC ANALYSIS

Introduction

The darter genus *Etheostoma* is one of the largest genera of freshwater fishes in North America. Within it the subgenus *Ulocentra*, the snubnose darters, has experienced numerous species descriptions over recent years. Currently comprised of twenty nominal species this subgenus has gained 12 members since 1982. The reason for this recent taxonomic explosion lies with a central characteristic of the members of the subgenus. Namely the members of the subgenus are characterized as possessing nearly identical cryptic coloration during a large portion of the year. However, when males of the group come into nuptial condition, beginning sometime in mid to late autumn, a profusion of species specific colorations are exhibited. These colors are the basis by which the members of the subgenus have been described and the color "dormancy" is responsible for the delay in the recognition and description of the most recent additions to the group.

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Within the subgenus *Ulocentra* several species are found in nature in syntopy in the Eastern United States. However, to date there have been only two documented cases (*E. duryi-E. flavum*, Etnier and Bailey, 1989 and *E. simoterum-E. atripinne*, Etnier and Starnes 1993, Porter et al., 2002) in which syntopy between sister species within this subgenus occurs.

The Cherokee darter (*Etheostoma scotti*) and the Coosa darter (*Etheostoma coosae*) are sister species within the *Ulocentra* subgenus. Prior to this study they have not been found to occur in syntopy although the two inhabit a similar range of streams and habitat in very close proximity to one another at the downstream extent (*Etheostoma scotti*) and the upstream extent (*Etheostoma coosae*) of their ranges. The analysis of the range of the Cherokee darter made by Bauer et al. (1995) in the description of the species found that the Cherokee darter occupied only Raccoon and Pumpkinvine Creeks downstream of Allatoona Dam with Raccoon Creek representing the furthest downstream extent of the species. The furthest upstream watershed occupied by the Coosa darter, noted by Bauer et al., was Pettit Creek in Bartow Co. This creek actually enters the

Etowah River at a point upstream of Raccoon Creek, thus making for an interesting pattern of distribution between the two species.

The intention of this study is to evaluate potential genetic variation and population structure of *Etheostoma scotti*. In addition, these results can be applied to an analysis of phylogeography from which the interaction of the evolutionary history of the species and the geologic history of the Etowah River basin can be extrapolated. Finally, this study then aims to correlate spawning male coloration variations across the range with the results of genetic analysis.

Methods

Samples of Cherokee darters from 37 localities were obtained from fish surveys conducted from 2001 to 2003 in the State of Georgia by Georgia Department of Natural Resources and the University of Georgia, Institute of Ecology (Table 1.1, 1.2 Appendix A.) (Figure 2.1). Many individuals included in this study were mortalities as a result of general collection efforts using backpack electrofishers and were obtained after attempts at revival failed in the field.

In addition, in an effort to increase the total sample size for various localities and to collect haplotype information from additional localities within the basin, caudal fin clips were taken from individuals at 12 localities under permission from a Federal collection permit. Fish from which tissue was collected were obtained with seines and/or backpack electrofishers. Upon capture the sex of the individual was noted, a standard length measurement taken and recorded, the posterior one-half to one-third of the caudal fin removed with surgical scissors and the individual then released.

All collection localities were noted and the collection results and notes entered into a long-term database administered by Byron J. Freeman at the University of Georgia, Institute of Ecology. Within this database micro locality data for each collection was retained and a unique Etowah site number assigned to each.

All collected whole fish and caudal fin samples were placed in 95 % ethanol in the field and the ethanol changed over a several day period as needed. Whole specimens were stored in glass jars or 50 ml Falcon® graduated tubes and fin clips were stored in 1.5 ml microcentrifuge tubes. All samples were stored at room temperature in the Genetics Department at the University of Georgia until extraction and analysis were complete, after which remaining tissue was archived in the Georgia Museum of Natural History DNA Archive.

The extraction of genomic DNA and PCR techniques followed methods outlined in Porter (1998). Genomic DNA was extracted from both muscle and fin tissue using similar methods. With whole specimens the musculature from the posterior one-third of the right side of each individual was removed with a razor blade. After removal the tissue was blotted dry of ethanol with Kimwipes, the integument was removed and discarded, and the remaining tissue was minced into small pieces with a clean razor. This tissue was then placed in a 1.5 ml microcentrifuge tube for digestion. Fin clips were left whole, but also blotted dry and placed in 1.5 ml microcentrifuge tubes for digestion.

Once in digestion tubes 500µl lysis buffer (0.1M Tris, 4M urea, 0.2M NaCl, 0.01 CDTA, 0.5% laurayl sarcosine) was added. To this 10µl proteinase K was added, the solution mixed, and left to digest at 55°C for a minimum of 6 hours.

After the digestion of the tissue was complete, the genomic DNA was extracted, by mixing 500µl of phenol chloroform (24:1) and ultra-centrifuging for 10 minutes. After centrifuging, the partitioned upper layer within the tube was removed using a disposable pipette and the material deposited in a clean 1.5ml microcentrifuge tube. To this tube 500µl of phenol chloroform isoamyl alchohol (25:24:1) was added, the solution mixed, and centrifuged for 2 minutes. After this centrifuge was complete the upper layer was again removed from the tube with a clean disposable pipette and the extracted material placed in another clean 1.5ml microcentrifuge tube. To this tube cold absolute ethanol was added until the total volume reached 1.5ml. The new solution was then mixed and placed in a cold room (3°C) where it was centrifuged for 30 minutes to pelletize the precipitated DNA. After centrifuging, the ethanol was poured off and the pellet and tube were rinsed with ~10 drops of 70% ethanol. After disposal of this ethanol-rinse the tube and genomic DNA were allowed to air dry, inverted for a minimum of several hours. After drying the genomic DNA was resuspended in 30µl (fin tissue) or 100µl (muscle tissue) water. This solution was then stored in a refrigerator for further use.

PCR was conducted with the use of three primers. Initially, the primers LPRO and 12Sa-rev (Table 1.1) were utilized in the PCR of both fin and muscle tissue templates. However, in the case of weak or no amplification with the original pair, the combination of primers BAPD1 and 12Sa-rev were utilized in PCR with the result of a shortened PCR product (when successful).

PCR was conducted in Perkin Elmer Cetuss thermocyclers under the following profiles: 2 min hot start at 93º followed by 32 cycles of 45 sec at 93º, 1 min at 58º and 2 min at 72º. For each template ~20-500ng genomic DNA template was used with 2.5mM MgCl2, 2mM of each dNTP, 0.5 units *Taq* DNA polymerase, 5pM of each primer (two utilized) and Promega® buffer to a final 1X concentration (Porter 1999). The resulting PCR product was subjected to electrophoreisis (120 amps) on a 2% argarose gel containing ethidium bromide for a minimum of 20 minutes, a photograph of the resulting gel run was taken, and the gel analyzed under UV light for the presence of a visible band (indicating successful PCR).

Successful PCR products were submitted for sequencing and quantification to the University of Georgia's Molecular Genetics Instrumentation Facility. Once sequenced all products were aligned to out-group individuals including *Etheostoma duryi* and *Etheostoma flavum* (Genbank AF404537, AF404560, AF404561, AF404562, AF404565, AF404563, AF404564) using the Eyeball Sequence Editor, ESEE3S ver. 3.0s (Cabot and Beckenback, 1989). A minimum of one individual from each unique locality was sequenced for the entire base pair area including the control region, tRNA phenalalanine gene, and a portion of the 12S rRNA gene (1034 aligned base pairs). Samples from additional individuals comprising population samples from each locality were amplified and sequenced through an ~ 800 base pair portion of the analyzed region using the primer BAPD1. After alignment of this region unique population samples differing by at least one base pair warranted the completion of the 1034 bp region to provide a full reference sequence for each unique haplotype in the population.

An analysis of nucleotide saturation was conducted with independent plots of all pairwise transition and transversion rates against total sequence divergence rates. Indel (insertion and deletion) gaps and uniformative bases were treated as missing data and pairwise deleted from the analysis.

Three phylogenetic analyses, neighbor-joining, maximum parsimony, and Bayesian likelihood, were conducted upon the resulting aligned sequences. Outgroup selections of *E. duryi and E. flavum* (other members of the *E. duryi* group) were based upon their previously examined sister relationship with the *E. coosae* clade (Porter et al., 2002).

For the neighbor joining analysis and tree construction a Kimura two-parameter genetic distance matrix was conducted with indels and ambiguous base characters pairwise deleted. Support for neighbor-joining nodes was calculated using 1000 bootstrap pseudoreplicates in MEGA2 (Kumar et al., 2001). Using the full haplotype dataset a pairwise distance matrix was generated in MEGA ver. 1.01 (Kumar et al., 1993) and mean distances were calculated for within and between genetic groups. The second of the phylogenetic analyses was conducted with maximum parsimony (MP) using PAUP* (Swofford 1998). Heuristic MP searches were performed with tree-bisection-reconstruction (TBR) branch swapping and 100 random addition sequence replications. Two such searches were performed, one with gaps deleted, and one with gaps included as a fifth character state. Support for MP nodes was performed by jackknifing the data with 50% of the characters deleted in each of the 100 pseudoreplications. The final analysis of phylogenetic structure was Bayesian maximum likelihood based on Markov chain Monte-Carlo (MCMC) method . Using the program MrBayes (Huelsenbeck and Ronquist, 2001) a general time reversible model of sequence evolution (GTR) and gamma rate parameters were used to estimate the likelihood of each tree. Four MCMC simulations were run in parallel for 1,500,000 generations, sampling every 100th tree. The MCMC converged on optimal maximum likelihood scores within the first 3,000 generations and were discarded (burn-in period). The resulting Bayesian trees and their associated maximum likelihood scores were imported into PAUP* (Swofford 1998) to construct a consensus tree with posterior probability support.

The phylogeographic method (Avise 2000) was used to overlay the genetic partitions upon spatial GIS data provided by the University of Georgia's Institute of Ecology. Haplotype groupings were delineated only if they were statistically supported by topology in all three phylogenetic methods. Polygons and circles encompassing these consistent haplotype groupings were visually overlaid with basin wide collection data and geologic layers using ESRI Arcview® ver. 3.2.

To capture possible morphological variations and to use this data in correlation with genetic results, field sampling of Cherokee and Coosa darters was conducted in the winter and spring of 2002. Cherokee and Coosa darters were collected from sites throughout their ranges with backpack electrofishers and/or seines. Once collected standard length was measured, individuals were photographed with 35 mm slide film in a photo tank or on a wax backdrop, and color notes taken.

Results

Primers Lpro and 12Sa-rev produced PCR products ~1600 bp in length. Problematic and unsuccessful templates (typically caudal fin clips) were subjected to an additional attempt of PCR using primers BAPD1. and 12Sa-rev. Of these attempts a small percentage (~10-20%) were successful and sufficient in concentration for sequencing and produced a more limited product (~1200bp). These smaller products were exclusively used in comparison with full haplotypes for the calculation of haplotype number.

The analysis and sequencing of the mtDNA control region, tRNA-Phe gene and partial 12 S rRNA gene revealed 41 unique haplotypes with partial sequence data from an additional 100 population samples. These data were aligned with 10 previously sequenced haplotypes from Genbank (AF404538, AF404537, AF404539, AF404560, AF404561, AF404562, AF404565, AF404563, AF404564) (Porter et al., 2002) for a total population genetic analysis of 118 *E. scotti* sequences from 42 localities and 27 *E. coosae* sequences from 14 localities (Table 1.3, Appendix A). In the process of sequence alignment of analyzed sequences the hypervariable and degraded repeat portion of the control region was excluded from the analysis as warranted through the potential for homology (Porter et al., 2002 and Faber and Stephien, 1997). The total aligned character set contained 1034bp. Only full haplotype sequences were utilized for phylogenetic analyses and all haplotypes (partial and full) were utilized in the calculation of the mean genetic diversity within and between genetically delineated groups.

The mean ratio of transversions to transitions was 2:1. The saturation test conducted upon both transversions (Figure 2.2) and transitions (Figure 2.3) shows an absence of saturation effect upon the data set analyzed.

Results of all phylogenetic analyses produced trees with very similar typologies. The rooted neighbor-joining tree (Figure 2.4) displays three distinct groups within *Etheostoma scotti*, each with bootstrap support of 97%. The first two *E. scotti* groups are sister to one another with 95% bootstrap support. The third major group is united with the first two with bootstrap support of 96%. These three groups of *E. scotti* are joined to a group of the most geographically proximate populations of *E. coosae* from the lower Etowah, Coosawattee, Conasauga, and Oostanaula.

The smallest group of *E. scotti* contains haplotypes from Camp Creek, an unnamed tributary to the upper Etowah, Palmer Creek, Burt Creek, and Shoal Creek. The latter two (three haplotypes) form a well-defined subgroup. The mean genetic distance as calculated through a pairwise genetic distance matrix of the 23 haplotypes (partial and full) was 0.35% with a standard error of 0.016%.

The second major group of *E. scotti* is comprised of haplotypes from 13 sites with Smithwick Creek samples as a basal split to all others. These other haplotypes are nearly identical in sequence characters making this group the least genetically diverse of the three groups. The mean genetic distance of this group, comprised of 42 haplotypes is 0.241% with a standard error of 0.079%.

The third major group of *E. scotti* consists of 12 haplotypes divided into three subgroups comprised of four full haplotypes each. The relationships of these subgroups are unresolved. The mean genetic distance within this group, comprised of 53 haplotypes (partial and full) is 0.414% with a standard error of 0.119%. The mean genetic distance between all 3 groups ranges from 1.3% (between the upper and the middle) to 1.86% (between the middle and the lower). Between any of these three groups and the *E. coosae* group the genetic distance ranges from 2.3% to 2.6% (Table 2.2).

The results of the maximum parsimony (MP) analysis reflected those found in the neighbor-joining analysis. This analysis revealed three major clades within *E. scotti* precisely consistent with the three groups delineated in the neighbor-joining phenogram. Comparison of MP trees with and without gaps (70 phylogenetically informative characters without) included within the analysis produced nearly identical results, however when gaps were treated as a fifth character state (74 PI characters) the analysis produced a slightly more resolved consensus tree (figure 2.5). Out of the 2,328,870 topological rearrangements attempted PAUP* (Swofford 1998) identified 50 equally parsimonious trees with 154 evolutionary steps. The consistency index was 0.714 and the retention index was 0.933.

The Bayesian maximum likelihood consensus tree was consistent in topology with both the neighbor-joining tree and nearly identical to the maximum parsimony consensus tree (Figure 2.6). A total of 15,001 trees from MrBayes (Huelsenbeck and Ronquist, 2001) were imported into PAUP*(Swofford 1998) and the first 3,000 trees were discarded as burn-in. The program MrBayes (Huelsenbeck and Ronquist, 2001) treated gaps as missing character states. A concensus of the remaining 12,001 trees has 147 evolutionary steps (same as the MP search without gaps) with a consistency index of 0.721 and retention index of 0.935.

Application of the phylogeographic method (Avise 2000) to the clades supported all three phylogenetic analyses, and uncovered unique population structure within the species (Figure 2.7). These clades were found to be geographically organized within distinct portions of the Etowah River. This pattern indicates three distinct units corresponding to the upper, middle, and lower reaches of the Etowah River basin. The upstream extent of the lower phylogeographic unit within the Etowah River system is bounded by Butler/Proctor/Allatoona system, Cobb Co. on the southern side of the Etowah and Stamp Creek, Bartow Co. on the northern side of the Etowah. The downstream range of this unit extends to the described downstream range of the

Cherokee darter at Raccoon Creek as outlined by Bauer et al. (1995). The middle phylogeographic unit extends from tributaries upstream of Stamp and Butler Creeks to Amicalola Creek and Settingdown Creek. The latter streams are not known to harbor Cherokee darters. Upstream of this break within their range, Cherokee darters fall within the upper phylogeographic unit.

The results of the color analysis correspond, in part with the above trends. Sites at which this analysis took place included; Shoal Cr., Dawson Co., McCanless Cr., Hickory Log Cr.; and Downing Cr., Cherokee Co., Butler Cr., Cobb Co., and Whitehead Creek, Paulding Co. In addition, Coosa darters (*Etheostoma coosae*) from sites within the Etowah basin were also collected, measured, and photographed. These sites included Two Run Creek and Pettit Creek, Bartow Co., Silver Creek and Spring Creek, Floyd Co., and Euharlee Creek, Polk Co.

Color variation was documented throughout the range of the Cherokee darter. Most importantly some color variation across the range of the species possibly reflects the genetic diversity found in the population. Coosa darters inhabiting streams in closest proximity to populations of Cherokee darters (from the lower phylogeographic unit) demonstrate nuptial colorations commonly found in other *Etheostoma coosae* populations throughout this species' range.

Nuptial male dorsal fin pigmentation in both the upper and middle clades or groups share common characteristics. Most males photographed possessed a diffuse red wash throughout the spinous dorsal fin with an intensity of pigmentation found through the middle of each membrane between each ray (as a band extending basally to distally). This pigmentation is noted within the description of the species and the photo image of the males within the paper (Bauer et al., 1995). Males that did not possess this diffuse red pigmentation, generally smaller males (< 40 mm), were characterized by having an area of red pigmentation between each membrane surrounded by an unpigmented region. This area of pigmentations between each ray is commonly taller than wide in the membrane. Of additional note, many of the observed and photographed males in this study were found to have a narrow (~1mm) distal margin throughout the spinous dorsal fin that was a light gray in color and on occasion a few individuals had blue pigment within the dorsal margin of one or two membranes. This was true for most localities, including the type locality at McCanless Creek (Figure 2.8).

While color traits separating males from the upper and middle groups were not plainly observed the difference between males of the lower group and those of the upper and middle were striking. Most notably males from the lower group localities documented, including Butler and Whitehead Creeks, possessed at least a short segment of a distal blue margin in the spinous dorsal fin. In Butler Creek most males documented and observed throughout the snorkel seasons possessed a complete blue distal dorsal fin margin. In contrast males in Whitehead Creek have a distal blue margin typically extending through just the first 2-5 membranes of the spinous dorsal fin. Typically, nuptial males in this lower group were characterized by a red pigmented area between each ray that is tall (~2/3 to 3/4 of membrane height) and surrounded by an unpigmented area (Figure 2.8).

Both color results and genetic results indicate a surprising occurrence within the lower reach of the range of the Cherokee darter. Mainly, those populations of Cherokee and Coosa darters in the area downstream of Allatoona Dam are narrowly syntopic in at least one locality, Pettit Creek (Figures 2.4-2.6, Table 1.1 Appendix A). Genetic results show that of 21 sequenced individuals from this locality 2 express haplotypes consistent with other *E. coosae* and all 19 others are consistent (and clade with) *E. scotti* from the lower group. One of these two individuals (the only male) displays a dorsal fin pattern not unlike the other *Ulocentra* (*E. scotti*) collected at the site. In addition, a morphological comparison at this site indicates possible hybridization between these two species as the dorsal fins of many of the collected and photographed males show a pattern distinct from *E. coosae* and *E. scotti* (color pattern summarized in Figure 2.9). This interesting result represents a presumed range extension for the Cherokee darter at Pettit Creek.

Discussion

Neighbor joining, maximum parsimony, and Bayesian analysis have revealed three distinct clades or groups within the range of the Cherokee darter. In addition, the phylogeographic application of this data shows partitioning of these clades into distinct regions within the Etowah River system. An examination of populations across the range of the species has revealed coloration patterns that correspond, in part to the genetic results. As evident from the trees produced by both phylogenetic methods the three clades form monophyletic units from which shared haplotypes are absent. Such results in combination with greater than 1% sequence variation (between groups) fulfill several criteria for the classification of the three "phylogeographic units" as ESUs (evolutionary significant units) as outlined by Moritz (1994, 1995). Despite the lack of nuclear DNA data within this analysis the results of this study readily support this classification.

The three ESUs found through this genetic analysis have important implications for the species. Conservation actions must consider these three units in management and consider that they are isolated from one another and are not replacements for one another. Indeed, results have not found apparent mixing between the ESUs suggesting forms of isolation that have been in place for at least tens of thousands of years. However, this isolation means that the replenishment of any given ESU is limited to streams within each. Habitat fragmentation at any level thus yields a very narrowed effect and impacts not only the species, but more importantly, each ESU.

The discovery of syntopy between *E. coosae* and *E. scotti* doesn't immediately suggest hybridization. Due to the maternally inherited nature of mtDNA the true paternal parentage of the analyzed individuals cannot be determined with the methods utilized in this study. In addition, a large percentage of the collected *Ulocentra* at these sites were females and were uninformative from a morphological standpoint due to their lack of nuptial pigmentation. However, of significance are the large number of Cherokee darter haplotypes present at Pettit Creek and the apparent intermediate coloration of nuptial males. Of twenty-one specimens analyzed only two expressed Coosa darter haplotypes. Such dominance of a haplotype suggests that either Coosa darters are extending their range into the Pettit Creek system or available male *Ulocentra* at this site select female Cherokee darters more frequently as mates and the two species have coexisted for some time. Another possibility exists that Cherokee darters simply outnumber Coosa darters at the collection locality due to effects of competition or environmental partitioning.

Table 2.1

Primers Used in PCR and Sequencing of the mtDNA Control Region, tRNA Phe. Gene,

and Partial 12S rRNA Gene in *E. scotti* and *E. coosae*

Primer	Sequence	Direction and use
BAPD1.	5'ATCTCGCATACCTCAAAATCTT3'	Light PCR/sequencing primer
LPRO	5'AACTCTCACCCCTAGCTCCCAAAG3'	Light PCR primer
TDKD	5'CCTGAAGTAGGAACCAGATG3'	Heavy sequencing primer
12Sa-rev	5'TAGTGGGGTATCTAATCCCAG3'	Heavy PCR primer

Table 2.2 Genetic Distance and Standard Error between *E. scotti* Phylogroups

and E. coosae

	Upper Group	Middle Group	Lower Group	*E. coosae*
Upper	0	*.00313	*.00349	*.00454
Middle	**0.01325**	0	*.00369	*..00469
Lower	**0.01753**	**0.0186**	0	*.00431
E. coosae	**0.02324**	**0.02369**	**0.02361**	0

*= Denotes standard error **Bold** = Denotes genetic distance

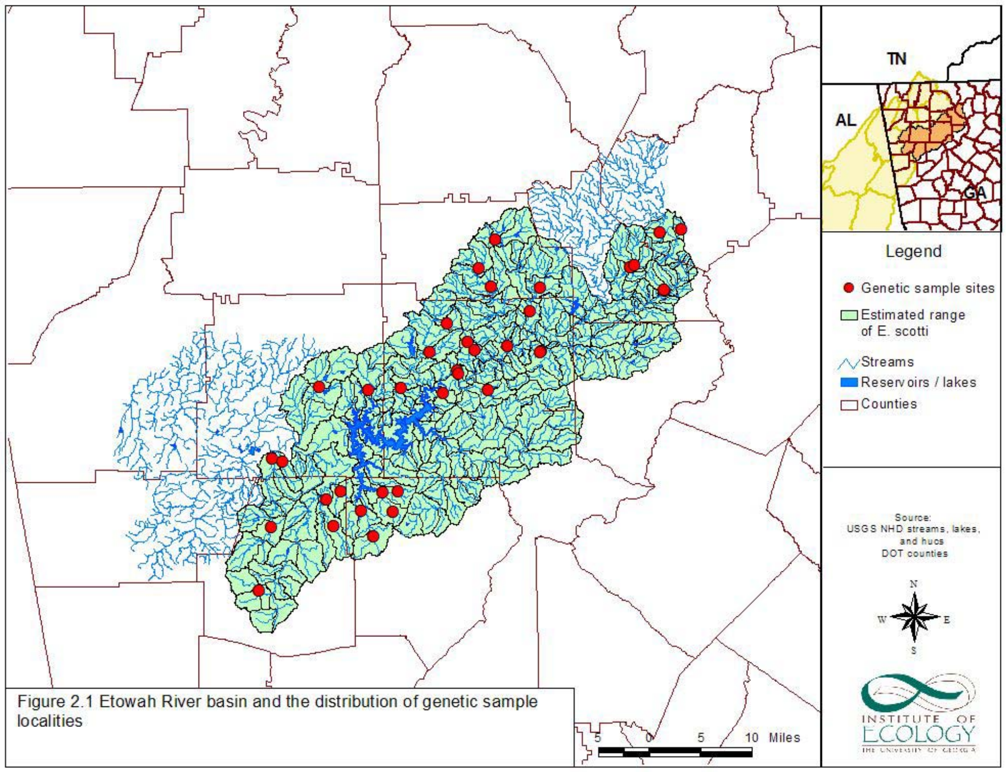

Figure 2.1 Distribution of genetic samples within the Etowah River basin and the estimated range of *E. scotti*

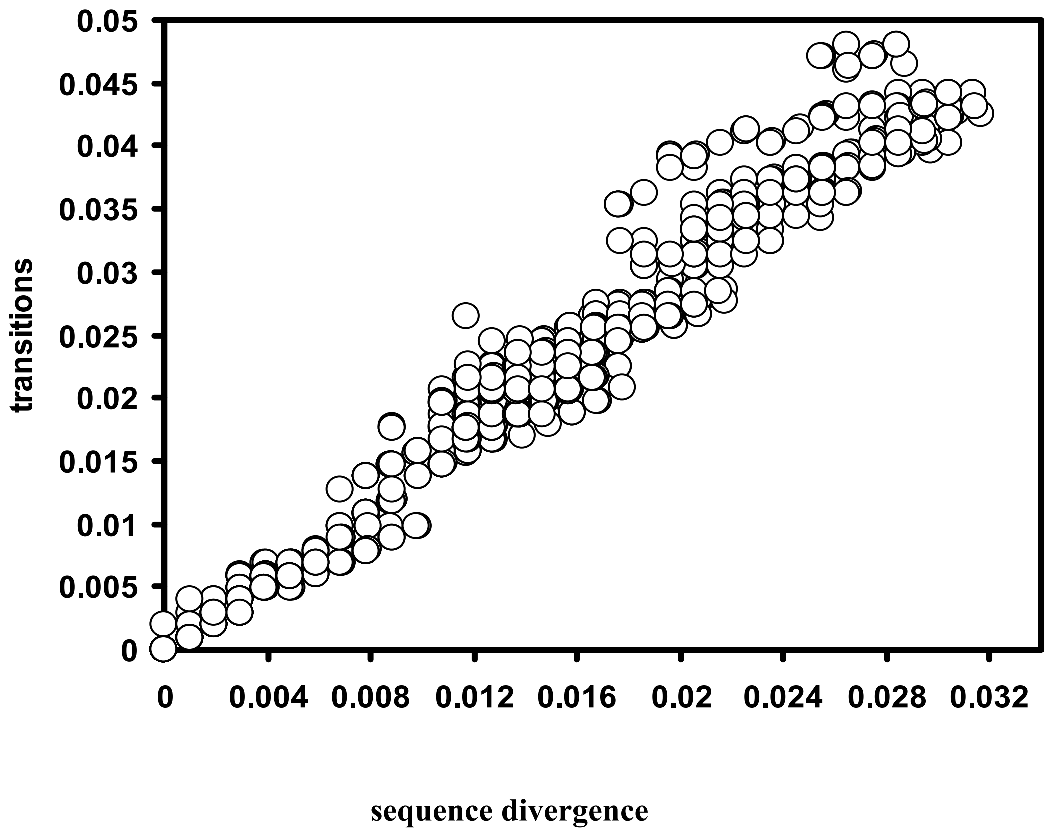

Figure 2.2 Plot of pairwise transition rates against total sequence divergence rates

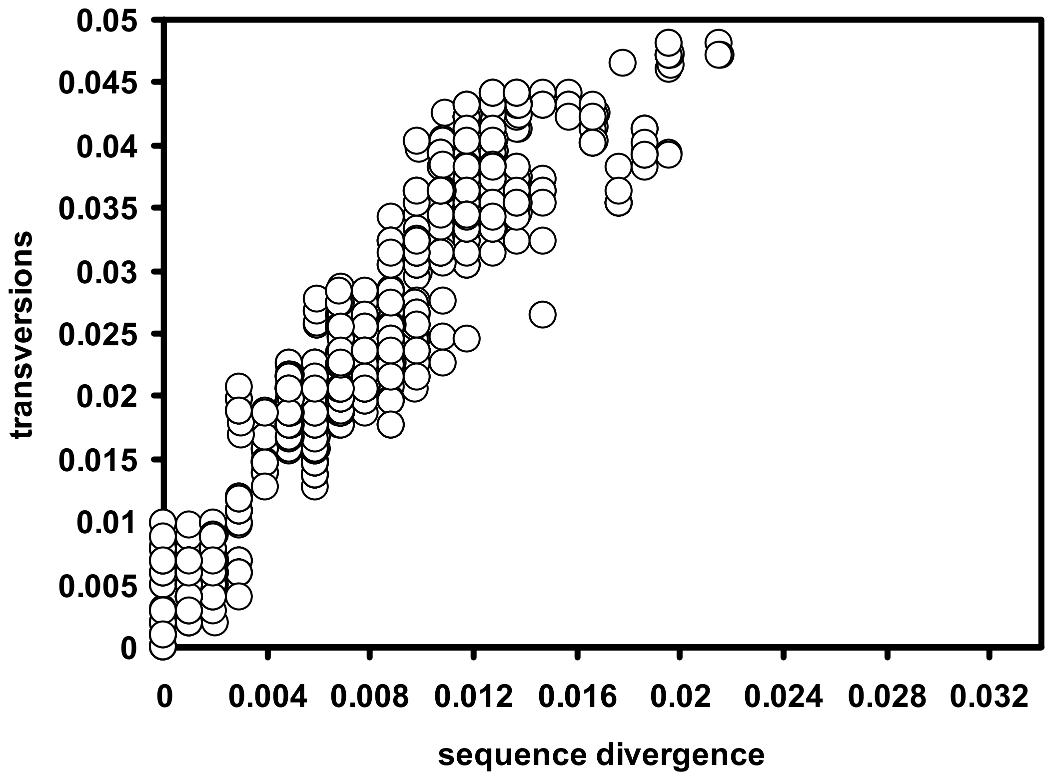

Figure 2.3 Plot of pairwise transversion rates against total sequence divergence rates

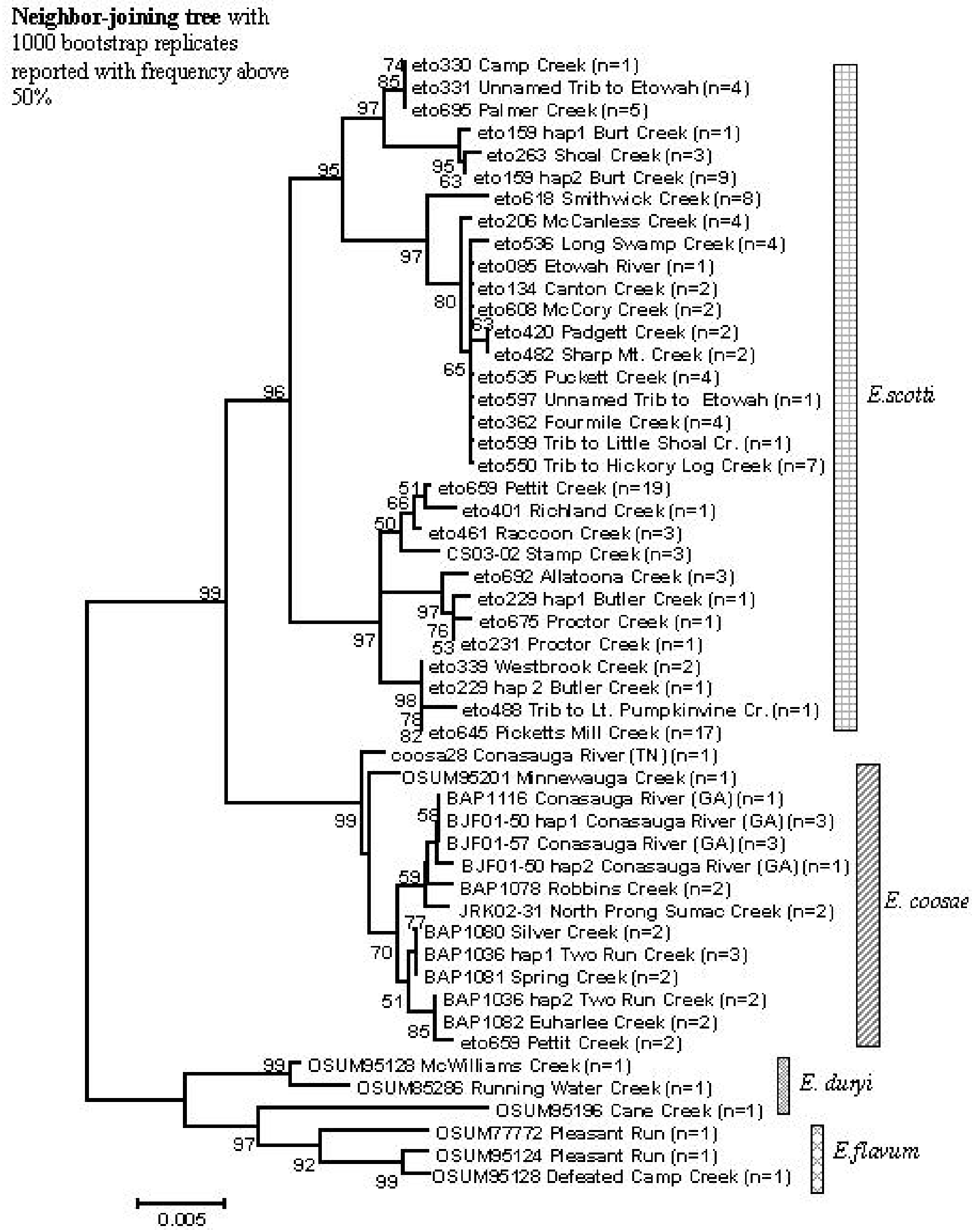

Figure 2.4 Neighbor-joining tree

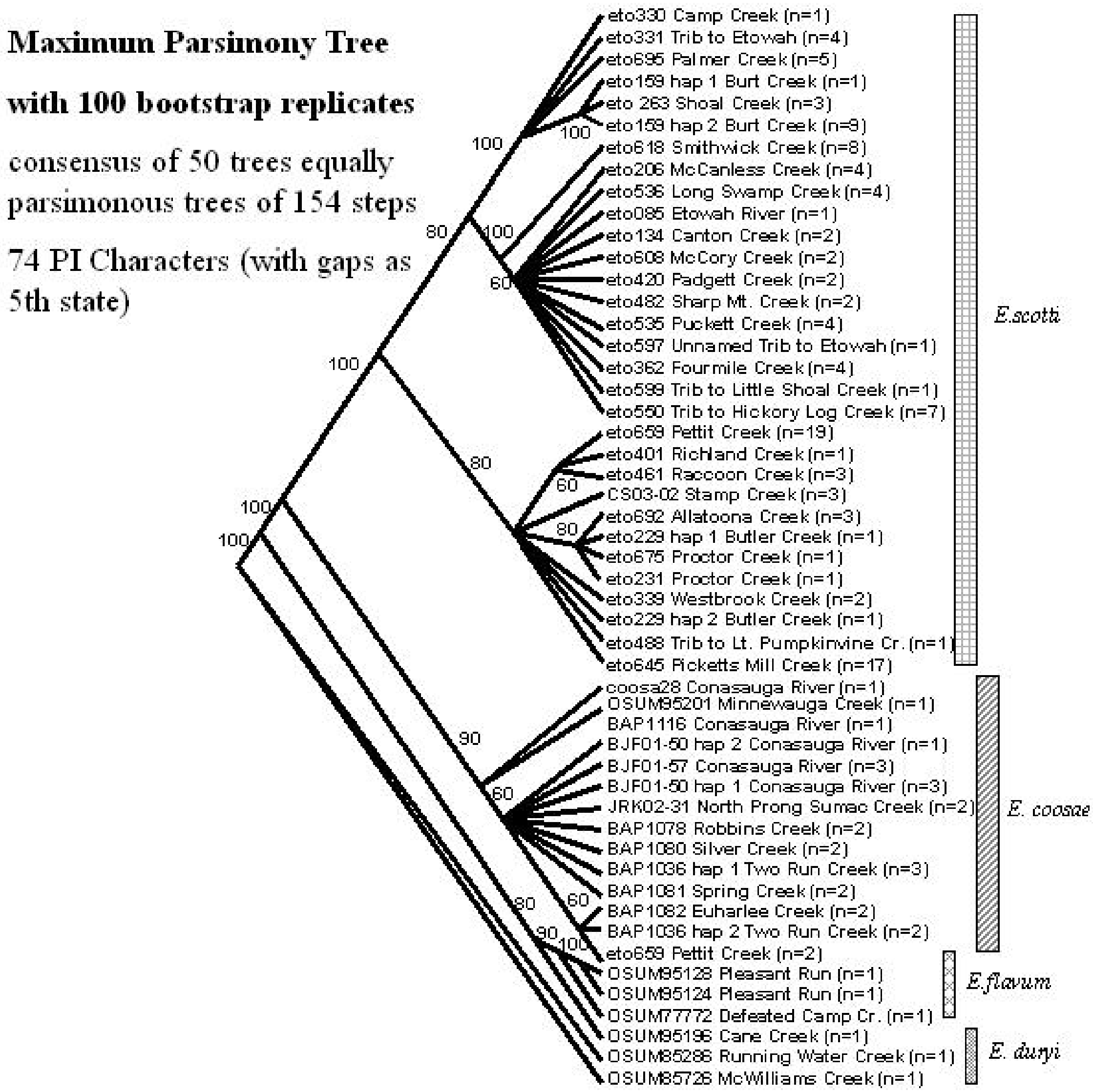

Figure 2.5 Maximum parsimony tree

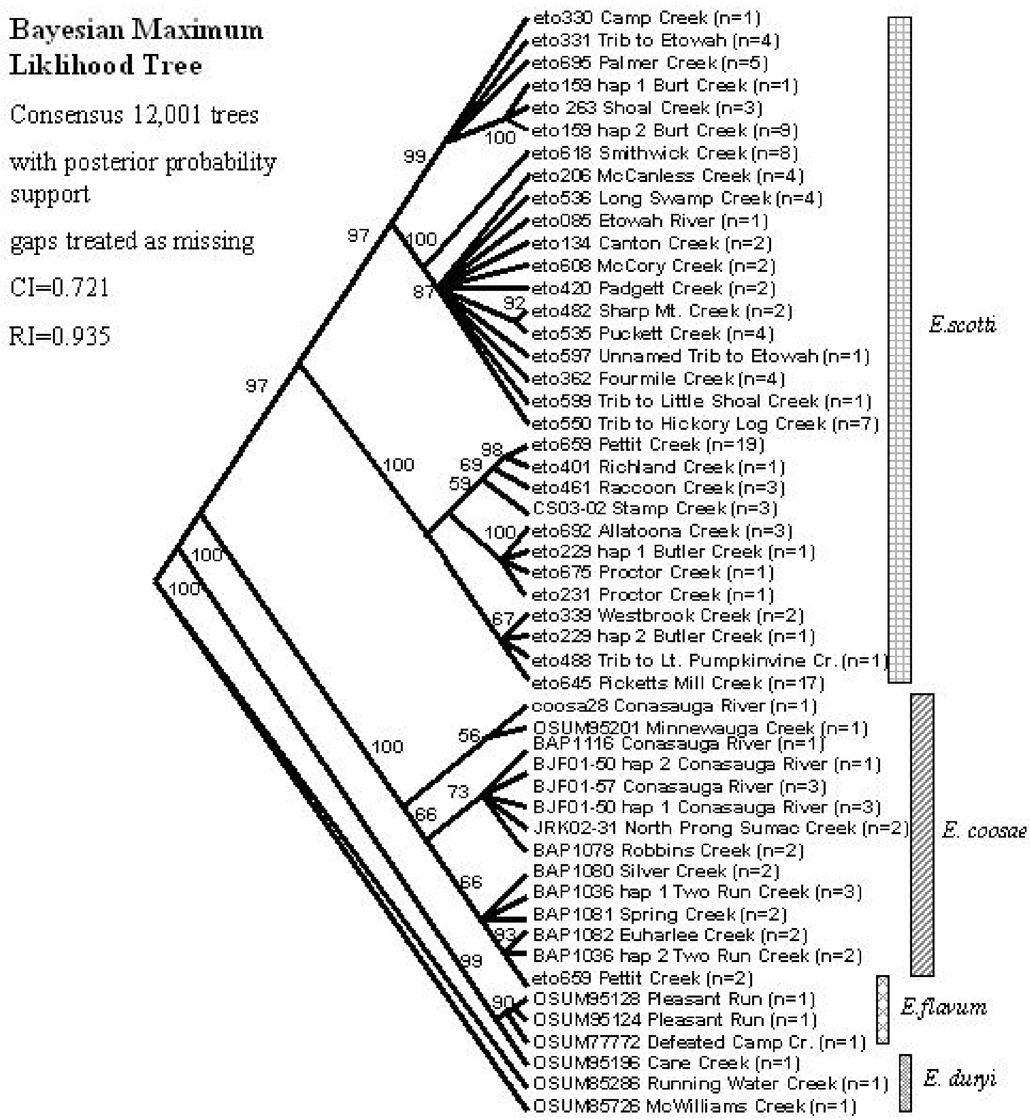

Figure 2.6 Bayesian likelihood tree

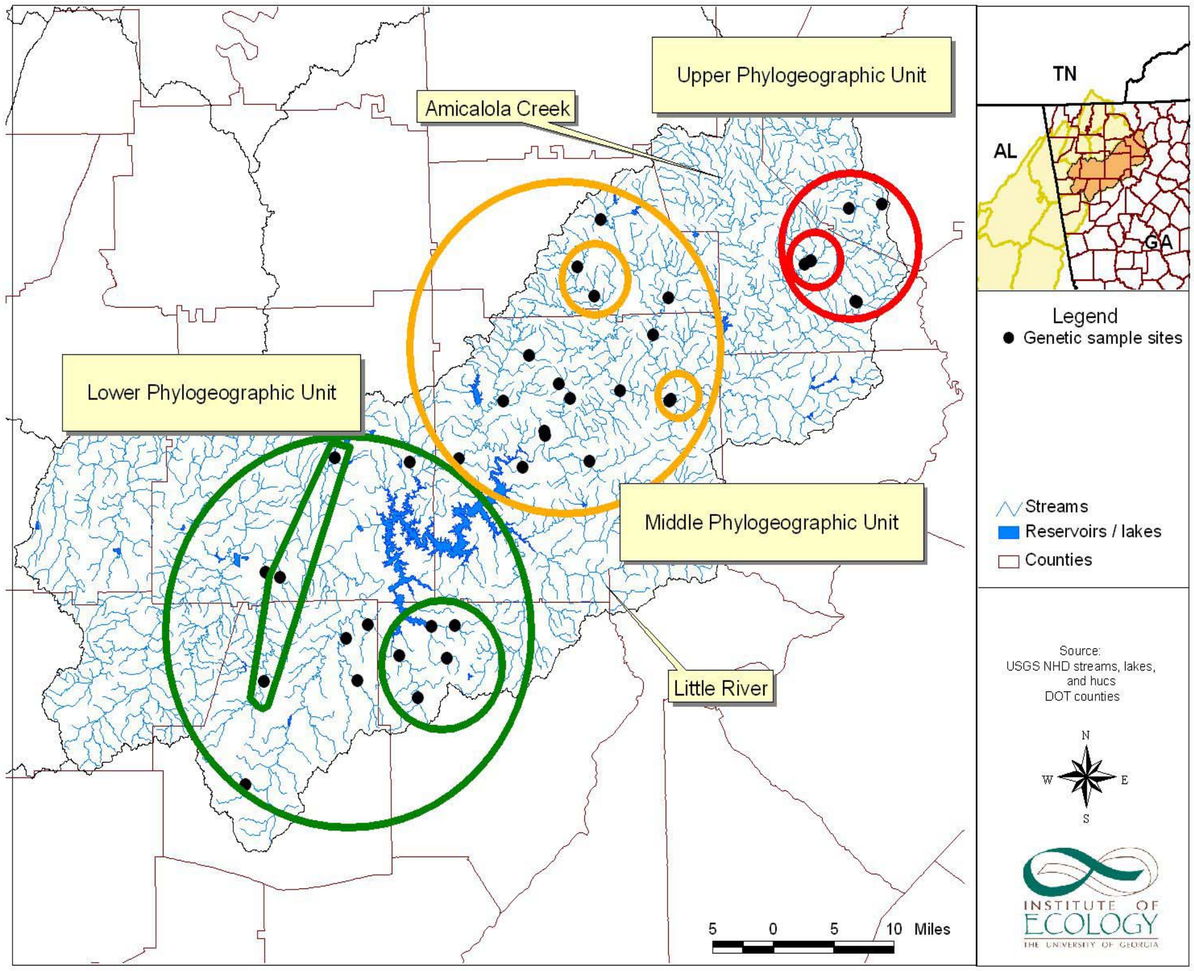

Figure 2.7 The Etowah River Basin and the Phylogeography of *E. scotti*

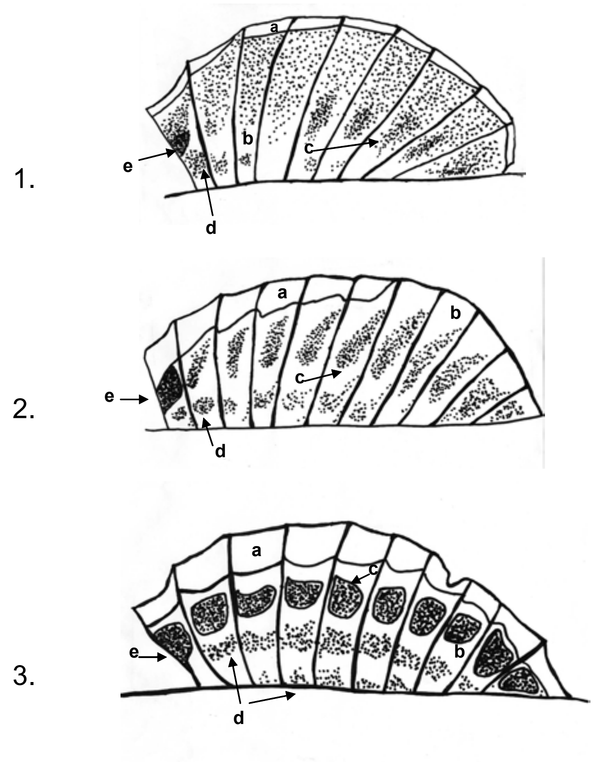

Figure 2.8 Nuptial male fin diagrams (a = blue or gray, b = clear, c = red, d = black or dark brown, e = oscellus)

1. Spinous dorsal fin depicting that of the upper and middle Etowah *E. scotti* group. Drawn from a 54mm (SL) male photographed at Downing Creek, Cherokee Co., GA (15 March, 2002)

2. Spinous dorsal fin depicting that of the lower Etowah E. scotti group. Drawn from a 50mm (SL) male from Butler Creek, Cobb Co., GA (15 March, 2002)

3. Spinous dorsal fin depicting that of the most geographically proximate populations of E. coosae. Drawn from a 45mm (SL) male from Two Run Creek, Bartow Co., GA (20 March, 2002)

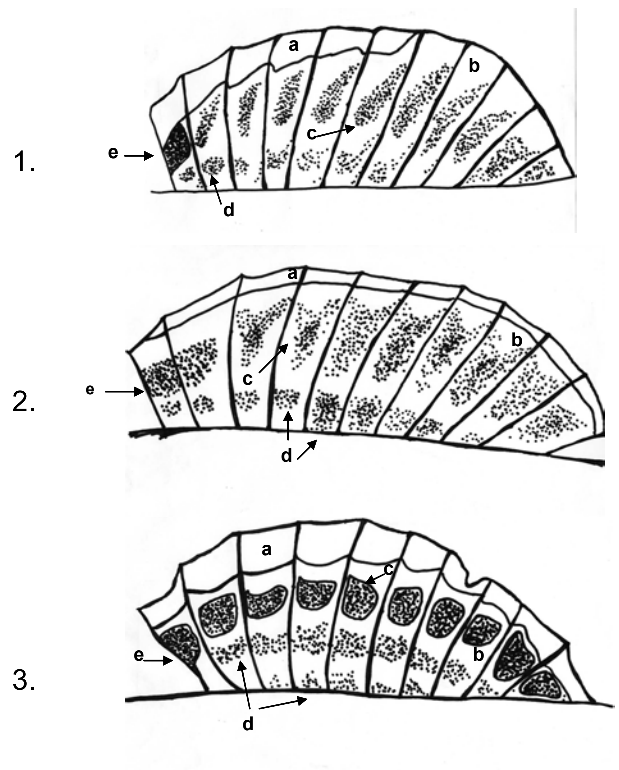

Figure 2.9 Nuptial male fin diagrams from lower phylogeographic unit, Pettit Creek, and E. coosae (a = blue or gray, b = clear, c = red, d = black or dark brown, e = oscellus)

1. Spinous dorsal fin depicting that of the lower Etowah E. scotti group. Drawn from a 50mm (SL) male from Butler Creek, Cobb Co., GA (15 March, 2002)

2. Spinous dorsal fin depicting that of individuals (males) from Pettit Creek. Drawn from a 47mm (SL) male from Pettit Creek, Bartow, Co., GA (20 March 2002)

3. Spinous dorsal fin depicting that of the most geographically proximate populations of E. coosae. Drawn from a 45mm (SL) male from Two Run Creek, Bartow Co., GA (20 March, 2002)

CHAPTER 3

ANALYSIS OF SPAWNING HABITAT PARAMETERS FOR THE CHEROKEE DARTER

(*Etheostoma scotti*)

Introduction

The spawning behavior of various members of the subgenus *Ulocentra* (family Percidae) has been noted as possibly the primary synapamorphy uniting this group of darters (Bailey and Etnier 1988, Porter et al., 2002). Spawning behavior described for various members of *Ulocentra*, including *E. coosae* (O'Neil 1981), *E. baileyi*, *E. etnieri* (Porterfield 1998), *E. flavum* (Keevin et al. 1989), *E. scotti* (Bauer et al. 1995), and *E. simoterum* (Page and Mayden 1981) - is consistent, with females typically depositing single eggs to rock substrate in a vertical position. The substrate selected has been noted to be of cobble and larger size class. Spawning temperatures and time of year vary between studied species within the subgenus, but have been noted to occur in April and May and in temperatures ranging from 10-20 degrees Celsius (Porterfield, 1998). Much of the seasonal and temperature variation of different species within the subgenus may be related to the broad geographic and latitudinal range of the species within *Ulocentra*. Occurring from the Green and Barren River system in Kentucky (*E. rafinesque*) to the coastal plain of Alabama (*E. colorosum*), the members of this subgenus experience a range of climate regimes.

I examined spawning behavior of the Cherokee darter *E. scotti*, a federally protected species endemic to the Etowah River system in north Georgia. In laboratory observations of spawning behavior, female Cherokee darters usually attached a single egg to a cobble, small boulder, or gravel substrate while in an inverted or vertical position (Bauer, et al., 1995). Although this behavior closely resembles that of other members of the group, aquaria spawning (Porterfield, 1998) may not accurately reflect the behavior of individuals in the wild.

The objective of this study is to verify the spawning observations made by Bauer et al. (1995) in aquaria with observations from the field to resolve possible discrepancies commonplace in aquaria observations (Porterfield, 1998). More importantly this study aims to identify critical environmental variables, conditions, and parameters required for reproduction by this species, which is threatened by degradation of small streams throughout its range.

<u>Methods</u>

To investigate variation in spawning parameters across the species' range, five observation localities were selected for this study. Sites were selected based on sampling conducted in the winter of 2002 at localities known to harbor Cherokee darters across a broad range of the Etowah River Basin. During this sampling period relative abundance and ease in collecting the species signified a potential observation site. Sites with high densities of Cherokee darters were chosen for a higher likelihood of observing spawning behavior.

Sites selected were Shoal Creek, Dawson Co., Georgia; McCanless Creek, and Hickory Log Creek, Cherokee Co., Georgia; Butler Creek, Cobb Co., Georgia; and

Whitehead Creek, Paulding Co., Georgia (Table 1.1 Appendix B) (Figure 3.1). The lengths of the study reaches varied from site to site dependent upon the densities of Cherokee darters, property boundaries, ease in accessing the reach, and habitat. The longest reach, Whitehead Creek was approximately 140 meters in length while the shortest reach (at Hickory Log Creek) was 40 meters in length. Stream widths ranged from approximately 3-6 meters at Butler Creek to 6-9 meters at Shoal Creek.

Spawning observations were made via snorkeling. Observations were made over a two-year period through the months of March, April, and May 2002, and March, April, May, and June 2003. In 2002, observations began on 22 March and ended on 15 May. In the 2003 field season observations began on 21 March and extended to 10 June, 2003. Setting the beginning of snorkel observations was based on literature review and weather conditions in an effort to capture conditions driving the onset of spawning. The observation period ended when spawning activity (including courting, spawning, and chasing) was no longer observed over several visits to each site or until a majority of observed individuals no longer displayed courting behavior. Through both seasons an attempt was made to visit all five sites at least once weekly, dependent on weather and habitat conditions. High turbidity and inclement weather required the postponement of field observations until conditions improved.

Prior to all snorkel observations water quality parameters were measured with a Hydrolab Datasonde® 4a and a Hach Turbidimeter model 2100P. Parameters measured included turbidity (NTUs), conductivity (µs), temperature (Celsius), pH, and dissolved oxygen (percent). All selected reaches were snorkeled in an upstream direction. All habitats observable through a snorkel mask (>0.2m) were visually scanned for the presence of Cherokee darters. Habitat too shallow to be observed through a snorkel mask was scanned from above for the presence of Cherokee darters. Cherokee darters were tallied as observed and individuals were watched for signs of spawning behavior. This behavior included the mounting of females by males, chasing of females by males, or pairing, in which a male and female were at rest immediately adjacent to one another on the streambed. The color condition of males and the reproductive condition of females were noted and described for each outing and each locality. Females were noted as slightly, moderately, or very gravid depending on the perceived volume of their ventral region. For males, the presence or absence of blue in the opercle, lateral bars, anal, caudal, and pelvic fins was noted. In addition the intensity of the red and the presence or absence of blue in the spinous dorsal was noted. When courting or spawning behavior was observed, pairs were followed until the activity ceased or the pair was lost. Spawn times and courting times observed were recorded. When spawns occurred the substrate upon which the egg was deposited was marked with a weight/float marker and left until the snorkel of the reach was complete. After the reach was completely snorkeled all spawning sites were measured. Specifically, the velocity and depth was measured at five points adjacent to the spawn site (immediately upstream, immediately downstream, adjacent right and left, and on top). The dimensions of the substrate upon which the spawning took place were measured to the nearest half centimeter and classified, based on the median axis measurement by Wentworth size category (Gordan et al. 1992), The depth of the area immediately surrounding the spawn was measured to the nearest centimeter. Depth and velocity measurements were taken with a Marsh-McBirney Inc. FLO-MATE ™ portable flow meter model 2000 - mounted on a top-setting wading rod with tenths of feet increments. All velocity measurements were taken at 60% depth. In 2003, the specific locations of observed spawns were mapped within the reach using distance from - flagging placed at the bank immediately adjacent to each observed spawn and from a predetermined landmark mid-channel. Sites in which a minimum of ten spawns were observed over the course of the 2003-spawning season were utilized in an effort to plot selected spawning habitat within the reach against a stream bed and surface profile. At the conclusion of spawning at these sites, total station surveying equipment was used to measure longitudinal variation in bed elevation and water surface elevation along the length of the reach. Elevation measurements were taken at 0.5 - 2 m (usually 1 m) intervals along the thalweg during base flow conditions, and were plotted to illustrate longitudinal variation in channel form. The bed elevation of the triangulated spawn localities was also measured and the results superimposed upon the graphed bed and surface elevations to examine occurrence relative to pool-run-riffle structure within the site. I also recorded size of dominant bed sediment at l m intervals, longitudinally along the mid-channel at these sites to provide a measure of spawning substrate availability.

Finally, in an effort to correlate diel as well as seasonal temperature patterns and spawning activity, ONSET Optic Stowaway Temp™ temperature loggers were deployed at each of the five spawning observation sites. Loggers were calibrated and deployed to read water temperature at fifteen-minute intervals. These temperature loggers were downloaded periodically throughout the spawning season and trends were examined in correlation with the spawning duration observed throughout each season.

Results

Over the observation period of two field seasons, spawning acts were observed across all field sites. In the first field season a total of 27.5 hours of field observations were made with 13 visits made between all sites (Table 1.1 Appendix B). In 2003 substantially more observations were made with a total of 60.6 observation hours and 36 visits. In 2002 several observations were made at the onset of the study in which spawns were not observed. However, spawns were observed on the beginning date of observations in 2003 (21 March). In addition, spawning activity was noted later in the season in 2003, extending the observation period (and the documented spawning period) by nearly a month. A summary of spawning dates and spawning activity can be seen in Table 3.1. In 2002 the earliest spawning event witnessed occurred on 23 April, while the last spawning event observed occurred on 30 April. In 2003 the first spawning event witnessed occurred much earlier, on 21 March, and the last spawning act for the season was witnessed on 10 June with observations terminated on this date due to a low activity rate of observed individuals (minus the single spawning pair).

A total of 63 spawning acts were observed throughout the study period over the two seasons of observations. Spawning occurred in a range of habitat conditions throughout the observation reaches and between sites. The most frequently selected substrate sizes noted during spawning acts were gravels and cobbles as defined through the Wentworth size class system. However, a range of substrates were utilized as attachment points for single eggs by females, including woody debris (Figure 3.2). A range of depths and velocities as well as habitat types were selected by females for suitable spawning sites. The mean depth and velocity at which eggs were deposited was

0.31 meters in depth, and 0.237 m/s velocity. The range of velocities measured across all spawning sites was 0-0.676 m/s, and the depth ranged from 0.088-0.594 meters (Figure 3.3). The majority of spawns witnessed occurred in run/glide habitat and often at the tail of pools, although spawns in riffle habitat and the margins of deep pools were also noted.

At the conclusion of the snorkel observations McCanless Creek, Cherokee Co., and Butler Creek, Cobb Co. were selected for total station analysis due to the numerous spawns observed at these sites during the 2003 season. The results of these surveys can be seen in Figure 3.4 (Butler Creek) and Figure 3.5 (McCanless Creek). Consistent with recorded field observations, the plotted spawn events demonstrate the use of glide or run habitat and pool tails as the primary sites selected for the deposition of eggs.

Spawning observations made of the Cherokee darter closely paralleled observations made of other members of the subgenus *Ulocentra*. Male Cherokee darters were often observed displaying perpendicularly to females with dorsal fins erect and nuptial colors at high intensity. Males often pursued receptive females, mounting them and periodically pecking at their nape to instigate spawning behavior. Females moved about habitat scanning substrate in what appeared to be an attempt to select a suitable egg deposition point. Often females would peck substrate in what appeared to be an attempt to signify to the male her readiness to spawn. This pecking often elicited quivering by the male as he mounted or positioned himself parallel or on top of the female. After this point the females would move about in search of appropriate egg deposition points with the male following very closely. Females appeared to prefer substrate free of algal growth or sediment coatings. Once suitable substrate was found the female would peck at a spot usually on the vertical surface of the substrate and at this point the male began to quiver and release milt. Very soon after pecking and while the male was still quivering the female briefly quivered and quickly moved forward with her ovipositor touching the exact point that she pecked. After this series of events a slightly opaque and colorless egg could be seen attached within micorcrevices upon the substrate selected. Frequently one spawn was quickly followed by another, often in close proximity to the previous. Two spawns were rarely (once) noted on the same substrata. In many situations, the removal of fine debris or algal growth was frequently observed with the peck of a female and at times, particularly in streams with dense algal growth, numerous pecks were made before the deposition of gametes. In addition, when frequent pecks were made the female would often abandon the site and continue the search for a more suitable and possibly a less densely coated piece of substrate.

In some circumstances a female would select substrate and would peck, upon which the male would begin to quiver. However, instead of placing her ovipositor over the cleaned crevice the female would abruptly change positions or move off the substrate and continue to search. Such behavior we have termed as pseudo-spawning. The exhibition of such behavior without the observation of a female quivering would signify that spawning was taking place on the date of the observation despite the actual observed deposition of eggs. This was useful in determining the duration of spawning and the environmental conditions in which spawning occurred. Throughout the spawning season, pseudo spawns occurred (Table 3.1) with females in various states of gravid condition. Another occurrence within the spawning behavior, noted towards the end of the spawning season was termed “dry spawning”. In this situation the entire courting and spawning ritual would be carried out by a pair, but after positioning of the ovipositor by the female an egg would not be observed in the spot. This behavior or occurrence was noted towards the end of the spawning season in 2003 at McCanless Creek (13 May, 2003) and Butler Creek (12 May, 2003). This behavior and the substrate upon which the egg was deposited was treated as a true spawn and the location plotted and measured as outline in the methods.

One observation made at Hickory Log Creek on April 9th, 2003 occurred with high turbidity levels created from recent heavy rains. The turbidity conditions were at the upper limits for observations, and no spawning related behavior was observed. Individuals were noted resting on the stream bottom and in protective areas. Despite the turbid conditions the flows were not abnormally high and the current no more swift than that observed several days prior. Several days prior to this observation, on the 1st of April courting and spawning behavior had been noted at the same locality.

Continuous temperature data from three sites were analyzed to correlate potential spawning trends. The sites selected were Shoal Creek, Whitehead Creek, and Hickory Log Creek. Mean daily temperatures as well the mean daily high and mean daily low temperatures for the spawning season were calculated at each site for 2002 and 2003. Deployment and retrieval dates varied between each site and all analyses were performed on the earliest start date and latest retrieval date in which the two years overlapped. At Shoal Creek temperatures were analyzed from 27 March through 26 May; at Whitehead Creek from 5 April to 29 May; and at Hickory Log Creek from 11 April to 26 May.

The mean of the daily averages and the mean of the daily highs were consistent across all sites in that both were higher in 2002 than in 2003. The difference between the years ranged from 0.44-1.03 degrees Celsius for the mean daily temperatures and 0.998-1.64 degrees Celsius for the mean of the daily highs. The mean of the daily lows was very similar at each site between years (Table 1.3 Appendix B).

Discussion

Peak spawning of Cherokee darters appears to take place in mid-April although spawning activity was noted throughout the study period (Table 3.1).

Spawning sites characteristically occurred in run and pool habitats, with moderate depths and velocities, and most often, on gravel substrates. The use of gravel for egg attachment contrasts with the use of cobble and boulder substrates frequently reported for other *Ulocentra* species (Porterfield 1998), but Cherokee darters also oviposited on a variety of bed sediments, from medium gravel to bedrock. In addition, after numerous observations it seems to be clear that the pecking performed by the female holds two purposes. The first is to stimulate the male and to signal the onset of egg deposition. The second, and arguably the most significant purpose of this behavior is to clear the site for strong adherence of the individual egg. Thus, availability of bed sediments that are relatively free of fine sediment and algal growth may strongly affect habitat suitability for spawning. My observations of the cessation of spawning activity coinciding with high turbidity levels indicates the potential importance of good visibility for successful courtship and spawning. Negative effects of high suspended sediment on spawning activity has similarly been reported for a cyprinid species that commonly co-occurs with the Cherokee darter and that also has well-developed nuptial coloration (Burkhead and Jelks 2001).

The extended observed spawning season in 2003 (in contrast to 2002) may be related to a cooler May and early June experienced in North Georgia in 2003. Indeed, temperature has been cited as a major factor contributing to the length of spawning season in percids (Hubbs 1985). Additionaly, cooler temperatures in 2003 may have also been responsible for the observed "dry" spawn behavior noted near the end of the spawning period. Appropriate or preferred water temperatures may stimulate spawning despite the inability of females to deposit or produce eggs.

My field observations correspond with laboratory observations of spawning by Cherokee darters by N. M. Burkhead (Bauer et al., 1995). For example, Burkhead observed females "visually scrutinizing" their surroundings, presumably in search of appropriate spawning substrata. Additionally, in the laboratory and in the field, male Cherokee darters tend to maintain a "roving territory" around the female with which they are courting. As the female may travel several meters or more through a variety of habitat the male maintains an area of territorial significance. When other males enter this loosely defined area the male will often give chase in the process losing track of the female or having another male take his place. Thus, the behavioral description from laboratory observations accurately reflects field behaviors. Field observations have permitted additional description of specific habitat conditions and spawning substrates used by Cherokee darters.

Table 3.1 Recorded activity of observed Cherokee darters at all sites (2002-2003)

Behavior Observed: M/M = Male chasing or displaying to other male, M/F = male chasing or displaying to female, PS = pseudo spawn, S = spawn, DS = Dry spawn, C = courting, NSA = no spawning related activity observed

Locality	Date	E. scotti observed.	Males Obs.	Females Obs.	Behavior Observed
McCanless Cr.	22-Mar-02	14	10	4	M/M
	23-Apr-02	NA	NA	NA	M/F, M/M, S
	14-May-02	23	11	12	M/F, PS, C
	21-Mar-03	37	19	18	M/M, C
	29-Mar-03	14	9	5	PS, C
	1-Apr-03	41	20	21	M/F
	13-Apr-03	32	19	13	S
	17-Apr-03	50	25	25	M/M, S, PS
	29-Apr-03	48	24	24	S
	13-May-03	35	19	16	M/F, S, DS, C
	27-May-03	26	13	13	NSA
Hickory Log Cr.	11-Apr-02	11	5	6	M/M
	23-Apr-02	25	NA	NA	M/F, S,
	14-May-02	12	2	10	NSA
	21-Mar-03	60	35	25	S, PS,
	29-Mar-03	84	27	57	M/M, C
	1-Apr-03	91	35	56	M/M, M/F
	9-Apr-03	40	14	26	NSA
	17-Apr-03	53	17	34	C
	28-Apr-03	30	17	15	S
	13-May-03	22	10	15	M/F
	27-May-03	17	9	8	S
	10-Jun-03	15	8	7	M/M, S
Shoal Creek	26-Mar-02	4	2	2	NSA
	11-Apr-02	4	2	2	M/F
	30-Apr-02	6	4	2	M/F, S, PS
	23-Mar-03	5	0	5	NSA
	1-Apr-03	17	8	9	NSA
	13-Apr-03	14	8	6	M/M, S
	28-Apr-03	9	5	4	S
	13-May-03	6	3	3	C, M/F
	27-May-03	6	2	4	NSA

Table 3.1 Continued

Behavior Observed: M/M = Male chasing or displaying to other male, M/F = male chasing or displaying to female, PS = pseudo spawn, S = spawn, DS = Dry spawn, C = courting, NSA = no spawning related activity observed

Locality	Date	E. scotti observed.	Males Obs.	Females Obs.	Behavior Observed
Butler Creek	4-Apr-02	4	1	3	NSA
	24-Apr-02	14	8	6	C, S, PS
	15-May-02	31	13	18	M/F, M/M
	25-Mar-03	38	17	21	S, PS
	31-Mar-03	14 ±	6 ±	8 ±	C, PS, M/F
	4-Apr-03	39	21	18	M/F, S, PS
	15-Apr-03	48	23	25	C, MF
	12-May-03	29	11	18	S, PS, DS
	30-May-03	22	14	8	M/F
Whitehead Cr.	4-Apr-02	4	3	2	M/M
	18-Apr-02	15	5	10	NSA
	25-Apr-02	11	5	6	M/M, C
	15-May-02	25	8	17	C, PS
	26-Mar-03	34	17	17	C, PS
	31-Mar-03	14	6	8	NSA
	4-Apr-03	24	10	14	C, PS
	15-Apr-03	24	10	14	C, S
	29-Apr-03	11 ±	3 ±	8 ±	NSA
	12-May-03	16	9	7	C
	30-May-03	9	7	2	NSA

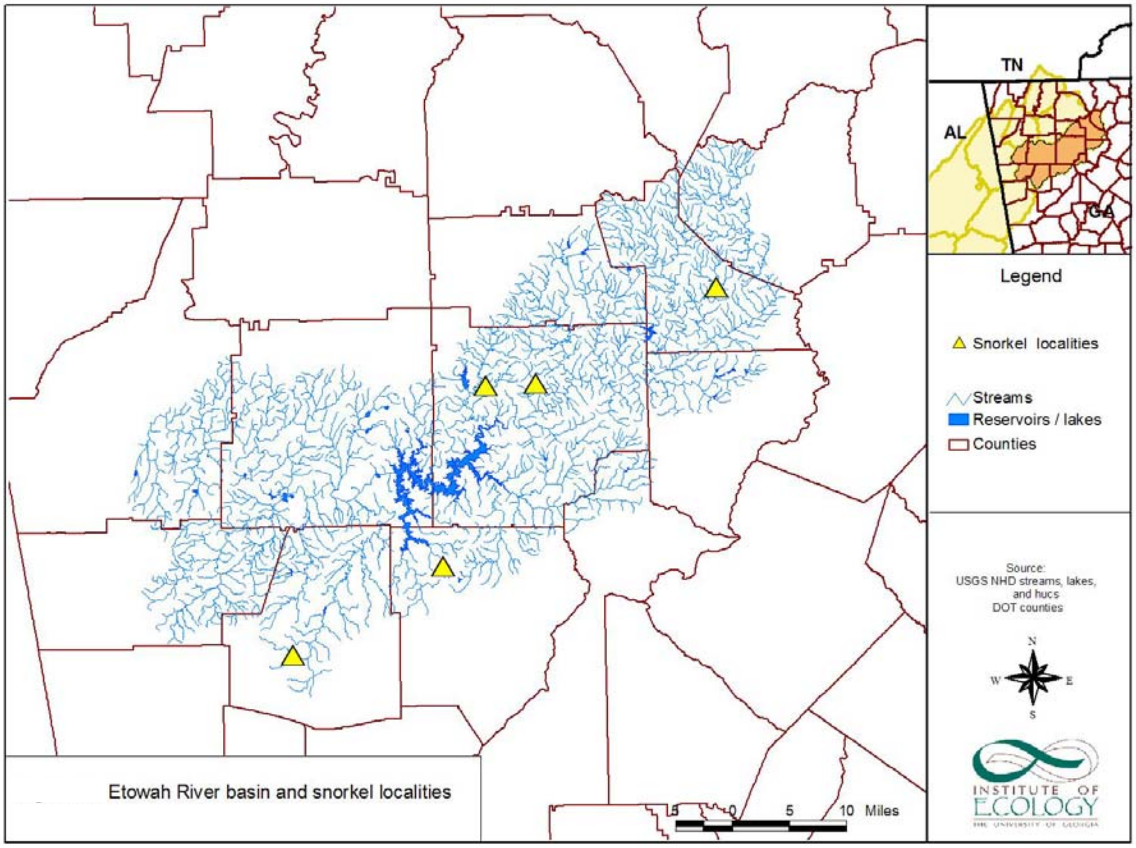

Figure 3.1 Etowah River basin and snorkel localities

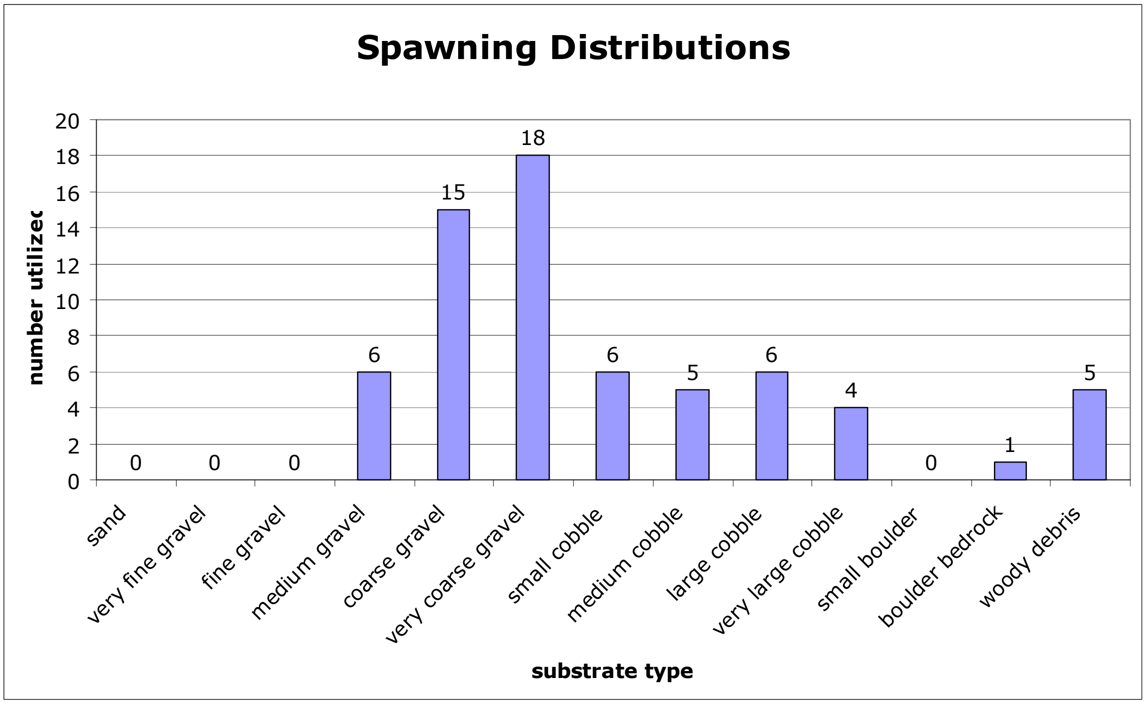

Figure 3.2 Spawning substrate utilization across all localities observed through seasons 2002 and 2003

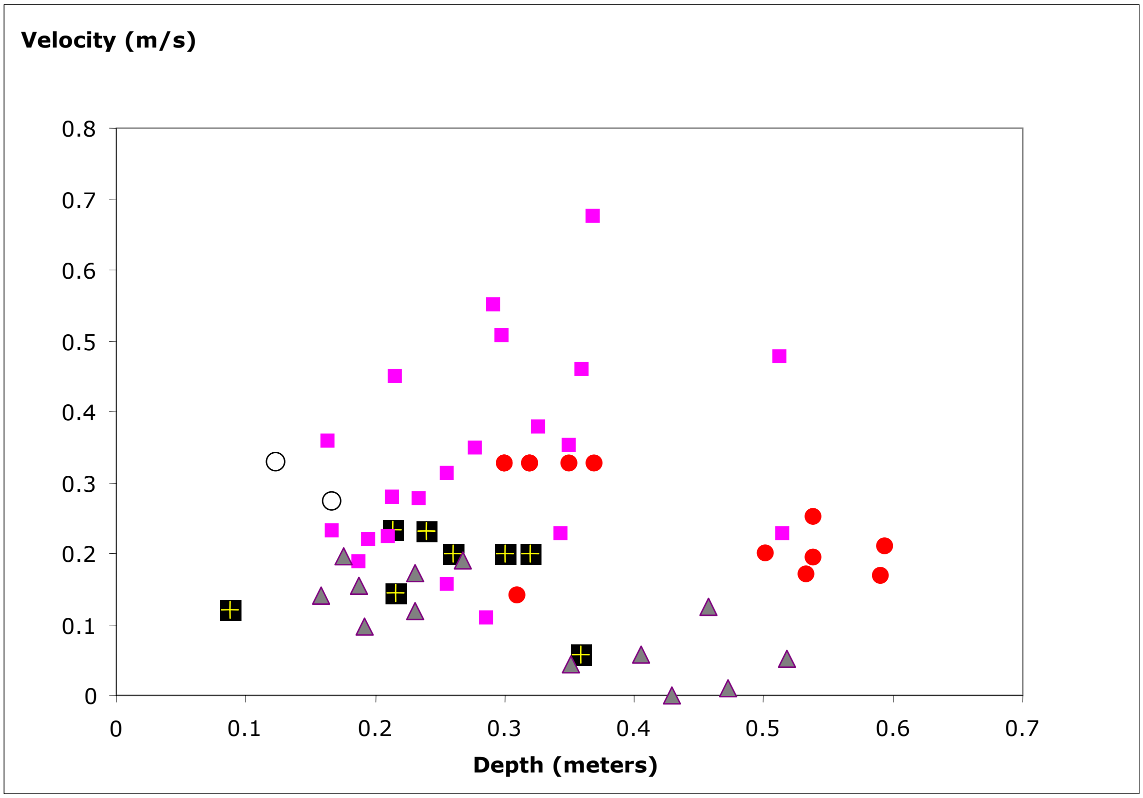

Figure 3.3 Plot of depth and velocity at observed spawns

Solid squares = McCanless Creek, squares with + = Hickory Log Creek, open circles = Whitehead Creek, solid circles = Shoal Creek, triangles = Butler Creek

Plot of measured spawn depths (meters) and velocities (meters/second) across all sites (2003).

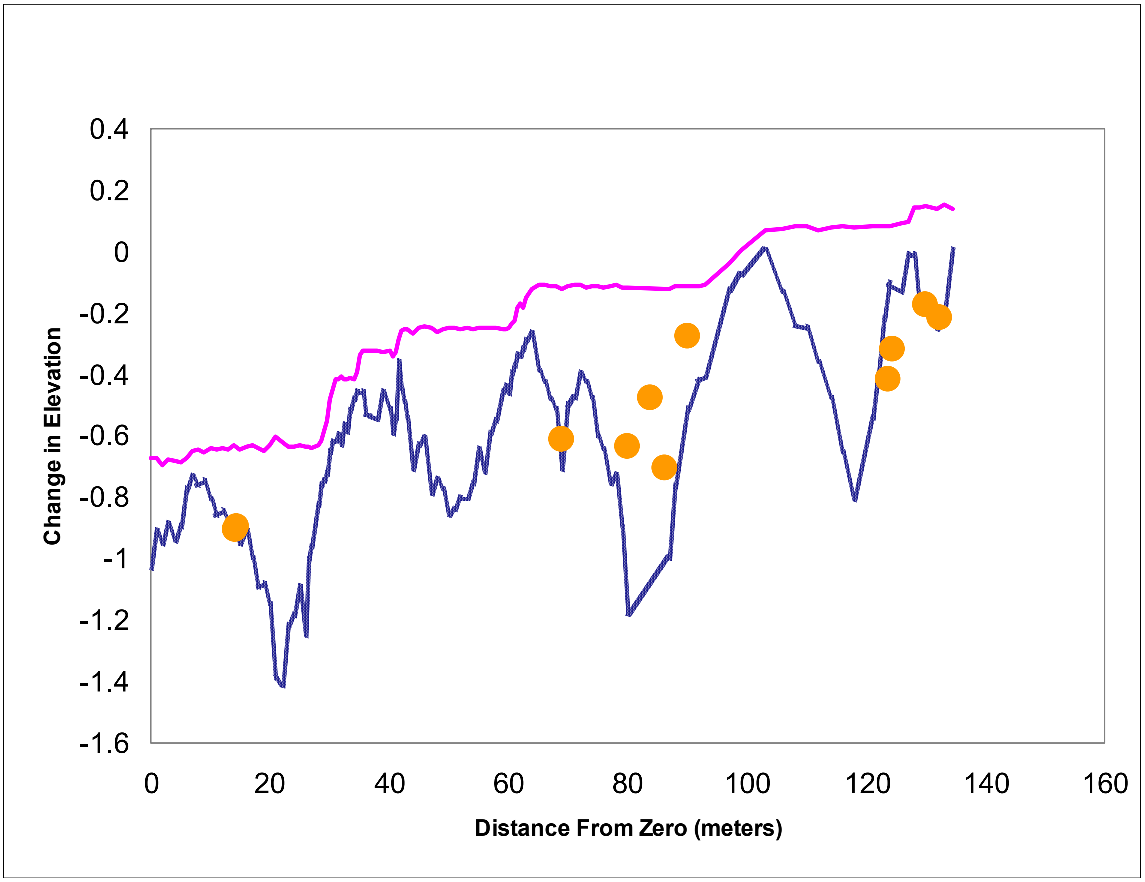

Figure 3.4 Total station analysis of water surface elevation (upper line), bed elevation (lower line), and marked spawn coordinates (points) for Butler Creek, Cobb Co., GA

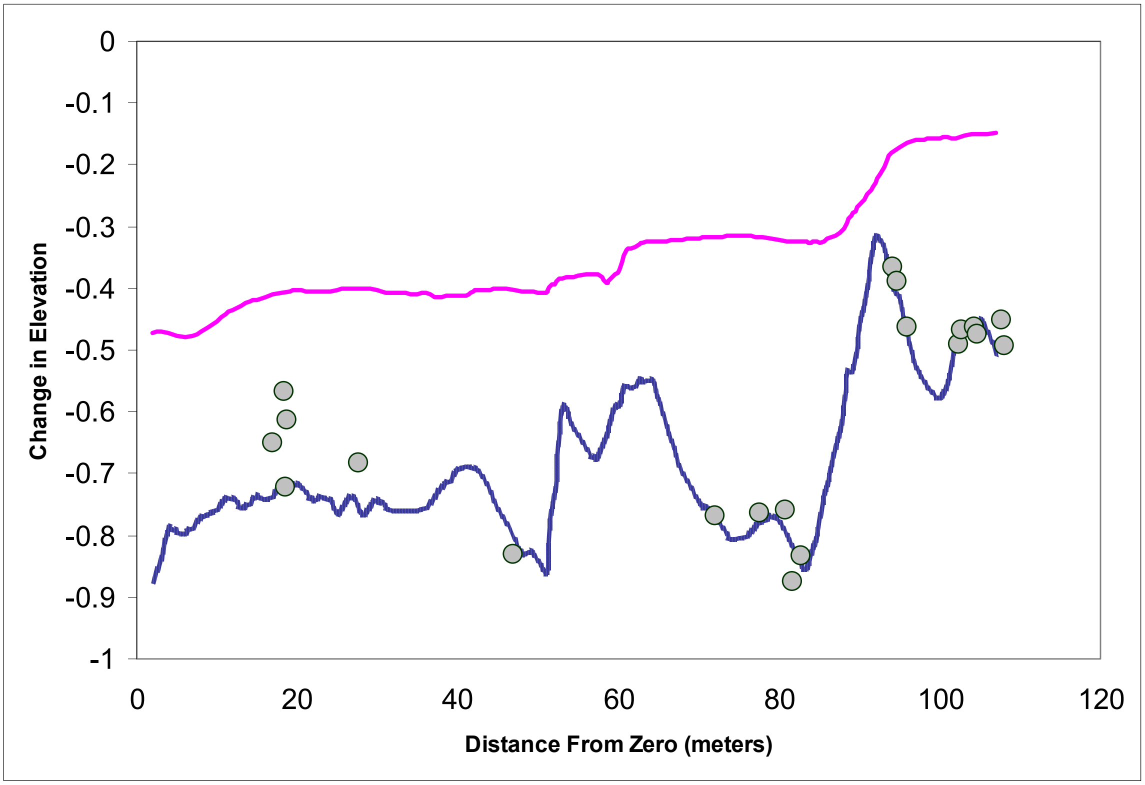

Figure 3.5 Total station analysis of water surface elevation (upper line), bed elevation (lower line), and marked spawn coordinates (points) for McCanless Creek, Cherokee Co., GA

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS FOR MANAGEMENT

Summary

Through the combined efforts of these studies a wide array of new information is known regarding the Federally Threatened Cherokee darter. Spawning studies suggest that sediment does in fact impact the spawning behavior of the species. This impact may be from deposition as well as - suspension of fine sediment in Cherokee darter habitat. Deposition of fine sediment may reduce the micro-crevices selected as sites of egg deposition as well as impairing the adherence of eggs to spawning substrates. Additionally, the pool and run habitats with moderate current velocities used for spawning by Cherokee darters are more susceptible to sediment deposition than swifter-flowing riffle habitats. Suspended sediment may interfere with spawning behavior, reducing courtship activity during episodes of high turbidity. Such reductions in activity may be substantial if combined with increased water temperatures (e.g., as a consequence of increasing runoff from impervious surfaces in the watershed) and a therefore shortened spawning season. This study supports the hypothesis that free-flowing streams providing flowing pool and run habitat, and with gravel sediments and low levels of sediment loading are essential for the reproduction and continued existence of the species. The spawning season for the Cherokee darter is longer than previously thought, or has the potential to be extended depending upon the weather conditions through the springtime period of reproductive potential. Spawning has been noted from middle March until early June with activity peaking in mid to late April. This period should be recognized as a critical time for reducing human activity in and around streams containing Cherokee darters.

The genetic analysis conducted across the Cherokee darter's range has confirmed assumptions of diversity within the species. This diversity consists of three monophyletic ESUs among which gene exchange is not evident. The variation between the ESUs is significant and is supported in part, by morphological variation of nuptial male coloration. While this morphological support is useful in distinguishing lower ESU males from males in the upper and middle ESUs, no color variation was evident between the middle and the upper ESUs. Individual males within these units are similar in nuptial coloration, suggesting that the variation lies within the genotype and not clearly in the phenotype. This is true as the results of the genetic analyses show that the two ESUs form a monophyletic group.

ESU divisions (boundaries) are not entirely resolved although breaks between these groups appear to fall close to significant geologic formations and/or watersheds in which Cherokee darters do not reside. The known extent of each ESU as depicted in Figure 2.7 show each to have a limited range within the range of the species. Genetic diversity coupled with limited distribution of individual ESUs highlights the importance of management on a fine scale and suggests increased complexity in dealing with the management of the species as a whole. For example, impacts become magnified when the range of a particular ESU is a third or less of the range of the species.

Finally, the apparent hybridization of *Etheostoma coosae* and *Etheostoma scotti* at Pettit Creek and the known occurrence of syntopy between these sister species represents one of the only documented cases of this situation in the subgenus *Ulocentra*. This occurrence may help in deciphering evolution of both species and of the subgenus.

References

Avise, J. C. 2000. “Phylogeography,” Harvard Univ. Press, London

Bauer, B. H., Etnier, D. A., and Burkhead, N.M. 1995. *Etheostoma* (*Ulocentra*) *scotti* (Osteichthyes: Percidae), a new darter from the Etowah River system in Georgia. *Bull. Alabama Mus. Nat. Hist.* **17:** 1-16

Bailey, R. M., and Etnier, D. A. (1988). Comments on the subgernera of darters (Percidae) with descriptions of two new species of *Etheostoma* (*Ulocentra*) from southeastern United States. Misc. Pub. Mus. Zool. Univ. Michigan **175**: 1-48

Burkhead, N. M. and H. L. Jelks. 2001. Effects of suspended sediment on the reproductive success of the tricolor shiner, a crevice-spawning minnow. *Transactions of the American Fisheries Society* **130:** 959-968.

Cabot, E. L., and Beckenbach, A.T. (1989). Simulataneous editing of multiple nucleic Acid and protein sequences with ESEE. *Comput. Appl. Biosci*. **5:** 233-234

Etnier, D. A. and Bailey, R. M. 1989. *Etheostoma* (*Ulocentra*) *flavum*, a new darter from The Tennessee and Cumberland drainages. *Occas. Pap. Michigan Mus. Zool*. **717:**1-24

Etnier, D. A., and Starnes, W. C. 1993. “The Fishes of Tennessee,” Univ of Tennessee Press, Knoxville, TN.

Faber, J. E., and Stepien, C. A. 1997. The utilized mitochondrial DNA control region sequences for analyzing phylogenetic relationships among populations, species, and genera of the Percidae. *In* “Molecular Systematics of Fishes” (T.D. Kocher and C.A. Stepien, Eds.). Academic Press, New York

Fraser, D. J., and Bernatchez, L. 2001. Adaptive evolutionary conservation: towards a Unified concept defining conservation units. *Molecular Ecology* **10:** 2741-2752

Gordon, N. D., T. A. McMahon and B. L. Finlayson. 1992. “Stream hydrology: an introduction for ecologists”. John Wiley and Sons, Chichester.

Huelsenbeck, J. P., and Ronquist, F. 2001. MRBAYES: Bayesian inference of Phylogeny. *Bioinformatics* **17:** 754-755

Hubbs, C. 1985. Darter Reproductive Seasons. Copeia **1:** 56-58

Keevin, T. M., Page, L. M., and Johnston, C.E. 1998. The spawning behavior of the saffron darter (*Etheostoma flavum*). *Trans. Ky. Acad*. Sci. **50:** 55-58

Kumar, S., Tamura, K., Jakobsen, I. B., Nei, M. 2001. MEGA2: Molecular Evolutionary Genetics Analysis software, Bioinformatics (submitted).

Kumar, S., Tamura, K., and Nei, M. 1993. “MEGA Molecular Evolutionary Genetic Analysis,” version 1.01. Pennsylvania State Univ., University Park, PA.

Moritz, C. 1994. Defining “Evolutionary Significant Units” for conservation. *Trends Ecol. Evol*. **9:**373-375

Moritz, C. 1995. Uses of molecular phylogenies for conservation. *Phil. Trans. R. Soc Lond*. B 349, 113-118

O’Neil, P. E. 1981. Life history of *Etheostoma coosae* (Pisces: Percidae) in Barbaree Creek, *Alabama. Tulane Stud. Zool. Bot*. **23:** 75-83

Porter, B. A., 1999. “Phylogeny, Evolution, and Biogeography of the Darter Subgenus *Ulocentra* (Genus *Etheostoma*, Family Percidae)” Ph.D. thesis, The Ohio State University, Columbus.

Porter, B.A., Cavender, T.M., and Fuerst, P.A. 2002. Molecular Phylogeny of the Snubnose Darters, Sugenuus *Ulocentra* (Genus Etheostoma, Family *Percidae*). *Molecular Phylogenetics and Evolution* **22:** 364-374.

Porterfield, J. C. 1997. Separation of spawning habitat in the sympatric snubnose darters *Etheostoma flavum* and *E. simoterum* (Teleostei, Percidae). *Trans. KY. Acad. Sci*. **58***:*4-8

Porterfield, J. C., 1998. Spawning behavior of snubnose darters (Percidae) in Natural and laboratory environments. *Environmental Biology of Fishes* **53:** 413-419

APPENDIX A

INFORMATION PERTINENT TO CHAPTER 2

Appendix A Table 1.1 Sample Origins

Institution#	Tree Number	Field Number	Specimens
E. scotti			
UGAMNH4141	CS 03-02	CS 03-02	4141.1, 4141.2
UGAMNH4142	NA	CS 02-60	4142.1
UGAMNH4143	eto027	DMW 01-26	4143.1
UGAMNH4144	NA	MMH 02-59	4144.3, 4144.4, 4144.6, 4144.7, 4144.11, 4144.12
UGAMNH4145	eto077	JRK 01-42	4145.1, 4145.2, 4145.4
UGAMNH4146	NA	JRK 02-94	4146.2-4146.5
UGAMNH4147	NA	CS 02-63	4147.5
UGAMNH4148	eto085	CS 02-75	4148.1
UGAMNH4149	eto134	JRK 01-43	4149.1, 4149.2
UGAMNH4150	eto159	JRK 02-88	4150.1*, 4150.2-4150.5 (*=hap1)
UGAMNH4151	eto206	CS 02-64	4151.6-8, 4151.10
OSUM85164	eto229 HAP2	NA	NA, NA, NA
OSUM85299	eto229 HAP1	NA	NA, NA, NA
UGAMNH4152	eto231	BAP 1070	4152.1
UGAMNH4153	eto675	JRK 02-33	4153.1
UGAMNH4154	eto695	JRK 02-89	4154.1, 4154.2, 4154.3
UGAMNH4155	NA	JRK 01-63	4155.1, 4155.2
UGAMNH4156	eto263	JRK 01-62	(4156.1, 4156.2, 4156.4**), 4156.3
UGAMNH4157	NA	CS 02-52	4157.1, 4157.2**
UGAMNH4158	NA	JRK 02-87	4158.1, 4158.3**
UGAMNH4159	eto330	JRK 01-67	4159.1
UGAMNH4160	eto331	JRK 01-65	4160.1, 4160.3-.5
UGAMNH4161	eto339	JCS 02-65	4161.1, 4161.4
UGAMNH4162	eto356	JCS 02-63	4162.1, 4162.3, 4162.4
UGAMNH4163	eto362	CS 02-62	4163.1, 4163.4, 4163.10, 4163.11
UGAMNH4164	eto370	JRK 01-102	4164.1
UGAMNH4165	NA	BAP1057	4165.1
UGAMNH4166	NA	CS 02-59	4166.6
UGAMNH4167	eto401	CS 02-66	4167.2
UGAMNH4168	eto420	JRK 02-85	4168.1, 4168.2
UGAMNH4170	eto482	CS 02-69	4170.1, 4170.2
UGAMNH4171	eto488	BJF 00-19	4171.1
UGAMNH4172	eto535	DMW 01-11	4172.1
UGAMNH4173	NA	DMW 01-15	4173.1-.3
UGAMNH4174	eto536	MMH 01-27	4174.1
UGAMNH4175	eto550	DMW 01-26	4175.1
UGAMNH4176	NA	MMH 02-59	4176.3-.7, 4176.11, 4176.12
UGAMNH4177	eto597	JRK 01-33	4177.1
UGAMNH4178	eto599	JRK 01-34	4178.1
UGAMNH4179	eto608	JRK 01-38	4179.2, 4179.3

Table 1.1 Continued

Institution#	**Tree Number**	**Field Number**	**Specimens**
E. scotti			
UGAMNH4180	eto618	JRK 01-42	4180.1, 4180.2, 4180.4
UGAMNH4181	NA	JRK 02-94	4181.2-.5
UGAMNH4182	NA	CS 02-63	4182.5
UGAMNH4183	eto692	CS 02-55	4183.1, 4183.13
UGAMNH4184	NA	JRK 01-44	4184.2
UGAMNH4185	eto645	JRK 01-103	4185.2-.10
UGAMNH4186	NA	BAP 1104	4186.1-.8
UGAMNH4187	eto659	BAP 1035	4187.1-.3, 4187.5-.21
E. coosae			
UGAMNH4169	BJF01- 50 hap1, hap2	BJF 01-50	(4169.1, 4169.3, 4169.5) 4169.6 (*=hap1)
UGAMN4188	BJF01- 57	BJF01- 57	4188.1-.3
UGAMN4189	BAP 1078	BAP 1078	4189.1, 4189.2
UGAMN4190	BAP 1036 hap1, hap2	BAP 1036	4190.1, 4190.2, 4190.4-4190.6
UGAMN4191	eto659	BAP 1035	4191.4, 4191.13
UGAMN4192	BAP 1116	BAP 1116	4192.1
UGAMN4193	BAP 1081	BAP 1081	4193.1, 4193.2
UGAMN4194	BAP 1082	BAP 1082	4194.1, 4194.2
UGAMN4195	BAP 1080	BAP 1080	4195.1, 4195.2
NA	coosae(28)*	NA	NA
OSUM 95201	OSUM 95201*	NA	NA
E. duryi			
OSUM 85726*	OSUM 85726	NA	NA
OSUM 85286*	OSUM 85286	NA	NA
OSUM 95196*	OSUM 95196	NA	NA
E. flavum			
OSUM 95128*	OSUM 95128	Logan, KY	NA
OSUM 95124*	OSUM 95124	Logan, KY	NA
OSUM 77772*	OSUM 77772	Hickman, TN	NA

Appendix A Table 1.1 Continued

Institution#	Tissue	Stream Name	Corresponding Haplotype
E. scotti			
UGAMNH4141	fin/muscle	Stamp Creek	4141
UGAMNH4142	muscle	Stamp Creek	4141
UGAMNH4143	muscle	Hickory Log Creek	4143
UGAMNH4144	muscle	Hickory Log Creek	4143
UGAMNH4145	muscle	Smithwick Creek	4145
UGAMNH4146	muscle	Smithwick Creek	4145
UGAMNH4147	fin	Smithwick Creek	4145
UGAMNH4148	fin	Etowah River	4148
UGAMNH4149	muscle	Canton Creek	4149
UGAMNH4150	muscle	Burt Creek	4150.1, 4150.2-.5
UGAMNH4151	fin/muscle	McCanless Creek	4151
OSUM85164	muscle	Butler Creek	85164
OSUM85299	muscle	Butler Creek	85299
UGAMNH4152	fin	Proctor Creek	4152
UGAMNH4153	muscle	Proctor Creek	4153
UGAMNH4154	muscle	Palmer Creek	4154
UGAMNH4155	muscle	Palmer Creek	4154
UGAMNH4156	muscle	Shoal Creek	4156, 4150.2-.5
UGAMNH4157	fin	Shoal Creek	4156, 4150.2-.5
UGAMNH4158	muscle	Shoal Creek	4156, 4150.2-.5
UGAMNH4159	muscle	Camp Creek	4159
UGAMNH4160	muscle	Trib to Etowah River	4160
UGAMNH4161	muscle	Westbrook Creek	4161
UGAMNH4162	muscle	Norton Creek	4174
UGAMNH4163	fin/muscle	Fourmile Creek	4163
UGAMNH4164	muscle	Raccoon Creek	4164
UGAMNH4165	muscle	Raccoon Creek	4164
UGAMNH4166	fin	Raccoon Creek	4164
UGAMNH4167	fin	Richland Creek	4167
UGAMNH4168	muscle	Padgett Creek	4168
UGAMNH4170	fin	Sharp Mountain Creek	4168
UGAMNH4171	muscle	Trib. to Little Pumpkinvine Crk.	4171
UGAMNH4172	muscle	Puckett Creek	4172
UGAMNH4173	muscle	Puckett Creek	4172
UGAMNH4174	muscle	Long Swamp Creek	4174
UGAMNH4175	muscle	Trib. to Hickory Log Creek	4175
UGAMNH4176	muscle	Hickory Log Creek	4175
UGAMNH4177	muscle	Trib to Etowah River	4177
UGAMNH4178	muscle	Trib to Little Shoal Creek	4178
UGAMNH4179	muscle	McCory Creek	4179
UGAMNH4180	muscle	Smithwick Creek	4180
UGAMNH4181	muscle	Smithwick Creek	4180
UGAMNH4182	fin	Smithwick Creek	4180

Appendix A Table 1.1 Continued

Institution#	Tissue	Stream Name	Corresponding Haplotype
E. scotti			
UGAMNH4183	fin	Allatoona Creek	4183
UGAMNH4184	muscle	Allatoona Creek	4183
UGAMNH4185	muscle	Picketts Mill Creek	4185
UGAMNH4186	muscle	Picketts Mill Creek	4185
UGAMNH4187	muscle	Pettit Creek	4187
E. coosae			
UGAMNH4169	muscle	Conasauga River	4169.1, 4169.6
UGAMN4188	muscle	Conasauga River	4188
UGAMN4189	muscle	Robbins Creek	4189
UGAMN4190	muscle	Two Run Creek	4190.4, 4190.6
UGAMN4191	muscle	Pettit Creek	4191
UGAMN4192	muscle	Conasauga River	4192
UGAMN4193	muscle	Spring Creek	4193
UGAMN4194	muscle	Euharlee Creek	4194
UGAMN4195	muscle	Silver Creek	4195
NA	muscle	Conasauga River	coosae(28)
OSUM 95201	muscle	Minnewauga Creek	OSUM 95201
E. duryi			
OSUM 85726*	muscle	McWilliams Creek	OSUM 85726
OSUM 85286*	muscle	Running Water Cr.	OSUM 85286
OSUM 95196*	muscle	Cane Creek	OSUM 95196
E. flavum			
OSUM 95128*	muscle	Pleasant Run	OSUM 95128
OSUM 95124*	muscle	Pleasant Run	OSUM 95124
OSUM 77772*	muscle	Defeated Camp Cr.	OSUM 77772

*From Porter et al.(2002)

Appendix A Table 1.2 Microlocality Data

Collection Field #s	Site	County	Tissue	Stream Name
CS 03-02/CS 02-60	CS 03-20	Bartow	fin/muscle	Stamp Creek
MMH 02-59	eto027	Cherokee	muscle	Hickory Log Creek
CS 02-63	eto077	Cherokee	fin	Smithwick Creek
CS 02-75	eto085	Cherokee	fin	Etowah River
JRK 01-43	eto134	Cherokee	muscle	Canton Creek
JRK 02-88	eto159	Dawson	muscle	Burt Creek
CS 02-64	eto206	Cherokee	fin/muscle	McCanless Creek
OSUM 85164, 85299	eto229	Cobb	muscle	Butler Creek
JRK 02-33	eto231	Cobb	fin	Proctor Creek
JRK 01-63	eto253	Dawson	muscle	Palmer Creek
JRK 01-62/JRK 02-87	eto263	Dawson	muscle	Shoal Creek
JRK 01-67	eto330	Lumpkin	muscle	Camp Creek
JRK 01-65	eto331	Lumpkin	muscle	Trib to Etowah River
JCS 02-65	eto339	Paulding	muscle	Westbrook Creek
JCS 02-63	eto356	Pickens	muscle	Norton Creek
CS 02-62	eto362	Pickens	fin/muscle	Fourmile Creek
CS 02-59/BAP 1057	eto370	Bartow	fin	Raccoon Creek
CS 02-66	eto401	Bartow	fin	Richland Creek
JRK 02-85	eto420	Pickens	muscle	Padgett Creek
CS 02-52	eto432	Dawson	fin/muscle	Shoal Creek
JRK 01-102	eto461	Paulding	muscle	Raccoon Creek
CS 02-69	eto482	Pickens	fin	Sharp Mountain Creek
BJF 00-19	eto488	Paulding	muscle	Trib. to Little Pumpkinvine Crk.
DMW 01-11	eto535	Cherokee	muscle	Puckett Creek
MMH 01-27	eto536	Cherokee	muscle	Long Swamp Creek
DMW 01-15	eto539	Cherokee	muscle	Puckett Creek
DMW 01-26	eto550	Cherokee	muscle	Trib. to Hickory Log Creek
JRK 01-33	eto597	Cherokee	muscle	Trib to Etowah River
JRK 01-34	eto599	Cherokee	muscle	Trib to Little Shoal Creek
JRK 01-38	eto608	Cherokee	muscle	McCory Creek
JRK 01-42 JRK 02-94	eto618	Cherokee	muscle	Smithwick Creek
JRK 01-44	eto621	Cobb	fin	Allatoona Creek
JRK 01-103/BAP 1104	eto645	Paulding	muscle	Picketts Mill Creek
BAP 1035	eto659	Bartow	muscle	Pettit Creek
BAP 1070	eto675	Cobb	muscle	Proctor Creek
CS 02-55	eto692	Cobb	fin	Allatoona Creek
JRK 02-89	eto695	Dawson	muscle	Palmer Creek

Appendix A Table 1.2 Microlocality Data Continued

Collection Field #s	Microlocality Data
CS 03-02/CS 02-60	at State Rte 20, 0.6 air miles E of Corbin, GA
MMH 02-59	at Co. Rte 766 (Fate Conn Rd.) 4.2 air miles NNE of Canton, GA
CS 02-63	0.55 air miles east of Creek crossing at County Route 154 4.4 air miles SE of Ball Ground, GA
CS 02-75	at County Route 782 (East Cherokee Drive) 2.9 air miles SSW of Ball Ground, GA
JRK 01-43	at County Route 288 (Scott Road) 3.0 air miles ESE of Canton, GA
JRK 02-88	Co. Rte 224 (Shoal Creek Rd.) 100m upstrm from the confl. with Shoal Cr. 0.9 air m. NNW of Dawsonville, GA
CS 02-64	at State Route 108 at the junction with County Route 30 (Darby Road) 1.75 air miles S of Waleska, GA
OSUM 85164, 85299	at County Route 102 (Mack Dobbs Road) 1.7 air miles WSW of Kennesaw, GA
JRK 02-33	at Main Street (Old Highway 41) in Acworth 1.25 air miles SW of Acworth, GA
JRK 01-63	at County Route 76 (Stower's Road / Etowah River Road) 3.7 air miles SE of Dawsonville, GA
JRK 01-62/JRK 02-87	at County Route 1 (Howser Mill Road) 1.2 air miles NW of Dawsonville, GA
JRK 01-67	at County Route 1 (Ben Higgins Road) 3.8 air miles SSW of Dahlonega, GA
JRK 01-65	Unnamed tributary of Etowah R. at County Route 109 (Pink Williams Road) 6.0 air miles SW of Dahlonega, GA
JCS 02-65	at County Route 72 (Cedarcrest Road) 4.1 air miles WSW of Acworth, GA
JCS 02-63	at County Route 6 (Old Burnt Mountain Road) 1.3 air miles NE of Jasper, GA
CS 02-62	at County Route 52 (Four Mile Church Road) 2.8 air miles NE of Nelson, GA
CS 02-59/BAP 1057	at State Route 113 (Main Street) 3.4 air miles SE of Euharlee, GA city center
CS 02-66	0.6 rm upstrm from State Rte. 113 off Co. Rte 23 (M Taylor Farm/Lucas Rd) 5.8 air miles SW of Cartersville, GA
JRK 02-85	Padgett Creek at County Route 307 (Mineral Springs Road), 2.7 air miles SW of Jasper, GA city center.
CS 02-52	at County Route 224 (Shoal Creek Road) 1.0 air miles NW of Dawsonville, GA
JRK 01-102	at County Route 105 (High Shoals Road) 3.6 air miles NE of Brasswell, GA
CS 02-69	at State Route 108 3.5 air miles NW of Nelson, GA
BJF 00-19	0.7 air m. SSW of the jct. of Co. Rte 473 (Cedar Crest Dr.)/ Co. Rte 84 (Graves Rd.) 6.3 air m. SW of Acworth
DMW 01-11	0.6 river miles upstream of County Route 24 (Old Shoal Creek Road), GA
MMH 01-27	100m downstream of trib. draining Blue Circle Gravel quarry 2.1 air miles NE of Ball Ground, GA
DMW 01-15	0.2 river miles upstream of County Route 24 (Old Shoal Creek Trail) 1.3 air miles NW of Canton, GA
DMW 01-26	2nd tributary from west end of County Rte 73 (Worley Rd.) 5.0 air m. NNE of Canton 1.7 air m. NNW Canton, GA
JRK 01-33	adja. and W of County Route 10 (Brooks Dr.) 1.1 rm upstr. of Lake Allatoona 2.6 air miles SW of Canton, GA
JRK 01-34	at State Route 108 7.4 air miles WSW Canton, GA
JRK 01-38	at State Route 108 2.5 air miles NE of Waleska, GA
JRK 01-42 JRK 02-94	1.4 river miles dwnstrm of Co. Rte 204 (Julius Bridge Rd.) 4.4 air m. SE of Ball Ground, GA
JRK 01-44	at County Route 53 (Stilesboro Lane) 10.6 air miles NW Dallas, GA
JRK 01-103/BAP 1104	0.1 river miles downstream of Co. Rte. 710 (Harmony Grove Church Road) 7.0 air miles SW of Acworth, GA
BAP 1035	at Co. Rd 532 (Industrial Park Road) 4.7 air miles NNE of Cartersville, GA city center.
BAP 1070	at Legacy Park Subdivision 2.2 air miles NNW of Kennesaw 2.8 air miles ESE of Acworth, GA
CS 02-55	at County Route 822 (Burnt Hickory Road) 5.1 air miles SW of Kennesaw, GA
JRK 02-89	200 m downstream of Co. Rte 76 (Stower's Rd. / Etowah River Rd.) 3.7 air miles SE of Dawsonville, GA

Appendix A Table 1.3 Haplotype, base-pair comparisons pertaining to Chapter 2

	1	1111111112	2222222223	3333333334	4444444445
	1234567890	1234567890	1234567890	1234567890	1234567890
ETO159H2	TAA-TACACG	TATGTATTAA	CACCATACAT	CTATATTAAC	CATATAAGGG
ETO263	...-......	....	....	....	....
ETO159H1	...-......	....	....	....	....
ETO331	...-......	....	....	T.........	....
ETO695	...-......	....	....	T.........	....
ETO330	...-......	....	....	T.........	....
ETO618	A..-.a..c.	....	.a........	T.........	....
ETO206	...-......	....	....	T.........	....
ETO536	...-......	....	.....?....	T.........	....
ETO134	...-......	....	....	T.........	....
ETO608	...-......	....	....	T.........	....
ETO535	...-......	....	....	T.........	....
ETO085	...-......	....	....	T.........	....
ETO550	...-......	....	....	T.........	....
ETO362	...-......	....	....	T.........	....
ETO599	...-......	....	....	T.........	....
ETO597	...-......	....	....	T.........	....
ETO482	...-......	....	....	T.........	....
ETO420	...-......	....	....	T.........	....
ETO659	A..-......	....	....	T.........	....
ETO401	A..-......	....	....	T.........	....
ETO461	A..-......	....	....	T.........	....
CS 03-02	A..-......	....	....	T.........	....
ETO675	A..-......	....	....	T.........	....
ETO231	A..-......	....	....	T.........	....
ETO229h1	A..-......	....	....	T.........	....
ETO692	A..-......	....	....	T.........	....
ETO339	A..-......	....	....	T.........	....
ETO645	A..-......	....	....	T.........	....
ETO488	A..-......	....	.....?....	T.........	....
ETO229h2	A..-......	....	....	T.........	....
OSUM95201	A..-......	....	....	T.........	....
coosa28	A..-......	....	....	T.........	....
JRK02-31	A..-......	....	....	T.........	....
BAP1080	A..-......	....	....	T.........	....
BAP1081	A..-......	....	....	T.........	....
BAP1082	A..-......	....	....	T.........	....
BAP1116	A..-......	....	....	T.........	....
BAP1078	A..-......	....	....	T.........	....
BJF0150h1	A..-......	....	....	T.........	....
BJF0150h2	A..-......	....	....	T.........	....
BJF01-57	A..-......	....	....	T.........	....
BAP1036h1	A..-......	....	....	T.........	....
BAP1036h2	A..-......	....	....	T.........	....
ETO659	A..-......	....	....	T.........	....
OSUM85726	...-......	........T.	....	T.........	....
OSUM85286	...-......	........T.	....	T.........	....
OSUM95196	A..-......	........T.	....	T.........	.....C....
OSUM95124	A..-......	........T.	....	T.........	....
OSUM77772	A..-......	........T.	....	T.........	....
OSUM95128	A..-......	........T.	....	T.........	....

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ETO159H2 GCATTCAAGT ACATATATGT TTAATCAACA TATCTAGGAT TAACACATTC
ETO263   .......... .......... .......... .......... ..........
ETO159H1 .......... .......... .......... .......... ..........
ETO331   .......... .......... .......... .......... ..........
ETO695   .......... .......... .......... .......... ..........
ETO330   .......... .......... .......... .......... ..........
ETO618   .......... .......... .......... C......A.. ..........
ETO206   .......... .......... .......... .......T.. ..........
ETO536   .......... .......... .......... .......T.. ..........
ETO134   .......... .......... .......... .......T.. ..........
ETO608   .......... .......... .......... .......T.. ..........
ETO535   .......... .......... .......... .......T.. ..........
ETO085   .......... .......... .......... .......T.. ..........
ETO550   .......... .......... ........?. .......T.. ..........
ETO362   .......... .......... .......... .......T.. ..........
ETO599   .......... .......... .......... .......T.. ..........
ETO597   .......... .......... .......... .......T.. ..........
ETO482   .......... .......... .......... .......T.. ..........
ETO420   .......... .......... .......... .......T.. ..........
ETO659   .........G .......... .......... .......... ..T.......
ETO401   .........G .......... .......... .......... ..T.......
ETO461   .........G .......... .......... .......... ..........
CS 03-02 .........G .......... .......... .......... ..........
ETO675   .........G .......... .......... .......... ..........
ETO231   .........G .......... .......... .......... ..........
ETO229h1 .........G .......... .......... .......... ..........
ETO692   .........G .......... .......... .......... ..........
ETO339   .........G .......... .......... .......... ..........
ETO645   .........G .......... ......??.. ...?...... ..........
ETO488   .........G .......... .......... .......... ..........
ETO229h2 .........G .......... .......... .......... ..........
OSUM95201.......... .......... .......... .......... .T........
coosa28  .......... .......... .......... .......... .T........
JRK02-31 .......... .......... .......... .......... .T........
BAP1080  .......... .......... .......... .......... .T........
BAP1081  .......... .......... .......... .......... .T........
BAP1082  .......... .......... .......... .......... .T........
BAP1116  .......... .......... .......... .......... .T........
BAP1078  .......... .......... .......... .......... .T........
BJF0150h1.......... .......... .......... .......... .T........
BJF0150h2.......... .......... .......... .......... .T........
BJF01-57 .......... .......... .......... .......... .T........
BAP1036h1.......... .......... .......... .......... .T........
BAP1036h2.......... .......... .......... .......... .T........
ETO659   .......... ....G..... .......... .......... .T........
OSUM85726.T........ .......... .......... .......... ..C.......
OSUM85286.T........ .......... .......... .......... ..C.......
OSUM95196.........G T......... ..T....... .......... .TC.......
OSUM95124.......... .......... .......... .......... .TC.......
OSUM77772.......... ....TC.... .......... .......... ..C.......
OSUM95128.......... .......... .......... .......... .TC.......
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ETO159H2  ATATATCACC TATACACTAA GGGTTACATA AAGCATCTAT AGATTTACTT
ETO263    .......... .......... .......... .......... ..........
ETO159H1  .......... .......... .......... .......... ..........
ETO331    .......... A......... .......... .......... ..........
ETO695    .......... A......... .......... .......... ..........
ETO330    .......... A......... .......... .......... ..........
ETO618    .......... A...T..... .......... .......... ..T.......
ETO206    .......... A......... .......... .......... ..T.......
ETO536    .......... A......... .......... .......... ..T.......
ETO134    .......... A......... .......... .......... ..T.......
ETO608    .......... A......... .......... .......... ..T.......
ETO535    .......... A......... .......... .......... ..T.......
ETO085    .......... A......... .......... .......... ..T.......
ETO550    .......... A......... .......... .......... ..T.......
ETO362    .......... A......... .......... .......... ..T.......
ETO599    .......... A......... .......... .......... ..T.......
ETO597    .......... A......... .......... .......... ..T.......
ETO482    .......... A......... .......... .......... ..T.......
ETO420    .......... A......... .......... .......... ..T.......
ETO659    .......... A......... .......... .......... ..G....T..
ETO401    .......... A......... .......... .......... ..G....T..
ETO461    .......... A......... .......... .......... ..G....T..
CS 03-02  .......... A......... .......... .......... ..G....T..
ETO675    .......... A......... .......... .......... ..G.......
ETO231    .......... A......... .......... .......... ..G.......
ETO229h1  .......... A......... .......... .......... ..G.......
ETO692    .......... A......... .......... .......... ..........
ETO339    .......... A......... .......... .......... .......T..
ETO645    .......... A......... .......... .......... .......T..
ETO488    .......... A......... .......... .......... .......T..
ETO229h2  .......... A......... .......... .......... .......T..
OSUM95201.......... AT........ .......... ......A... .......TC.
coosa28   .......... AT........ .......... ......A... .......TC.
JRK02-31  .......... AT........ .......... ......A... .......TC.
BAP1080   .......... AT........ .......... ......A... .......TC.
BAP1081   .......... AT........ .......... ......A... .......TC.
BAP1082   .......... AT........ .......... ......A... ..G....TC.
BAP1116   .......... AT........ .......... ......A... .......TC.
BAP1078   .......... AT........ .......... ......A... ..G....TC.
BJF0150h1.......... AT........ .......... ......A... .......TC.
BJF0150h2.......... AT........ .......... ......A... .......TC.
BJF01-57  .......... AT........ .......... ......A... .......TC.
BAP1036h1.......... AT........ .......... ......A... .......TC.
BAP1036h2.......... AT........ .......... ......A... ..G....TC.
ETO659    .......... AT........ .......... ......A... ..G....TC.
OSUM85726.......... AT..T..... .......... .......... ..G....TC.
OSUM85286.......... AT..T..... .......... .......... ..G....TC.
OSUM95196.....A.... AT........ .......... .......... ..G....TC.
OSUM95124.......... AT.G...... .......... ......G... ..G....TC.
OSUM77772.......... AT..T..... .......... .......... .....C.TC.
OSUM95128.......... AT........ .......... ......G... ..G....TC.
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ETO159H2  AACAA-TAT- ATTAAATCTT GGACAGGCGA CATTTAAGAC CGAACACAAA
ETO263    .....-...- .......... .......... .......... ..........
ETO159H1  .....-...- .......... .......... .......... ..........
ETO331    .....-...- .......... .......... .......... ........T.
ETO695    .....-...- .......... .......... .......... ........T.
ETO330    .....-...- .......... .......... .......... ........T.
ETO618    .....-...- .......... .....A.... .......... ........T.
ETO206    .....-...- .......... .....A.... .......... ........T.
ETO536    .....-...- .......... .....A.... .......... ........T.
ETO134    .....-...- .......... .....A.... .......... ........T.
ETO608    .....-...- .......... .....A.... .......... ........T.
ETO535    .....-...- .......... .....A.... .......... ........T.
ETO085    .....-...- .......... .....A.... .......... ........T.
ETO550    .....-...- .......... .....A.... .......... ........T.
ETO362    .....-...- .......... .....A.... .......... ........T.
ETO599    .....-...- .......... .....A.... .......... ........T.
ETO597    .....-...- .......... .....A.... .......... ........T.
ETO482    .....-...- .......... ...T.A.... .......... ........T.
ETO420    .....-...- .......... ...T.A.... .......... ........T.
ETO659    .....-...- ........C. .......... .......... ........T.
ETO401    .....-...- ........C. .......... .......... ........T.
ETO461    .....-...- ........C. .......... .......... ........T.
CS 03-02  .....-...- .tt.....C. .......... .......... ........T.
ETO675    .....-...- ........C. .......... .......... ........T.
ETO231    .....-...- ........C. .......... .......... ........T.
ETO229h1  .....-...- ........C. .......... .......... ........T.
ETO692    .....-...- ........C. .......... .......... ........T.
ETO339    .....-...- ........C. .......... .......... ........T.
ETO645    .....-...- ........C. .......... .......... ........T.
ETO488    .....-...- ........C. .......... ........G. ........T.
ETO229h2  .....-...- ........C. .......... .......... ........T.
OSUM95201.....-...- .......... .......... A......... ........T.
coosa28   .....-...- .......... .......... A......... ........T.
JRK02-31  .....-...- .......... .......... A......... ........T.
BAP1080   .....-...- .......... .......... .......... ........T.
BAP1081   .....-...- .......... .......... .......... ........T.
BAP1082   .....-...- .......... .......... .......... ........T.
BAP1116   .....-...- .......... .......... A......... ........T.
BAP1078   .....-...- .......... .......... A......... ........T.
BJF0150h1.....-...- .......... .......... A......... ........T.
BJF0150h2.....-...- .......... .......... A......... ........T.
BJF01-57  .....-...- .......... .......... A......... ........T.
BAP1036h1.....-...- .......... .......... .......... ........T.
BAP1036h2.....-...- .......... .......... .......... ........T.
ETO659    .....-...- .......... .......... .......... ........T.
OSUM85726....--...T .......... .......... .......... ........T.
OSUM85286....--...T .......... .......... .......... ........T.
OSUM95196....--.T.T .......... .......... .......... ........T.
OSUM95124....--...T .......... .......... .......... ........T.
OSUM77772....--...T .......... .......... .......... ........T.
OSUM95128....--...T .......... .......... .......... ........T.
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ETO159H2  TACTCATAAG TTAAGTTATA CCTTTACCCA ACATCTCGTC ATACCTCAAA
ETO263    .......... .......... .......... .......... ..........
ETO159H1  .......... .......... .......... .......... ..........
ETO331    .......... .......... .......... .......... ..........
ETO695    .......... .......... .......... .......... ..........
ETO330    .......... .......... .......... .......... ..........
ETO618    .C........ .......... .......... .......... ..........
ETO206    .C........ .......... .......... .......... ..........
ETO536    .C........ .......... .......... .......... ....?..?..
ETO134    .C........ .......... .......... .......... ..........
ETO608    .C........ .......... .......... .......... ..........
ETO535    .C........ .......... .......... .......... ..........
ETO085    .C........ .......... .......... .......... ..........
ETO550    .C........ .......... .......... .......... ..........
ETO362    .C........ .......... .......... .......... ..........
ETO599    .C........ .......... .......... .......... .........?
ETO597    .C........ .......... .......... .......... ..........
ETO482    .C........ .......... .......... .......... ..........
ETO420    .C........ .......... .......... .......... ..........
ETO659    .C........ .......... .......... .......... ...T......
ETO401    .C........ .......... .......... .......... ...T......
ETO461    .C........ .......... .......... .......... ...T......
CS 03-02  .C........ .......... .......... a.a....?.. ...T...???
ETO675    .C........ .......... .......... .......... ...T......
ETO231    .C........ .......... .......... .......... ...T......
ETO229h1  .C........ .......... .......... .......... ...T......
ETO692    .C........ .......... .......... .......... ...T....G.
ETO339    .C........ .......... .......... .......... ..........
ETO645    .C........ .......... .......... .......... ..........
ETO488    .C........ .......... .......... ac..c..... ..........
ETO229h2  .C........ .......... .......... .......... ..........
OSUM95201.......... .......... .......... .......... ..........
coosa28   .......... .......... .......... .......... ..........
JRK02-31  .......... .......... .......... .......... ..........
BAP1080   .......... .......... .......... .......... ..........
BAP1081   .......... .......... .......... .......... ..........
BAP1082   .......... .......... .......... .......... ..........
BAP1116   .......... .......... .......... .......... ..........
BAP1078   .......... .......... .......... .......... ..........
BJF0150h1.......... .......... .......... .......... ..........
BJF0150h2.......... .......... .......... .......... ..........
BJF01-57  .......... .......... .......... .......... ..........
BAP1036h1.......... .......... .......... .......... ..........
BAP1036h2.......... .......... .......... .......... ..........
ETO659    .......... .......... .......... .......... ..........
OSUM85726.C........ .......... .......... .......... ..........
OSUM85286.C........ .......... .......... .......... ..........
OSUM95196.C........ .......... .......... .......... ..........
OSUM95124.C........ .......... .......... ........C. .C........
OSUM77772.C........ .......... .......... .......... ..........
OSUM95128.C........ .......... .......... ........C. .C........
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ETO159H2  ATCTTAATGT AGTAAGAGCC TACCATCAGT TGATTTCTTA ATGTCAACGG
ETO263    .......... .......... .......... .......... ..........
ETO159H1  .......... .......... .......... .......... ..........
ETO331    .......... .......... .......... ..?......? ..........
ETO695    .......... .......... .......... .......... ..........
ETO330    .......... .......... .......... .......... ..........
ETO618    .......... .......... .......... .......t.. ....T.....
ETO206    .......... .......... .......... .......... ....T.....
ETO536    ?......... .......... .......... .......... ....T.....
ETO134    .......... .......... .......... .......... ....T.....
ETO608    .......... .......... .......... .......... ....T.....
ETO535    .......... .......... .......... .......... ....T.....
ETO085    .......... .......... .......... .......... ....T.....
ETO550    .......... .......... .a........ .......... ....T.....
ETO362    .......... .......... .......... .......... ....T.....
ETO599    .......... .......... .......... .......... ....T.....
ETO597    .......... .......... .......... .......... ....T.....
ETO482    .......... .......... .......... .......... ....T.....
ETO420    .......... .......... .......... .......... ....T.....
ETO659    .......... .......... .......... .......... ..........
ETO401    .......... .......... .......... .......... ......a...
ETO461    .......... .......... .....?.... .......??? ..........
CS 03-02  .t........ .......... .......... .......... ..........
ETO675    .......... .......... .......... .......... ..........
ETO231    .......... .......... .......... .......... ..........
ETO229h1  .......... .......... .......... .......... ..........
ETO692    .......... .......... .......... .........a ......a...
ETO339    .......... .......... .......... .......... ..........
ETO645    .......... .......... .......... .......... ..........
ETO488    .......... .......... .......... .......... ..........
ETO229h2  .......... .......... .......... .......... ..........
OSUM95201.......... .......... .......... .......... ...C......
coosa28   .......... .......... .......... .......... ...C......
JRK02-31  .......... .......... .......... .......... ...C......
BAP1080   .......... .......... .......... .......... ...C......
BAP1081   .......... .......... .......... .......... ...C......
BAP1082   .......... .......... .......... .......... ...C......
BAP1116   .......... .......... .......... .......... ...C......
BAP1078   .......... .......... .......... .......... ...C......
BJF0150h1.......... .......... .......... .......... ...C......
BJF0150h2.......... .......... .......... .......... ...C......
BJF01-57  .......... .......... .......... .......... ...C......
BAP1036h1.......... .......... .......... .......... ...C......
BAP1036h2.......... .......... .......... .......... ...C......
ETO659    .......... .......... .......... .......... ...C......
OSUM85726.......... .......... .......... .......... ...CT.....
OSUM85286.......... .......... .......... .......... ...CT.....
OSUM95196.......... .......... .......... .......... ...C......
OSUM95124.......... .......... .......... .......... ...C......
OSUM77772.......... .......... .......... .......... ...C......
OSUM95128.......... .......... .......... .......... ...C......
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ETO159H1 .......... .......... .......... .......... ..........
ETO331   .......... .......... .......... .......... ..........
ETO695   .......... .......... .......... .......... ..........
ETO330   .......... .......... .......... .......... ..........
ETO618   .......... .......... .......... .......... ..........
ETO206   .......... ......a... .......... .......... ..........
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ETO134   .......... .......... .......... .......... ..........
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ETO535   .......... .......... .......... .......... ..........
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ETO420   .......... .......... .......... .......... ..........
ETO659   .......... .......... .......... ...CG..... ..........
ETO401   .......... .......... .......... ...CG..... ..........
ETO461   .......... .......... .......... ...CG..... ..........
CS 03-02 .......... .....g??.. ......t... ...CG..... ..........
ETO675   .......... .......... .......... ...CG..... ..........
ETO231   .......... .......... .......... ...CG..... ..........
ETO229h1 .......... .......... .......... ...CG..... ..........
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ETO488   .......... .......... .......... ...CG..... ..........
ETO229h2 .......... .......... .......... ...CG..... ..........
OSUM95201.......... .......... .......... .......... ..........
coosa28  .......... .......... .......... .......... ..........
JRK02-31 .......... .......... .......... .......... ..........
BAP1080  .......... .......... .......... .......... ..........
BAP1081  .......... .......... .......... .......... ..........
BAP1082  .......... .......... .......... .......... ..........
BAP1116  .......... .......... .......... .......... ..........
BAP1078  .......... .......... .......... .......... ..........
BJF0150h1.......... ......a... .......... .......... ..........
BJF0150h2.......... .......... .......... .......... ..........
BJF01-57 .......... .......... .......... .......... ..........
BAP1036h1.......... .......... .......... .......... ..........
BAP1036h2.......... .......... .......... .......... ..........
ETO659   .......... .......... .......... .......... ..........
OSUM85726.......... .......... .......... .......... ..........
OSUM85286.......... .......... .......... .......... ..........
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OSUM95128.......... .......... .......... .......... ..........
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ETO695   .......... .......... .......... .......... ..........
ETO330   .......... .......... .......... .......... ..........
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ETO206   .......... .......... .......... .......... ..........
ETO536   .......... .......... .......... .......... ..........
ETO134   .......... .......... .......... .......... ..........
ETO608   .......... .......... .......... .......... ..........
ETO535   .......... .......... .......... .......... ..........
ETO085   .......... .......... .......... .......... ..........
ETO550   .......... .......... .......... .......... ..........
ETO362   .......... .......... .......... .......... ..........
ETO599   .......... ...t...... .......... .......... ..........
ETO597   .......... .......... .......... .......... ..........
ETO482   .......... .......... .......... .......... ..........
ETO420   .......... .......... .......... .......... ..........
ETO659   .......... .......... .......... .......... ..........
ETO401   .......... .......... .......... .......... ..........
ETO461   .......... .......... .......... .......... ..........
CS 03-02 .......a.. .......... .......... .......... ..........
ETO675   .......... .......... .......... .......... ..........
ETO231   .......... .......... .......... .......... ..........
ETO229h1 .......... .......... .......... .......... ..........
ETO692   .......... .......... .......... .......... ..........
ETO339   .......... .......... .......... .......... ..........
ETO645   .......... .......... .......... .......... ..........
ETO488   .......... .......... .......... .......... ..........
ETO229h2 .......... .......... .......... .......... ..........
OSUM95201.......... .......... .......... .A........ ..........
coosa28  .......... .......... .......... .A........ ..........
JRK02-31 .......... .......... .......... .A........ ..........
BAP1080  .........t .......... .......... .A........ ..........
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BAP1082  .......... .......... .......... .A........ ..........
BAP1116  .......... .......... .......... .A........ ..........
BAP1078  .......... .......... .......... .A........ ..........
BJF0150h1.......... .......... .......... .A........ ..........
BJF0150h2.......... .......... .......... .A........ ..........
BJF01-57 .......... .......... .......... .A........ ..........
BAP1036h1.......... .......... .......... .A........ ..........
BAP1036h2.......... .......... .......... .A........ ..........
ETO659   .......... .......... .......... .A........ ..........
OSUM85726.......... .......... .......... .......... ..........
OSUM85286.......... .......... .......... .A........ .........C
OSUM95196.......... .......... .......... .......... ..........
OSUM95124.......... .......... .......... .C........ ..........
OSUM77772.......... .......... .......... .......... ..........
OSUM95128.......... .......... .......... .C........ ..........
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ETO159H2  CACTTTCATC GACGCTGACA TAAGTTAATG GTGGAATACA TACTCCTCGT
ETO263    .......... .......... .......... .......... ..........
ETO159H1  .......... .......... .......... .......... ..........
ETO331    .......... .......... .......... .......... ..........
ETO695    .......... .......... .......... .......... ..........
ETO330    .......... .......... .......... .......... ..........
ETO618    .......... .......... .......... .......... ..........
ETO206    .......... .......... .......... .....G.... ..........
ETO536    .......... .......... .......... .....G.... ..........
ETO134    .......... .......... .......... .....G.... ..........
ETO608    .......... .......... .......... .....G.... ..........
ETO535    .......... .......... .......... .....G.... ..........
ETO085    .......... .......... .......... .....G.... ..........
ETO550    .......... .......... .......... .....G.... .........?
ETO362    .......... .......... .......... .....G.... ..........
ETO599    .......... .......... .......... .....G.... ..........
ETO597    .......... .......... .......... .....G.... ..........
ETO482    .......... .......... .......... .....G.... ..........
ETO420    .......... .......... .......... .....G.... ..........
ETO659    .......... .......... .......... .......... ..........
ETO401    .......... .......... .......... .......... ..........
ETO461    .......... .......... .......... .......... ..........
CS 03-02  .......... .......... .......... .......... ..........
ETO675    .......... .......... .......... .......... ..........
ETO231    .......... .......... .......... .......... ..........
ETO229h1  .......... .......... .....-.... .......... ..........
ETO692    .......... .......... .......... .......... ..........
ETO339    .......... .......... .......... .......... ..........
ETO645    .......... .......... .......... .......... ..........
ETO488    .......... .......... .......... .......... ..........
ETO229h2  .......... .......... .....-.... .......... ..........
OSUM95201.......... .......... .....-.... .......... ..........
coosa28   .......... .......... .....-.... .......... ..........
JRK02-31  .......... .......... .......... .......... ..........
BAP1080   .......... .......... .......... .......... ..........
BAP1081   .......... .......... .......... .......... ..........
BAP1082   .......... .......... .......... .......... ..........
BAP1116   .......... .......... .......... .......... ..........
BAP1078   .......... .......... .......... .......... ..........
BJF0150h1.......... .......... .......... .......... ..........
BJF0150h2.......... .......... .......... .......... ..........
BJF01-57  .......... .......... .......... .......... ..........
BAP1036h1.......... .......... .......... .......... ..........
BAP1036h2.......... .......... .......... .......... ..........
ETO659    .......... .......... .......... .......... ..........
OSUM85726.......... .......... .....-.... .......... ..........
OSUM85286.......... ......T... .....-.... .......... ..........
OSUM95196.......... .......... .....-.... .......... ..........
OSUM95124.......... .......... .....-.... .......... ..........
OSUM77772.......... .......... .....-.... .......... ..........
OSUM95128.......... .......... .....-.... .......... ..........
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ETO159H2  TACCCACCAA GCCGGGCATT CTTTCTACGG CGCATGGGGT TTTTTATTTT
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ETO159H1  .......... .......... .......... .......... ..........
ETO331    .......... .......... .......... .......... ..........
ETO695    .......... .......... .......... .......... ..........
ETO330    .......... .......... .......... .......... ..........
ETO618    .......... .......... .......... .......... ..........
ETO206    .......... .......... .......... .......... ..........
ETO536    .......... .......... .......... .......... ..........
ETO134    .......... .......... .......... .......... ..........
ETO608    .......... .......... .......... .......... ..........
ETO535    .......... .......... .......... .......... ..........
ETO085    .......... .......... .......... .......... ..........
ETO550    .......... .......... .......... .......... ..........
ETO362    .......... .......... .......... .......... ..........
ETO599    .......... .......... .......... .......... ..........
ETO597    .......... .......... .......... .......... ..........
ETO482    .......... .......... .......... .......... ..........
ETO420    .......... .......... .......... .......... ..........
ETO659    .......... .......... .......... .......... ..........
ETO401    .......... .......... .......... .......... ..........
ETO461    .......... .......... .......... .......... ..........
CS 03-02  .......... .......G.. .......... .......... ..........
ETO675    .......... .......G.. .......... .......... ..........
ETO231    .......... .......G.. .......... .......... ..........
ETO229h1  .......... .......G.. .......... .......... ..........
ETO692    .......... .......G.. .......... .......... ..........
ETO339    .......... .......G.. .......... .......... ..........
ETO645    .......... .......G.. .......... .......... ..........
ETO488    .......... .......G.. .......... .......... ..........
ETO229h2  .......... .......G.. .......... .......... ..........
OSUM95201.......... .......G.. .....C..A. .......... ..........
coosa28   .......... .......G.. .....C..A. .......... ..........
JRK02-31  .......... .......G.. .....C..A. .......... ..........
BAP1080   .......... .......G.. .....C..A. .......... ..........
BAP1081   .......... .......G.. .....C..A. .......... ..........
BAP1082   .......... .......G.. .....C..A. .......... ..........
BAP1116   .......... .......G.. .....C..A. .......... ..........
BAP1078   .......... .......G.. .....C..A. .......... ..........
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BJF0150h2.......... .......G.. .....C..A. .......... ..........
BJF01-57  .......... .......G.. .....C..A. .......... ..........
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BAP1036h2.......... .......G.. .....C..A. .......... ..........
ETO659    .......... .......G.. .....C..A. .......... ..........
OSUM85726.......... .......... .......... .....A.... ..........
OSUM85286.......... .......... ........A. .....A.... ..........
OSUM95196.......... .......G.. ........A. .....A..T. ..CC.T....
OSUM95124.......... .......G.. .......... .....A..T. ..........
OSUM77772.......... .......G.. .......... .....A..T. ..........
OSUM95128.......... .......G.. .......... .....A..T. ..........
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ETO159H2 TTTTT-CCTT TCACTTGGCA TTTCACAGTG CACACGGGAA -TAACAGATA
ETO263   .....-.... .......... .......... .......... -.........
ETO159H1 .....-.... .......... .......... .......... -.........
ETO331   .....-.... .......... .......... .......... -.........
ETO695   .....-.... .......... .......... .......... -.........
ETO330   .....-.... .......... .......... .......... -.........
ETO618   .....-.... .......... .......... .......... -.....A...
ETO206   .....-.... .......... .......... .......... -.........
ETO536   .....-.... .......... .......... .......... -.....A...
ETO134   .....-.... .......... .......... .......... -.....A...
ETO608   .....-.... .......... .......... .......... -.....A...
ETO535   .....-.... .......... .......... .......... -.....A...
ETO085   .....-.... .......... .......... .......... -.....A...
ETO550   .....-.... .......... .......... .......... -.....A...
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ETO482   .....-.... .......... .......... .......... -.....A...
ETO420   .....-.... .......... .......... .......... -.....A...
ETO659   .....-.... .......... .......... .......... -.........
ETO401   .....-.... .......... .......... .......... -.........
ETO461   .....-.... .......... .......... .......... -.........
CS 03-02 .....-.... .......... .......... .......... -.........
ETO675   .....-.... .......... .......... .......... -.........
ETO231   .....-.... .......... .......... .......... -.........
ETO229h1 .....-.... .......... .......... .......... -.........
ETO692   .....-.... .......... .......... .......... -.........
ETO339   .....-.... .......... .......... .......... -.........
ETO645   .....-.... .......... .......... .......... -.........
ETO488   .....-.... .......... .......... .......... -.........
ETO229h2 .....-.... .......... .......... .......... -.........
OSUM95201.....-.... .......... .......... .......... -.........
coosa28  .....-.... .......... .......... .......... -.........
JRK02-31 .....-.... ...TC..... .......... .......... -.........
BAP1080  .....-.... ...TC..... .......... .......... -.........
BAP1081  .....-.... ...TC..... .......... .......... -.........
BAP1082  .....-.... ...TC..... .......... .......... -.........
BAP1116  .....-.... ...TC?.... .......... .......... -.........
BAP1078  .....-.... ...TC..... .......... .......... -.........
BJF0150h1.....-.... ...TC..... .......... .......... -.........
BJF0150h2.....-.... ...TC..... .......... .......... -.........
BJF01-57 .....-.... ...TC..... .......... .......... -.........
BAP1036h1.....-.... ...TC..... .......... .......... -.........
BAP1036h2.....-.... ...TC..... .......... .......... -.........
ETO659   .....-.... ...TC..... .......... .......... -.........
OSUM85726.....-.... .......... .......... .......... -.........
OSUM85286.....-.... .......... .......... .......... -.........
OSUM95196.....-.... ...GC..... .......... .......... -.........
OSUM95124.....-.... ..G.C..... .......... .......... -.........
OSUM77772.....-.... ..G.C..... .......... .......... -.........
OSUM95128.....-.... ..G.C..... .......... .......... -.?.......
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ETO159H2 AGGGTGTACA TTTTTCTT-G CCCCA-GCGT ATAGTATGTG TGATGAAAAG
ETO263   .......... ........-. .....-.... .......... ..........
ETO159H1 .......... ........-. ....G-.... .......... ..........
ETO331   .......... ........-. ....G-.... ........A. ..G.......
ETO695   .......... ........-. ....G-.... ........A. ..G.......
ETO330   .......... ........-. ....G-.... ........A. ..G.......
ETO618   .......... ........-. ....G-.... ........A. ..G.......
ETO206   .......... ........-. .....-.... ........A. ..G.......
ETO536   .......... ........-. .....-.... ........A. ..G.......
ETO134   .......... ........-. .....-.... ........A. ..G.......
ETO608   .......... ........-. .....-.... ........A. ..G.......
ETO535   .......... ........-. .....-.... ........A. ..G.......
ETO085   .......... ........-. .....-.... ........A. ..G.......
ETO550   .......... ........-. .....-.... ........A. ..G.......
ETO362   .......... ........-. .....-.... ........A. ..G.......
ETO599   .......... ........-. .....-.... ........A. ..G.......
ETO597   .......... ........-. .....-.... ........A. ..G.......
ETO482   .......... ........-. .....-.... ........A. ..G.......
ETO420   .......... ........-. .....-.... ........A. ..G.......
ETO659   .......... ........-. .....-.... ........A. ..G.......
ETO401   .......... ........-. ....G-.... ........A. ..G.......
ETO461   .......... ........-. ....G-.... ........A. ..G.......
CS 03-02 .......... ........-. ....G-.... ........A. ..G.......
ETO675   .......... ........-. ....G-...C ........A. ..G.......
ETO231   .......... ........-. ....G-...C ........A. ..G.......
ETO229h1 .......... ........-. ....G-...C ........A. ..G.......
ETO692   .......... ........-. ....G-...C ........A. ..G.......
ETO339   .......... ........-. .....-.... ........A. ..G.......
ETO645   .......... ........-. .....-.... ........A. ..G.......
ETO488   .......... ........-. .....-.... ........A. ..G.......
ETO229h2 .......... ........-. .....-.... ........A. ..G.......
OSUM95201.......... ........-. .....-.... ........A. ..G.......
coosa28  .......... ........-. .....-.... ........A. ..G.......
JRK02-31 .......... ........-. .....-.... ........A. ..G..G....
BAP1080  .......... ........-. .....-.... ........A. ..G.......
BAP1081  .......... ........-. .....-.... ........A. ..G.......
BAP1082  .......... ........-. .....A.... ........A. ..G.......
BAP1116  .......... ........-. .....-.... ........A. ..G..G....
BAP1078  .......... ........-. .....-.... ........A. ..G..G....
BJF0150h1.......... ........-. .....-.... ........A. ..G..G....
BJF0150h2.......... ........-. .....-.... ........A. ..G..G....
BJF01-57 .......... ........-. .....-.... ........A. ..G..G....
BAP1036h1.......... ........-. .....-.... ........A. ..G.......
BAP1036h2.......... ........-. .....A.... ........A. ..G.......
ETO659   .......... ........-. .....-.... ........A. ..G.......
OSUM85726.......... ........-. ....G-.A.. ........AA ..G.......
OSUM85286.......... ........-. ....G-.A.. ........AA ..G.......
OSUM95196.......... ........-. ....G-.A.. ........AA ..G.......
OSUM95124.......... ........-. ....G-.A.. ........AA ..G.......
OSUM77772.......... ........-. ....G-.A.. ........AA ..G.......
OSUM95128.......... ........-. ....G-.A.. ........AA ..G.......
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ETO159H2  ATTTTATACA AAGAACCACA TATTAGGATA TCATGTGCAT AAGTAGTGGT
ETO263    .......... .......... .......... .......... ..........
ETO159H1  .......... .......... .......... .......... ..........
ETO331    .......... .......... .......... .......... ..........
ETO695    .......... .......... .......... .......... ..........
ETO330    .......... .......... .......... .......... ..........
ETO618    .......... .......... .......... .......... ..........
ETO206    .......... .......... .......... .......... ..........
ETO536    .......... .......... .......... .......... ..........
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ETO535    .......... .......... .......... .......... ..........
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ETO550    .......... .......... .......... .......... ..........
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ETO599    .......... .......... .......... .......... ..........
ETO597    .......... .......... .......... .......... ..........
ETO482    .......... .......... .......... .......... ......t...
ETO420    .......... .......... .......... .......... ..........
ETO659    .......... .......... .......... .......... ........AC
ETO401    .......... .......... .......... .......... ........AC
ETO461    .......... .......... .......... .......... ........AC
CS 03-02  .......... .......... .......... .......... ........AC
ETO675    .......... .......... .......... .......... ........A.
ETO231    .......... .......... .......... .......... ........A.
ETO229h1  .......... .......... .......... .......... ........A.
ETO692    .......... .......... .......... .......... ........A.
ETO339    .......... .......... .......... .......... ........AC
ETO645    .......... .......... .......... .......... ........AC
ETO488    .......... .......... .......... .......... ........AC
ETO229h2  .......... .......... .......... .......... ........AC
OSUM95201..?....... .......... .......... .....A.... ..........
coosa28   .......... .......... .......... .......... ........A.
JRK02-31  .......... .......... .......... .......... ..........
BAP1080   .......... .......... .......... .......... ..........
]BAP1081    .......... .......... .......... .......... ..........
BAP1082   .......... .......... .......... .......... ..........
BAP1116   .......... .......... .......... ...?...... ........A.
BAP1078   .......... .......... .......... .......... ..........
BJF0150h1.......... .......... .......... .......... ........A.
BJF0150h2.......... .......... .......... .......... ........A.
BJF01-57  .......... .......... .......... .......... ........A.
BAP1036h1.......... .......... .......... .......... ..........
BAP1036h2.......... .......... .......... .......... ..........
ETO659    .......... .......... .......... .......... ..........
OSUM85726.......... .......... .......... .......... ......A...
OSUM85286.......... .......... .......... .......... ......A...
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OSUM95128.......... .......... .......... .......... ..........
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ETO331    .......... .......... .......... ..--...... ..........
ETO695    .......... .......... .......... ..--...... ..........
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ETO618    .......... .......... .......... ..--...... ..........
ETO206    .......... .......... .......... ..--...... ..........
ETO536    .......... .......... .......... ..--...... ..........
ETO134    .......... .......... .g.g...... ..--...... ..........
ETO608    .......... .......... .......... ..--...... ..........
ETO535    .......... .......... .......... ..--...... ..........
ETO085    .......... .......... .......... ..--...... ..........
ETO550    .......... .......... .......... ..--...... ..........
ETO362    .......... .......... .......... ..--...... ..........
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ETO420    .......... .......... .......... ..--...... ..........
ETO659    ....T..... .......... .......... ..--...... ..........
ETO401    ....T..... .......... .......... ..--...... ..........
ETO461    ....T..... .......... .......... ..--...... ..........
CS 03-02  .......... .......... .......... ..--...... ..........
ETO675    G...T.C... .......... .......... ..--...... ..........
ETO231    G...T.C... .......... .......... ..--...... ..........
ETO229h1  G...T.C... .......... .......... ..--...... ..........
ETO692    G...T.C... .......... .......... ..--...... ..........
ETO339    ....T.C... .......... .......... ..--...... ..........
ETO645    ....T.C... .......... .......... ..--...... ..........
ETO488    ....T.C... .......... .......... ..--...... ..........
ETO229h2  ....T.C... .......... .......... ..--...... ..........
OSUM95201....T..... .......... .......... ..--...... ..........
coosa28   ....T..... .......... .......... ..--...... ..........
JRK02-31  ....T..... .......... .......... ..--...... ..........
BAP1080   ....T..... .......... .......... ..--...... ..........
BAP1081   ....T..... .......... .......... ..--...... ..........
BAP1082   ....T..... .......... .....T.... ..--...... ..........
BAP1116   ....T..... .......... .......... ..--...... ..........
BAP1078   ....T..... .......... .....T.... ..--...... ..........
BJF0150h1....T..... .......... .......... ..--...... ..........
BJF0150h2....T..... .......... .....T.... ..--...... ..........
BJF01-57  ....T..... .......... .......... ..--...... ..........
BAP1036h1....T..... .......... .......... ..--...... ..........
BAP1036h2....T..... .......... .....T.... ..--...... ..........
ETO659    ....T..... .......... .....T.... ..--...... ..........
OSUM85726....T.C... .....A.... .......... ..--...... ..........
OSUM85286....T.C... .....A.... .......... ..--...... ..........
OSUM95196....T.C... .....A.... .......... ..--...... ..........
OSUM95124....T.C... .....A.... .....T.... ..--...... ..........
OSUM77772....T.C... .....A.... .....T.... ..--...... ..........
OSUM95128....T.C... .....A.... .....AT... ..--...... ..........
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[         1234567890 1234567890 1234567890 1234567890 1234567890 ]
ETO159H2 TTTCTTTGCG TAAAACCCCC C-TACCCCCC TAAACTCCTG AGATAGTTAT
ETO263   .......... .......... .-........ .......... ..........
ETO159H1 .......... .......... .-........ .......... ..........
ETO331   .......... .......... .-........ .......... ..........
ETO695   .......... .......... .-........ .......... ..........
ETO330   .......... .......... .-........ .......... ..........
ETO618   .......... .......... .-........ .......... ..........
ETO206   .......... .......... .-........ .......... ..........
ETO536   .......... .......... .-........ .......... ..........
ETO134   .......... .......... .-........ .......... .g........
ETO608   .......... .......... .-........ .......... ..........
ETO535   .......... .......... .-........ .......... ..........
ETO085   .......... .......... .-........ .......... ..........
ETO550   .......... .......... .-........ .......... ..........
ETO362   .......... .......... .-........ .......... ..........
ETO599   .......... .......... .-........ .......... ..........
ETO597   .......... .......... .-........ .......... ..........
ETO482   .......... .......... .-........ .......... ..........
ETO420   .......... .......... .-........ .......... ..........
ETO659   .......... .......... .-........ .......... ..........
ETO401   .......... .......... .-........ .......... ..........
ETO461   .......... .......... .-........ .......... ..........
CS 03-02 .......... .......... .-........ .......... ..........
ETO675   .......... .......... .-........ .......... ..........
ETO231   .......... .......... .-........ .......... ..........
ETO229h1 .......... .......... .-........ .......... ..........
ETO692   .......... .......... .-........ .......... ..........
ETO339   .......... .......... .-........ .......... ..........
ETO645   .......... .......... .-........ .......... ..........
ETO488   .......... .......... .-........ .......... ..........
ETO229h2 .......... .......... .-........ .......... ..........
OSUM95201.......... .......... .-........ .......... ..........
coosa28  .......... .......... .-........ .......... ..........
JRK02-31 .......... .......... .-........ .......... ..........
BAP1080  .......... .......... .-........ .......... ..........
BAP1081  .......... .......... .-........ .......... ..........
BAP1082  .......... .......... .-........ .......... ..........
BAP1116  .......... .......... .-........ .......... ..........
BAP1078  .......... .......... .-........ .......... ..........
BJF0150h1.......... .......... .-........ .......... ..........
BJF0150h2.......... .......... .-........ .......... ..........
BJF01-57 .......... .......... .-........ .......... ..........
BAP1036h1.......... .......... .-........ .......... ..........
BAP1036h2.......... .......... .-........ .......... ..........
ETO659   .......... .......... .-........ .......... ..........
OSUM85726.......... .......... .-........ .......... ..........
OSUM85286.......... .......... .-........ .......... ..........
OSUM95196.......... .......... .-........ .......... ......C...
OSUM95124.......... .......... .-........ .......... ......C...
OSUM77772.......... .......... .-........ .......... ......C...
OSUM95128.......... .......... .-........ .......... ......C...
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ETO159H2 CAATCCTGAA AACCCCCCGG AAACAGGAAA ATCTCTAGAA ATA-TTTTTA
ETO263   .......... .......... .......... .......... ...-......
ETO159H1 .......... .......... .......... .......... ...-......
ETO331   .......... .......... .......... .......... ...-......
ETO695   .......... .......... .......... .......... ...-......
ETO330   .......... .......... .......... .......... ...-......
ETO618   .......... .......... .......... .......... ...-......
ETO206   .......... .......... .......... .......... ...-......
ETO536   .......... .......... .......... .......... ...-......
ETO134   .......... .......... .......... .......g.. ...-......
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ETO535   .......... .......... .......... .......... ...-......
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ETO597   .......... .......... .......... .......... ...-......
ETO482   .......... .......... .......... .......... ...-......
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ETO659   .......... .......... .......... .......... ...-......
ETO401   .......... .......... .......... .......... ...-......
ETO461   .......... .......... .......... .......... ...-......
CS 03-02 .......... .......... .......... .......... ...-......
ETO675   .......... .......... .......... .......... ...-......
ETO231   .......... .......... .......... .......... ...-......
ETO229h1 .......... .......... .......... .......... ...-......
ETO692   .......... .......... .......... .......... ...-......
ETO339   .......... .......... .......... .......... ...-......
ETO645   .......... .......... .......... .......... ...-......
ETO488   .......... .......... .......... .......... ...-......
ETO229h2 .......... .......... .......... .......... ...-......
OSUM95201.......... .......... .......... .C........ ...-.....G
coosa28  .......... .......... .......... .C........ ...-.....T
JRK02-31 .......... .......... .......... .C........ ...-.....G
BAP1080  .......... .......... .......... .C........ ...-.....G
BAP1081  .......... .......... .......... .C........ ...-.....G
BAP1082  .......... .......... .......... .C........ ...-.....G
BAP1116  .......... .......... .......... .C........ ...-.....G
BAP1078  .......... .......... .......... .C........ ...-.....G
BJF0150h1.......... .......... .......... .C........ ...-.....G
BJF0150h2.......... .......... .......... .C........ ...-.....G
BJF01-57 .......... .......... .......... .C........ ...-.....G
BAP1036h1.......... .......... .......... .C........ ...-.....G
BAP1036h2.......... .......... .......... .C........ ...-.....G
ETO659   .......... .......... .......... .C........ ...-.....G
OSUM85726.......... .......... .......... .......... G..-.....T
OSUM85286.......... .......... .......... .......... G..-.....T
OSUM95196.......... .......... .......... .......... G..-.....T
OSUM95124.......... .......... .......... .......... G..-.....T
OSUM77772.......... .......... .......... .......... G..-.....T
OSUM95128.......... .......... .......... .......... G..-.....T
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[         0000000001 1111111112 2222222223 3333333334 4444444445 ]
[         1234567890 1234567890 1234567890 1234567890 1234567890 ]
ETO159H2 GGGG-AATCA ATTTGCATCT ATTTACATTA TTAAAATGAT GTATTTGCTA
ETO263   ....-..... .......... .......... .......... ..G.......
ETO159H1 ....-..... .......... .......... .......... ..........
ETO331   ....-..... .......... .......... .......... ..G.......
ETO695   ....-..... .......... .......... .......... ..G.......
ETO330   ....-..... .......... .......... .......... ..G.......
ETO618   ....-..C.. .......... .......... .......... ..........
ETO206   ....-..C.. .......... .......... .......... ..........
ETO536   ....-..C.. .......... .......... .......... ..........
ETO134   ....-..C.. .......... .......... .......... ..........
ETO608   ....-..C.. .......... .......... .......... ..........
ETO535   ....-..C.. .......... .......... .......... ..........
ETO085   ....-..C.. .......... .......... .......... ..........
ETO550   ....-..C.. .......... .......... .......... ..........
ETO362   ....-..C.. .......... .......... .......... ..........
ETO599   ....-..C.. .......... .......... .......... ..........
ETO597   ....-..C.. .......... .......... .......... ..........
ETO482   ....-..C.. .......... .......... .......... ..........
ETO420   ....-..C.. .......... .......... .......... ..........
ETO659   ....-..C.. .......... .......... .......... ..........
ETO401   ....-..C.. .......... .......... .......... ..........
ETO461   ....-..C.. .......... .......... .......... ..........
CS 03-02 ....-..C.. .......... .......... .......... ..........
ETO675   ....-..C.. .......... .......... .......... ..........
ETO231   ....-..C.. .......... .......... .......... ..........
ETO229h1 ....-..C.. .......... .......... .......... ..........
ETO692   ....-..C.. .......... .......... .......... ..........
ETO339   ....-..C.. .......... .......... .......... ..........
ETO645   ....-..C.. .......... .......... .......... ..........
ETO488   ....-..C.. .......... .......... .......... ..........
ETO229h2 ....-..C.. .......... .......... .......... ..........
OSUM95201....-..C.. .......... .......... .......... ..........
coosa28  ....-..C.. .......... .......... .......... ..........
JRK02-31 ....-..C.. .......... .......... .......... ..........
BAP1080  ....-..C.. .......... .......... .......... ..........
BAP1081  ....-..C.. .......... .......... .......... ..........
BAP1082  ....-..C.. .......... .......... .......... ..........
BAP1116  ....-..C.. .......... .......... .......... ..........
BAP1078  ....-..C.. .......... .......... .......... ..........
BJF0150h1....-..C.. .......... .......... .......... ..........
BJF0150h2....-..C.. .......... .......... .......... ..........
BJF01-57 ....-..C.. .......... .......... .......... ..........
BAP1036h1....-..C.. .......... .......... .......... ..........
BAP1036h2....-..C.. .......... .......... .......... ..........
ETO659   ....-..C.. .......... .......... .......... ..........
OSUM85726....-..C.. .......... .......... .......A.. ..G.A.....
OSUM85286....-..C.. .......... .......... .......A.. ..G.A.....
OSUM95196....-..C.. .......... .......... .......A.. ..G.A.....
OSUM95124....-..C.. .......... .......... .......A.. ..G.A.....
OSUM77772....-..C.. .......... .......... .......A.. ..G.A.....
OSUM95128....-..C.. .......... .......... .......A.. ..G.A.....
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[        5555555556 6666666667 7777777778 8888888889 9999999990 ]
[        1234567890 1234567890 1234567890 1234567890 1234567890 ]
ETO159H2 GCGTAGCTTA ACTAAAGCAT AACACTGAAG ATGTTAAGAT GGGCCCTAGA
ETO263   .......... .......... .......... .......... ..........
ETO159H1 .......... .......... .......... .......... ..........
ETO331   .......... .......... .......... .......... ..........
ETO695   .......... .......... .......... .......... ..........
ETO330   .......... .......... .......... .......... ..........
ETO618   .......... ..C....... .......... .......... ..........
ETO206   .......... ..C....... .......... .......... ..........
ETO536   .......... ..C....... .......... .......... ..........
ETO134   .......... ..C....... .......... .......... ..........
ETO608   .......... ..C....... .......... .......... ..........
ETO535   .......... ..C....... .......... .......... ..........
ETO085   .......... ..C....... .......... .......... ..........
ETO550   .......... ..C....... .......... .......... ..........
ETO362   .......... ..C....... .......... .......... ..........
ETO599   .......... ..C....... .......... .......... ..........
ETO597   .......... ..C....... .......... .......... ..........
ETO482   .......... ..C....... .........g .......... ........g.
ETO420   .......... ..C....... .......... .......... ..........
ETO659   .......... .......... .......... .......... ..g.......
ETO401   .......... .......... .......... .......... .....c....
ETO461   .......... .......... .......... .......... ..........
CS 03-02 .......... .......... .......... .......... ..........
ETO675   .......... .......... .......... .......... ........A.
ETO231   .......... .......... .......... .......... ..........
ETO229h1 .......... .T........ .......... .......... ..........
ETO692   .......... .......... .......... .......... ..........
ETO339   .......... .......... .......... .......... ..........
ETO645   .......... .......... .......... .......... ..........
ETO488   .......... .......... .......... .......... ..........
ETO229h2 .......... .......... .......... .......... ..........
OSUM95201.......... .......... .......... .......... ..........
coosa28  .......... .......... .......... .......... ..........
JRK02-31 .......... .......... .......... .......... ..........
BAP1080  .......... .......... .......... .......... ..........
BAP1081  .......... .......... .......... .......... ..........
BAP1082  .......... .......... .......... .......... ..........
BAP1116  .......... .......... .......... .......... ..........
BAP1078  .......... .......... .......... .......... ..........
BJF0150h1.......... .......... .......... .......... ..........
BJF0150h2.......... .......... .......... .......... ..........
BJF01-57 .......... .......... .......... .......... ..........
BAP1036h1.......... .......... .......... .......... ..........
BAP1036h2.......... .......... .......... .......... ..........
ETO659   .......... .......... .......... .......... ..........
OSUM85726.......... .T........ .......... .......... .A........
OSUM85286.......... .T........ .......... .......... .A........
OSUM95196.......... .T........ .......... .......... .A........
OSUM95124.......... .T........ .......... .......... .A........
OSUM77772.......... .T........ .......... .......... .A........
OSUM95128.......... .T........ .......... .......... .A........
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[         1234567890 1234567890 1234567890 1234567890 1234567890 ]
ETO159H2 AAGCTCCGCA AGCACAAAGG CTTGGTCCTG ACTTTACTAT CAACTCTAGC
ETO263   .......... .......... .......... .......... ..........
ETO159H1 .......... .......... .......... .......... ..........
ETO331   .......... .......... .......... .......... ..........
ETO695   .......... .......... .......... .......... ..........
ETO330   .......... .......... .......... .......... ..........
ETO618   .......... .......... .......... .......... ........g.
ETO206   .......... .......... .......... .......... ..........
ETO536   .......... .......... .......... .......... ..........
ETO134   .......... .......... .......... .......... ..........
ETO608   .......... .......... .......... .......... ..........
ETO535   .......... .......... .......... .......... ..........
ETO085   .......... .......... .......... .......... ..........
ETO550   .......... .......... .......... ?......... ..........
ETO362   .......... .......... .......... .......... ..........
ETO599   .......... .......... .......... .......... ..........
ETO597   .......... .......... .......... .......... ..........
ETO482   .......... .......... .......... .......... ..........
ETO420   .......... .......... .......... .......... ..........
ETO659   .......... .......... .......... .......... ..........
ETO401   .a........ ?......?.? ..t..?.cg. ....t..... ..a.......
ETO461   .......... .......... .......... .......... ..........
CS 03-02 .......... .......... .......... .......... ..........
ETO675   .......... .......... .....t.... .......... ..........
ETO231   .......... .......... .......... .......... ..........
ETO229h1 .......... .......... .......... .......... ..........
ETO692   .......... .......... .......... .......... ..........
ETO339   .......... .......... .......... .......... ..........
ETO645   .......... .......... .......... .......... ..........
ETO488   ....C..... .......... .......c.. .......... ..........
ETO229h2 .......... .......... .......... .......... ..........
OSUM95201.......... .......... .......... .......... ..........
coosa28  .......... .......... .......... .......... ..........
JRK02-31 .......... .......... .......... .......... ..........
BAP1080  .......... .......... .......... .......... ..........
BAP1081  .......... .......... .......... .......... ..........
BAP1082  .......... .......... .......... .......... ..........
BAP1116  .......... .......... .......... .......... ..........
BAP1078  .......... .......... .......... .......... ..........
BJF0150h1.......... .......... .......... .......... ..........
BJF0150h2.......... .......... .......... .......... ..........
BJF01-57 .......... .......... .......... .......... ..........
BAP1036h1.......... .......... .......... .......... ..........
BAP1036h2.......... .......... .......... .......... ..........
ETO659   .......... .......... .......... .......... ..........
OSUM85726.......... .......... .......... .......... ..........
OSUM85286.......... .......... .......... .......... ..........
OSUM95196.......... .......... .......... .......... ..........
OSUM95124.........G G......... .......... .......... ..........
OSUM77772.........G G......... .......... .......... ..........
OSUM95128.........G G......... .......... .......... ..........
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[        5555555556 6666666667 7777777778 8888888889 9999999990 ]
[        1234567890 1234567890 1234567890 1234567890 1234567890 ]
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ETO263   .......... .......... .......... ..?....... ..........
ETO159H1 .......... .......... .......... .......... ..........
ETO331   .......... .......... .......... .......... ..........
ETO695   .......... .......... .......... .......... ..........
ETO330   .......... .......... .......... .......... ........?.
ETO618   .......... .......... .......... ..t..g.... ..........
ETO206   .......... .......... .......... .......... ..........
ETO536   ..G....... .......... .......... .......... ..........
ETO134   .......... .......... .......... .......... ..........
ETO608   .......... .......... .......... .......... ..........
ETO535   .......... .......... .......... ..t....... ..........
ETO085   .......... .......... .......... .......... ..........
ETO550   .......... .......... .......... .......... ..........
ETO362   .......... .......... .......... .......... ..........
ETO599   .......... .......... .......... .......... ..........
ETO597   .......... .......... .......... .......... ..........
ETO482   .......... .......... .......... ..t....... ..........
ETO420   .......... .......... .......... .......... ..........
ETO659   .......... .......... .......... .......... ..........
ETO401   ...?..t..? ca....a... .......... c.t..g.?t. ..ct..a..t
ETO461   .......... .......... .......... ..??...... ..........
CS 03-02 .......... .......... .....G.... .......... ..........
ETO675   .......... .......... .......... c.t....... ..........
ETO231   .......... .......... .......... .......... ..........
ETO229h1 .......... .......... .......... .......... ..........
ETO692   .......... .......... .......... .......... ..........
ETO339   .......... .......... .......... .......... ..........
ETO645   .......... .......... .......... .......... ..........
ETO488   .......... .......... .......... ..t....... ..........
ETO229h2 .......... .......... .......... .......... ..........
OSUM95201.......... .......... .......... .......... ..........
coosa28  .......... .......... .......... .......... ..........
JRK02-31 .......... .......... .....C.... .......... ..........
BAP1080  ..G....... .......... .......... .......... ..........
BAP1081  ..G....... .......... .......... .......... ..........
BAP1082  ..G....... .......... .......... .......... ..........
BAP1116  .......... .......... .......... .......... ..........
BAP1078  .......... .......... .......... .......... ..........
BJF0150h1.......... .......... .......... .......... ..........
BJF0150h2.......... .......... .......... .......... ..........
BJF01-57 .......... .......... .......... .......... ..........
BAP1036h1..G....... .......... .......... ..?....... ..........
BAP1036h2..G....... .......... .......... .......... ..........
ETO659   ..G....... .......... .......... .......... ..........
OSUM85726.......... .......... ..C....... .......... ..........
OSUM85286.......... .......... ..C....... .......... ..........
OSUM95196..G.C..... .......... ..C...C... .......... ..........
OSUM95124..G....... .......... ..C....... .......... ..........
OSUM77772..G....... .......... ..C....... .......... ..........
OSUM95128..G....... .......... ..C....... .......... ..........
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[         0000000001 1111111112 2222222223 3333]
[         1234567890 1234567890 1234567890 1234]
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ETO263    .......... .......... .......... ....
ETO159H1  .......... .......... .......... ....
ETO331    .......... .......... .......... ....
ETO695    .......... .......... .......... ....
ETO330    .......... .......... .......... ...?
ETO618    .......c.. g........g ....?????? ????
ETO206    .......... .......... .......... ....
ETO536    .......... .......... .......... ....
ETO134    .......... .......... .......... ....
ETO608    .......... .......... .......... ....
ETO535    .......... .......... .......... ....
ETO085    .......... .......... .......... ....
ETO550    .......... .......... .......... ....
ETO362    .......... .......... .......... ....
ETO599    .......... .......... .......... ....
ETO597    .......... .......... ....?..... ....
ETO482    .......... .......... .......... ....
ETO420    .......... .......... .......... ....
ETO659    .......... .......... .......... ....
ETO401    ..ct...... ...c.a.g.. c...ta.c.. .c..
ETO461    .......... .......... .......... ....
CS 03-02  .......... .......... .......... ....
ETO675    ........g. .......?.. ....??.... ....
ETO231    .......... .......... .......... ....
ETO229h1  .......... .......... .......... ....
ETO692    .......... .......... .......... ....
ETO339    .......... .......... .......... ....
ETO645    .......... .......... .......... ....
ETO488    .......... .......... .......... ....
ETO229h2  .......... .......... .......... ....
OSUM95201.......... .......... .......... ....
coosa28   .......... .......... .......... ....
JRK02-31  .......... .......... .......... ....
BAP1080   .......... .......... .......... ....
BAP1081   .......... .......... .......... ....
BAP1082   .......... .......... .......... ....
BAP1116   .......... .......... .......... ....
BAP1078   .......... .......... .......... ....
BJF0150h1.......... .......... .......... ....
BJF0150h2.......... .....a.... .......... ....
BJF01-57  .......... .......... .......... ....
BAP1036h1.......... .......... .......... ....
BAP1036h2.......... .......... .......... ....
ETO659    .......... .......... .......... ....
OSUM85726.......... .......... .C........ ....
OSUM85286.......... .......... .C........ ....
OSUM95196.......... .......... .......... ....
OSUM95124.......... .......... .......... ....
OSUM77772.......... .......... .......... ....
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OSUM95128.......... ......

APPENDIX B

INFORMATION PERTINENT TO CHAPTER 3

Appendix B Table 1.1

Snorkel observation sites, dates and length of observations (observation time = minutes) across all sites (2002-2003)

Stream Name	Microlocality Data	Easting	Northing
Hickory Log Cr.	at Co. Rte 766 (Fate Conn Rd.) 4.2 air miles NNE of Canton, GA	733340	3797477
Whitehead Cr.	at Siera Mt. Rd. off of Hulsey Town Rd. ca. 3.5 air miles ENE of Beulah, GA	692112.94	3751584
McCanless Cr.	at St. Rte 108 at the jct with County Route 30 (Darby Rd.) 1.75 air miles S of Waleska, GA	724792.98	3797189.1
Shoal Cr.	at Co. Rte 224 (Shoal Creek Rd.) 1.0 air miles NW of Dawsonville, GA	763795.71	3813825.2
Butler Cr.	at County Route 102 (Mack Dobbs Road) 1.7 air miles WSW of Kennesaw, GA	717613.6	3766601.7

Snorkel Localities	**Date**	**Observation Time**
McCanless Cr.	22-Mar-2002	134
@ GA Hwy 108 and Darby Rd.	23-Apr-2002	140
	14-May-2002	117
	21-Mar-2003	105
	29-Mar-2003	64
	1-Apr-2003	110
	13-Apr-2003	113
	17-Apr-2003	119
	29-Apr-2003	100
	13-May-2003	102
	27-May-2003	45
Shoal Cr. (Dawson Co.)	26-Mar-2002	50
@ Confl. W/ Burt Cr.	11-Apr-2002	60
	30-Apr-2002	107
	23-Mar-2003	139
	1-Apr-2003	101
	13-Apr-2003	113
	28-Apr-2003	121
	13-May-2003	67
	27-May-2003	116
	12-May-2003	117
	30-May-2003	102

Appendix B (cont.) Table 1.1

Snorkel observation dates and length of observations (observation time = minutes) across all sites (2002-2003)

Snorkel Localities	Date	Observation Time
Whitehead Cr. @ Siera Mt. Rd.	4-Apr-2002	60
	18-Apr-2002	130
	25-Apr-2002	136
	15-May-2002	112
	26-Mar-2003	160
	31-Mar-2003	156
	4-Apr-2003	128
	15-Apr-2003	143
	29-Apr-2003	100
	12-May-2003	117
	30-May-2003	102
Hickory Log Cr. @ Fate Conn Rd.	11-Apr-2002	55
	23-Apr-2002	210
	14-May-2002	97
	21-Mar-2003	133
	29-Mar-2003	69
	1-Apr-2003	85
	9-Apr-2003	62
	17-Apr-2003	64
	28-Apr-2003	78
	13-May-2003	46
	27-May-2003	47
Butler Cr. @ Mack Dobbs Rd.	4-Apr-2002	30
	25-Apr-2002	124
	15-May-2002	91
	25-Mar-2003	161
	31-Mar-2003	106
	4-Apr-2003	118
	15-Apr-2003	112
	12-May-2003	112
	30-May-2003	84

Appendix B Figure 1.2 Water quality data from all sites (2002-2003)

*=Recorded With a Hach © Turbidimeter
**= Recorded with an alchohol termometer

Locality	Date	D.O.	pH	Cond	Temp ©	Turb
Hickory Log Cr.	11-Apr-2002	9.69	6.95	34	15.22	8.5
	23-Apr-2002	10.88	7.14	35	14.49	7.4
	14-May-2002	10.38	7.44	36	14.7	9.7
	21-Mar-2003	10.81	7.27	33.5	13.6	NA
	29-Mar-2003	10.13	7.12	8.1	15.25	NA
	1-Apr-2003	11.4	7.15	7	11.71	2.99
	9-Apr-2003	10.75	8.08	36	11.85	11.5*
	17-Apr-2003	10.21	7.94	38	13.9	3.83*
	28-Apr-2003	9.51	7.09	37	14.62	5.84*
	13-May-2003	9.43	7.46	9.43	16.93	5.9/6.35*
	27-May-2003	9.47	7.38	34	16.7	5.1
	10-Jun-2003	NA	NA	NA	18.1**	6.11*
Shoal Creek	26-Mar-2002	10.7	6.8	19	12.64	3.5
	11-Apr-2002	9.94	7.4	20.1	15.22	7.2
	30-Apr-2002	10.45	6.97	20	16.5	6.5
	23-Mar-2003	10.59	7.27	20.6	13.7	25.5
	1-Apr-2003	10.3	7.08	NA	14.32	NA
	13-Apr-2003	11.32	7.03	26	11.47	3.4*
	28-Apr-2003	10.57	7.8	25	13.38	4.41*
	13-May-2003	10.31	7.11	19	13.02	4.6/5.67*
	27-May-2003	10.44	6.19	20	14.63	2.1
McCanless Cre.	22-Mar-2002	12.08	7.95	35.9	9.37	1.7
	23-Apr-2002	10.02	7.58	52	18.09	0.3
	14-May-2002	10.52	7.66	51	16.76	6.6
	21-Mar-2003	10.01	7.74	44.9	16.06	NA
	29-Mar-2003	10.4	7.69	23.3	17.05	NA
	1-Apr-2003	11.6	7.22	24	9.31	3.39*
	13-Apr-2003	10.64	8.24	49	16.09	2.78*
	17-Apr-2003	10.9	8.24	52	15.01	2.55*
	29-Apr-2003	9.3	8.11	50	16.27	3.56*
	13-May-2003	10.7	6.09	44	14.42	4.2/4.6*
	27-May-2003	9.8	7.23	47	17.61	2.2

Appendix B Figure 1.2 (cont.)

Water quality data from all sites (2002-2003)

*=Recorded With a Hach © Turbidimeter
**= Recorded with an alchohol termometer

Locality	Date	D.O.	pH	Cond	Temp ©	Turb
Butler Cr.	4-Apr-2002	11.16	7.54	78	14.56	7.2
	25-Apr-2002	9.19	7.16	89	16.9	9.4
	15-May-2002	9.65	7.47	100	14.23	6.1
	25-Mar-2003	11.29	7.91	109	13.52	NA
	31-Mar-2003	11.12	7.19	95.2	11.12	3.54*
	4-Apr-2003	10.32	7.45	106	16.8	4.5*
	15-Apr-2003	9.6	7.57	113	16.77	2.7*
	12-May-2003	8.84	7.47	113	16.45	4.1
	30-May-2003	9.77	7.22	110	15.95	NA

Locality	Date	D.O.	pH	Cond	Temp ©	Turbidity
Whitehead Cr.	4/4/2002	NA	NA	34	12.94	NA
	4/18/2002	9.7	7.36	47	19.75	4.8
	4/25/2002	10.21	7.39	10	18.92	3
	5/15/2002	9.52	7.53	47	16.51	3.8
	3/26/2003	9.64	7.66	41.1	17.08	NA
	3/31/2003	12.09	6.92	18.3	8.91	4.38*
	4/4/2003	10.14	7.37	45	14.59	3.4*
	4/15/2003	10.07	8.17	46	14.05	3.01*
	4/29/2003	9.1	7.41	47	18.32	4.46
	5/12/2003	9.6	7.3	41	18.77	4.2
	5/30/2003	9.51	7.11	41	17.1	NA

Appendix B Table 1.3

Shoal Creek

27 March - 26 May	2002	2003
mean daily temperature through spawning season	15.14755	14.70331
daily max (average through season)	17.0218	16.02344
daily min (average through season)	13.42623	13.43197
accumulated mean	924.0007	896.9022
2002-2003 difference	avg.	0.44
	max.	0.998

Whitehead Creek

april 5 - may 29

	2002	2003
mean daily temperature through spawning season	16.68788	15.65787
daily max (average through season)	19.006	17.366
daily min (average through season)	14.648	14.18655
accumulated mean	917.8336	861.1826
2002-2003 difference	avg.	1.03
	max.	1.64

Hickory Log

11 April - 26 May	2002	2003
mean daily temperature through spawning season	16.46323	15.86969
daily max (average through season)	18.02978	17.02783
daily min (average through season)	15.10826	14.78739
accumulated mean	757.3088	730.0058
2002-2003 difference	avg.	0.594
	max.	1