CONFRONTING INCOMPLETE DETECTION TO ADDRESS QUESTIONS ABOUT DISTRIBUTION AND REPRODUCTIVE SEASON FOR FOUR IMPERILED STREAM FISHES

by

GREGORY B. ANDERSON

(Under the Direction of Mary C. Freeman)

ABSTRACT

Geographical and ecological restrictions are the primary drivers of freshwater fish imperilment within the southeastern United States. To effectively manage existing populations and implement regulatory mechanisms of protection, information on the ecology and distributional patterns of imperiled taxa is needed. In this study, reproductive aspects including spawning behavior, microhabitat use and phenology were recorded for four imperiled percid taxa of the Upper Etowah watershed: the Etowah and Amicalola holiday darters *(Etheostoma* sp. cf. *E. brevirostrum* A and B), the Etowah darter (*Etheostoma etowahae*), and the bridled darter (*Percina kusha*). While accounting for incomplete detection, the occurrence of the spawning events was modeled according to visit characteristics to determine the duration of the spawning season. Additionally, patterns of spatial variation of the two holiday darter species were studied in an attempt to refine the known geographic range of these species and to identify factors influencing variations in occupancy and detection.

INDEX WORDS: Percidae, *Etheostoma*, *Percina*, *Ulocentra*, *Nothonotus*, *Alvordius*, Holiday darter, Etowah darter, Bridled darter, Amicalola Creek, Etowah River, Occupancy modeling, Incomplete detection, Information theoretic, Land use

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CHAPTER ONE

Introduction and Literature Review

The ichthyofauna of the southeastern United States is incredibly diverse and has high levels of endemism (Burr and Mayden 1992, Warren et al. 1997, Warren et al. 2000), but imperilment of taxa within this region is second only to arid western fishes (Warren and Burr 1994, Warren et al. 1997). Restricted geographic ranges and isolated endemism make many fish taxa within the southeast vulnerable to extirpation or extinction by very localized degradation or habitat fragmentation (Burr and Mayden 1992, Warren and Burr 1994, Angermeier 1995, Burkhead et al. 1997, Warren et al. 1997). Undoubtedly, benthic communities are the first to witness the adverse effects of many types of stream degradation (Etnier 1997, Warren et al. 1997), and rheophilic freshwater fishes that utilize this habitat type face disproportionate levels of imperilment when compared to other taxa (Angermeier 1995, Etnier 1997, Warren et al. 1997).

Effective conservation and management strategies for threatened and endangered taxa require information on the distribution and autecological patterns of the species (Warren et al. 2000, Boschung and Mayden 2004). However, this information is lacking for many species already listed as threatened or endangered. Moreover, because new taxa are still being discovered within the region, newly described fish are often on the threshold of extinction, and information on species requirements and life history characteristics is not included within recovery efforts (Warren et al. 2000). Ecological information can aid in the successful recovery of this aquatic biodiversity and can be essential in the identification of extinction prone species (Angermeier 1995, Warren et al. 1997).

In the recent years, species distribution models have become increasingly effective tools in the conservation and management of taxa (Manel et al. 2001, Guisan and Thuiller 2005). The uses for such models can be found within ecology, conservation biology, biogeography, evolution and research on climate change (see Manel et al. 2001 and Guisan and Thuiller 2005 for relevant reviews).

Occupancy models relate dichotomous spatial occupancy patterns (e.g., presence/absence) to environmental predictor variables (MacKenzie et al. 2006). The results from these models can guide discovery of additional populations of threatened species (Pfad and Witozski 1997), identify discontinuities within the distributional ranges (Wiser et al. 1998), aid in the creation of managed or protected areas (Bradbury et al. 2000), identify areas of possible reintroductions (Pearce and Lindenmayer 1998, Yanez and Floater 2000) and can identify populations that have a high risk of extirpation (Gates and Donald 2000). However, models that do not account for incomplete detection when modeling species distributions can underestimate the proportion of areas occupied by species of concern and can lead to biased models of species occurrence (MacKenzie et al. 2006). In Chapter Two, I adopt a strategy that accounts for incomplete detection to model the occupancy and detection of the two holiday darter taxa, *Etheostoma* sp. cf. *E. brevirostrum* A and B, within the Etowah River system of north Georgia (see Appendix A for information on the Etowah River system and the *E. brevirostrum* species complex). Furthermore, I use the distribution patterns of these taxa to discriminate among competing hypotheses regarding the factors (e.g., hydrogeomorphic, land use, etc) influencing variations in occupancy.

Life history characterizations enable the identification of extinction-prone species (Angermeier 1995), and can be essential information for the conservation of imperiled species (Burkhead et al. 1997). Such information is also important within the field of systematics, and sometimes can be the key factor in the placement of a species within a phylogeny of other species (see Page 1985). My third chapter's focus is on the reproductive aspects of four imperiled taxa of the Etowah River system: *Etheostoma* sp. cf. *E. brevirostrum* A and B (Amicalola and Etowah holiday darter), *E. etowahae* (Etowah darter) and *Percina kusha* (bridled darter; see Appendix A for information on target species). These four taxa have been identified as high priorities for life history studies based on their restricted ranges and their threatened or endangered status. For each of the target species, I summarize the reproductive behaviors observed, describe the typical spawning habitat and create a known spawning phenology. Moreover, I address two questions: over what period did the species of concern likely spawn given that detection was not perfect, and could the occurrence of spawning be better predicted by water temperature or day length (two factors hypothesized to influence onset and cessation of spawning in darters).

Together, these two studies provide much needed information for the conservation of these species. With knowledge about the habitat requirements and reproductive attributes of these species, we can begin to understand why these species face imperilment and develop management practices that incorporate their distribution and autecological characteristics.

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CHAPTER TWO

Occupancy Modeling and Estimation of the *Etheostoma brevirostrum* species complex within the Etowah River System

Introduction

Information on the factors that influence the distribution of organisms is of utmost importance in the field of ecology (Andrewartha and Birch 1954, Lawton 1996, Gaston and Blackburn 1999). Effective conservation management strategies depend on the credible knowledge of how species are distributed and their resource requirements (Warren et al. 2000). Based on dichotomous spatial occupancy patterns (e.g., presence/absence), occupancy of species within a site can be related to the site characteristics (e.g., conductivity, stream-size, etc.; Diamond 1975, MacKenzie et al. 2006). Therefore, it is possible to predict both the chance of occurrence at a particular location and the ways in which a species might respond to a change in a hypothesized covariate of occurrence (e.g., habitat type). However, species distribution models that do not account for imperfect detection can underestimate the proportion of sites occupied and the strength of the relationship of a hypothesized covariate (Tyre et al. 2003, MacKenzie et al. 2006).

Etheostoma brevirostrum, the holiday darter, is a sparsely distributed species found within the Coosa River system of Tennessee, Georgia and Alabama (Suttkus and Etnier 1991). Its populations are hypothesized to contain several distinct species unique to each sub-system of the Coosa River system (Etnier and Starnes 1993). Because of its restricted range, the species complex within the state of Georgia is listed as endangered by the Georgia Department of Natural Resources, Wildlife Resource Division (2008). The holiday darter is thought to occur in bedrock habitats and pool areas in streams ranging from small creeks to moderately sized rivers, and is typically found in cooler streams with “lush growths” of riverweed (*Podostemum ceratophyllum*; Etnier and Starnes 1993, Boschung and Mayden 2004).

Within the Etowah River system of northern Georgia two distinct forms of holiday darters are thought to represent separate taxa: *Etheostoma* sp. cf. *E. brevirostrum* A [Amicalola] and *E.* sp. cf. *E. brevirostrum* B [Etowah] (Freeman et al. 2005). The detection of these taxa has been based on the discovery of subtle but distinct differences between male nuptial coloration patterns. Burkhead et al. (1997) hypothesized that these differences are “analogous to those of the greenbreast darter group” analyzed by Wood and Mayden (1993), which has resulted in the description of three new cryptic species including the federally endangered Etowah darter, *E. etowahae*.

Little is known about the two holiday darter taxa of the Etowah River system. The two species are restricted to the Amicalola Creek watershed and the watershed of the Etowah headwaters (see Figure A.2 from Appendix A). Based on records available through the Georgia Museum of Natural History (GMNH), the Amicalola Creek form has been collected at 17 sites throughout the Amicalola catchment, and the Etowah River form has been collected at 13 sites within the Etowah headwaters catchment. Because their ranges are extremely limited, these taxa are believed to be highly susceptible to extirpation and moreover, extinction. The objectives of this study were 1) to refine the known geographic range of the holiday darter taxa within the Etowah River system, 2) to identify any discontinuities within this range, and 3) to collect data to discriminate among competing hypotheses regarding factors that lead to variation in the occupancy and detection of the holiday darter within both the larger context of a watershed and the smaller scale of a single site.

Methods

Study Sites

Site selection was limited to an area within a USGS 10-digit HUC of the Amicalola Creek watershed and a modified USGS 10-digit HUC of the Etowah River headwaters (hereafter, Amicalola Creek watershed and Etowah headwaters watershed). The Etowah headwaters watershed excluded the Shoal Creek system and any part of the watershed below State Route 136, which was a natural break in the hydrologic drainage pattern of the system. These two watersheds encompassed the known range of the holiday darter species. All easily accessible stream sites (e.g., road crossings, trails, easy boat access) were plotted based on a 1:100,000 national hydrography dataset (hereafter, nhd). If more than one access point was available on a stream segment, one point was randomly chosen to be included within the larger random selection. I did this to avoid randomly selecting two sites within close proximity. In total, I plotted 83 sites within the Amicalola Creek watershed and 65 sites within the Etowah headwaters watershed. Within each of the eight 12-digit HUC watersheds (hereafter, small watersheds) where either *Etheostoma* sp. cf. *E. brevirostrum* A or B had previously been collected, I randomly selected five accessible sites to be surveyed using a stratified random selection based on the Shreve (1966) method of stream classification (link magnitude). This approach accounts for subtle changes in stream-size and discharge that have no influence on stream order. Thus, each small watershed had one 1-2-link stream, three 3-10-link streams and one greater-than-10-link stream chosen at random. If one size-class was not represented within the small watershed, the number of missing sites was randomly assigned to other size-classes. This method of stratification was chosen based on occupancy records available through the GMNH, which indicated that holiday darters of the Etowah River were detected only in streams with a link magnitude greater than three. Only four survey sites were randomly selected in two of the five small watersheds within the Etowah headwaters watershed due to the scarcity of *E.* sp. cf. *E. brevirostrum* B (there were no records of detections of this species in one of these small watersheds and only a single record at the upstream-most portion of the other). This study design was intended to allow for sampling from various stream-sizes and habitat types while still ensuring that the chosen sites were a relatively unbiased representation of the watershed as a whole. In total, I selected 42 sites: 19 from the Amicalola Creek watershed and 23 from the Etowah headwaters watershed (Table 2.1 and Figure 2.1). Due to access problems, I was not able to sample two sites within the Etowah headwaters watershed. Of the sampled sites, nine had historical records of holiday darter presence, eleven had been previously sampled without detection of holiday darters, and twenty were new sites that had never been sampled according to the GMNH records.

Data Collection

Before each survey, I collected *in situ* site level metrics. Water temperature, conductivity, dissolved oxygen and pH were measured using a multi-probe (Hydrolab Datasonde® 4a). Turbidity was measured using a portable turbidity meter (Hach Turbidimeter® model 2100). Discharge was measured using a Marsh-McBirney Inc. FLO-MATE^{TM} Model 2000 portable flow meter with a top-setting wading rod (see Table B.2 from Appendix B for values).

All sampling was conducted during the growing season at baseflow conditions. Fishes were collected using a Smith Root Model 12B POW backpack electrofisher and one 8'x 6' seine (1/8" mesh) in conjunction with kick-seining and seine-hauling. Sampling began at a randomly selected distance from the access point of the stream, and at least 30 kicksets and seinehauls (hereafter, quadrats) were systematically placed (e.g. in a zigzag pattern) from downstream to upstream, with more quadrats added as needed to thoroughly sample all available habitat. Each quadrat was approximately four square meters. I recorded depth (to the nearest tenth of a foot), velocity (meters per second), presence of *Podostemum ceratophyllum* and substrate type at each quadrat. Substrate types were sand (<2mm), gravel (2-65mm), cobble (64-256mm) and boulder (256-2048mm). Additionally, I enumerated all species collected in each quadrat, and I measured the standard length of individual holiday darters to the closest millimeter. I re-sampled four randomly selected sites on a second visit in an effort to account for possible temporal differences in occupancy and detection as the growing season progressed.

Link magnitude, downstream link (the link magnitude of the next downstream confluence), elevation, link slope (the slope between the preceding upstream confluence and the next downstream stream confluence), catchment-size and land use characteristics were measured using ArcView 3.3 and ArcGIS 9.2 (ESRI, Redlands, California). I calculated link magnitude and downstream link (Osborne and Wiley 1992) using a 1:24,000 national hydrography dataset. Elevation and link slope were calculated using a USGS digital elevation model (10-meter cell). I delineated the upstream catchment of each sampling site using a digital raster graphic, and I calculated land use characteristics using the 2001 National Land Cover Dataset (Homer et al., 2004).

Data Analysis

The sampling data were used to estimate site occupancy (ψ) and detection probability (*p*) based on a modeling approach developed by MacKenzie et al. (2002). This method, which utilizes maximum likelihood theory, separates the probability of detecting an individual given that it is within the site (*p*) from the probability that at least one individual is present at the site at that time (ψ). Following the adaptation developed by Albanese et al. (2007), each quadrat was treated as a separate survey unit within a site. Thus, the detection probability was an estimate of the probability of detecting a species in a quadrat given that it was within the site. The benefit of this approach was that both microhabitat data and site level parameters could be modeled as covariates of the probability of detection.

Several assumptions were made in the modeling approach adapted for this study. First, the system was assumed to be demographically closed to any changes in occupancy during the sampling period (i.e., during a site visit). Because this study aimed to model a relatively immobile fish species (i.e., holiday darters are assumed not to travel long distances within a period of 1-2 hours), it was believed that the data satisfied this assumption. Secondly, this approach assumed that species were not falsely detected. Thirdly, occupancy and detection probability were assumed to independent across all sites.

Before analysis began, the sampling data were conditioned for modeling. I transformed enumerations of holiday darters within each quadrat at each site into a single vector composed of ones (detected) and zeros (not-detected). I treated replicate visits as additional quadrat information, and a binary indicator variable was assigned to represent quadrats that were sampled during the repeat visit (i.e., original visit=0, repeat visit=1). Each site had two sets of covariates: site (e.g., link magnitude, downstream link, link slope, etc.) and quadrat (e.g., depth, velocity, bed sediment type, etc.) The three bed sediment variables distinguished four substrate types: silt and sand, sand to fine gravel, coarse gravel to small cobble, and bedrock/boulder, with silt and sand as the reference habitat (i.e., 0,0,0). I also used a binary indicator variable to represent the existence of course bed-sediments within a quadrat. Covariates were scaled to bring mean values close to zero. The probability of occupancy was only modeled as a function of site level covariates, whereas detection probability was modeled as a function of both the quadrat microhabitat parameters and site level parameters.

Modeling detection probability within a quadrat as a function of site level parameters potentially addressed factors that influence the abundance of the target species. Royle and Nichols (2003) suggest that abundance is the most significant source of heterogeneity of detection probability. As the density of a species increases, so does the probability of detecting at least one individual. However, it is very hard to separate true detection or capture efficiency from abundance-induced changes in detection probability. Modeling detection as a function of site covariates might provide insight into the forces influencing the number of times a species is detected at a site. This information can then be used in future studies as *a priori* hypotheses with study designs that can quantify differences in actual abundances of a species.

Covariates represented *a priori* hypotheses regarding occupancy patterns. Each hypothesis represented one or more specific ecological mechanisms (Table 2.2). Based on the collection records of the GMNH and anecdotal observations, it was predicted that occupancy would be positively related to stream-size, elevation and slope, and negatively related to the proportion of non-forest within the catchment. In addition, based on anecdotal observations, it was predicted that detection probability would be positively related to depth and coarse sediments (coarse gravel to cobble) and negatively related to velocity. Based on records available through the GMNH, I hypothesized that the Etowah holiday darter would have a smaller proportion of area occupied and lower detection probabilities than the Amicalola holiday darter. To test this hypothesis, a binary indicator (watershed) was used to signify Amicalola holiday darters. Finally, it was hypothesized that darter abundance and consequently detection probability would also be strongly positively related to the site parameters of stream size, elevation, and slope, and negatively related to proportion of non-forest within the catchment. Due to the small range of values observed in conductivity over the sample sites, conductivity was not used as covariate of occupancy or detection.

Models were constructed using the R (R Development Core Team 2008) package RMark (Laake and Rexstad 2008), which accesses Program Mark's occupancy-estimation procedure (White and

Burnham 1999). In this modeling procedure, the logit link uses maximum likelihood estimation to model detection (p) and occupancy (ψ) probabilities as linear functions of covariates. Before the analysis, I tested for linear correlations between covariates. Any two covariates that had a Pearson coefficient (r) with an absolute value greater than 0.5 were not used within the same model structure for either detection or occupancy. However, correlated variables were used as separate covariates for detection and occupancy within the same model. In each detection probability model, the intercept and slopes were held constant because the probability of detecting a species in a particular quadrat or temporal change within a visit to a site was not important in this study. Instead, my goal was to examine how detection probability in general varied with chosen *a priori* covariates. I used a simple intercept-only model (i.e., $p=\beta_0$ and $\psi= \beta_1$) to test the relative support of models with more complex structure (i.e., covariate models). All possible linear additive combinations (i.e., no interactions) of both detection probability and occupancy models with their respective covariates were used. Due to the small sample size of the data, the number of parameters was limited to three covariates and intercepts for ψ and p (i.e., a model could have up to 8 total parameters).

Models were ranked using Akaike's Information Criterion (AIC) as corrected for small sample size (AICc; Akaike 1973, Hurvich and Tsai 1989, Burnham and Anderson 2002). AIC represents an estimate of the relative distance between the fitted model and the true mechanism (unknown) that actually generated the observed outcome. This value is calculated such that models that overfit the data are penalized by the number of terms used to estimate the model. In this approach it is not the size of the AIC value that it important, but rather the differences in values (Δ_i) from the best-supported model (Burnham and Anderson 2002). The larger the difference, the less plausible it is that the fitted model is the best model in the candidate set given the observed data. A fitted model that has a relative difference of less than two (ΔAIC< 2) indicates substantial support for the model. Models with this amount of relative difference are essentially ranked the same, and the relative weak support for a single best model suggests that there would be differences in the rankings given a different sample of similar size (Burnham and Anderson 2002). Moreover, models with a relative difference of greater than 10 ($\Delta AIC > 10$) essentially have no support and might be omitted from further consideration (Burnham and Anderson 2002). Based on the relative difference in fitted models, Akaike weights (w_i) were computed as $w_i = \exp(-1/2\Delta_I)/ \sum \exp(-1/2\Delta_I)$ where the denominator is a sum of $\exp(-1/2\Delta_I)$ for all models in the candidate set (Akaike 1978). A given w_i is the weight of evidence of the fitted model being the actual best model in the set. Evidence then can be judged by the ratio of Akaike weights (w_1/w_j) where model 1 is the best-supported model and j is the model of consideration (Burnham and Anderson 2002). Although AIC can be useful in selecting the best supported model within a candidate set, this ranking is not useful if no models within the set fit the observed data (Burnham and Anderson 2002).

To test the relative fit of the models, I utilized a goodness-of-fit test described by Williams et al. (2002). First, a dispersion parameter ($\hat{c}$) was calculated as the deviance divided by the degrees of freedom for the best-supported model. A parametric bootstrap approach was then used to calculate the mean ($\hat{c}$) for 10,000 bootstrap models. I simulated bootstraps using random numbers between 0 and 1. Each site had one random number for ψ, and n (the number of quadrats) random numbers for the detection probability in each quadrat. If the random ψ was less than ψ_i (estimated from site-specific model parameters) then the site was considered unoccupied and values for all quadrats were set to 0. Otherwise, a quadrat was given a value of 1 if its random number for p was more than p_i (also estimated by site-specific parameters). These bootstrap datasets mimicked the structure of the real dataset (i.e., the bootstrap had the same number of missing values at each site as the original data). The dispersion parameter ($\hat{c}$) was then divided by the mean ($\hat{c}$) of the 10,000 bootstraps to assess the relative fit. Values greater than 1 indicated overdispersion, whereas values less than 1 indicated underdispersion within the data.

I used parameters from the best-supported models ($\Delta AIC_C < 2$) to plot the geographic patterns of occupancy and site detection probability for all stream segments (1:100,000 nhd) within each occupied large watershed. Site detection was estimated based on an effort of 30 quadrats at a site and was calculated as $1-(1-p)^{30}$. Downstream link was calculated using a 1:24,000 nhd. Elevation and link slope were calculated using a 10-meter resolution digital elevation model, and the mean elevation of a stream segment was used as the value for the entire link. I used the catchments defined by the USEPA and USGS National Hydrography Dataset (Plus) based on a 1:100,000 nhd. These catchments were defined just upstream of each link confluence. Catchments were used to estimate forest cover for each link using the NLCD (2001), and this value was assumed to be constant for the entire stream link. Parameter estimates for detection for the best-supported models were averaged using the procedure outlined by Burnham and Anderson (2002) because values were similar and represented the same relationships.

Results

A total of 1,525 quadrats were sampled at 40 sites from June 25 to September 26, 2008. Over 11,200 fish representing 8 families, 20 genera and 38 species were collected. Holiday darters were detected in 15 of the 40 sites sampled (37.5%). Within the Amicalola Creek watershed, holiday darters were detected at 8 sites: four historically occupied, one historically unoccupied, and three sites that had not been sampled, according to the GMNH records. Within the upper Etowah River headwaters watershed, holiday darters were detected at 7 sites: three historically occupied, two historically unoccupied and two sites that had not been sampled, according to the GMNH records. Holiday darters were detected in 87 (5.7%) of the total quadrats sampled and were detected in 13.8% of the quadrats at sites where there was at least one detection. The mean number of detections in quadrats at a site was 5.8 with a maximum of 13 detections occurring in the Etowah River at State Route 52. Including the detections made in this study, holiday darters have been historically detected at 38 sites within the Etowah River watershed: 21 within the Amicalola watershed and 17 within the upper Etowah headwaters watershed (see Figure B.1 of Appendix B for map of historical and newly detected sites).

Based on the goodness-of-fit test employed in this study, there was no reason to conclude that the modeling technique did not fit the data. The dispersion parameter ($\hat{c}$) was estimated as 0.89, indicating only slight under-dispersion. Additionally, there was essentially no support for detection models that used a binary indicator to represent replicate visits to a site, indicating that there was no temporal change in detection over the sampling period. However, due to the small sample size of these replicate visits (*n*=4), the temporal effect on occupancy probability could not be estimated.

Several models based on the *a priori* hypotheses had relatively strong support as predictors of occupancy patterns and detection probability (Table 2.3). Three models had substantially more support ($\Delta AIC_C < 2$) than the other models within the candidate set. All three best-supported models suggest the proportion of area occupied by these two species was 0.525, which is 40% higher than the naive estimate (0.375). These three best-supported models included the same covariates for detection (elevation, link slope and proportion of non-forest within the catchment) and similar parameter estimates (Figure 2.2). According to the three models, detection increased with elevation and decreased as link slope and the proportion of non-forest within a watershed increased (see Table 2.4 for range of values observed within study). Conversely, there was substantial support for three different models regarding occupancy probability; however each of these models had a parameter that accounted for stream network position (downstream link). According to the parameter estimates from the best-supported model (ψ (d-link) *p* (elevation, slope, pnf)), the probability of occupancy was positively related to downstream link (Figure 2.3). The next most highly ranked model (1.5 times less likely based on evidence ratio) also suggested that the probability of occupancy was related to downstream link, but sites within the Amicalola watershed had higher occupancy probabilities at sites with similar downstream links. Based on the parameter estimates from this model, the proportion of area occupied by the Amicalola holiday darter (0.582) was higher than the Etowah holiday darter (0.477). The third model (1.75 times less likely than the best-supported model) suggested that the occupancy probability was positively related to both link slope and downstream link. Downstream link was positively related to link magnitude (r=0.58) and negatively related to elevation (r=0.73). However models using link magnitude and elevation were not well-supported ((ΔAIC_C>2)

In all candidate models with a difference in AIC_C of less than 10 there was both a detection parameter that accounted for the proportion of non-forest within the catchment and an occupancy parameter that accounted for the stream position within the stream network (downstream link). There was also considerable support for several additional parameters for both detection and occupancy (Table 2.4). Additional positive covariates of occupancy included link slope, link magnitude and a binary variable indicating the site was within the Amicalola watershed, whereas the proportion of non-forest within the catchment was negatively related to occupancy probability. Other positive covariates of detection included elevation at the site and link magnitude, whereas link slope and downstream link were negatively associated with detection. There was also support for a curvilinear relationship between occupancy probability and downstream link, however estimates within these models had wide credible intervals and the effect size for the squared term was quite small. There was relatively no support ($\Delta AIC_C > 10$) for microhabitat parameters (e.g., velocity of quadrat) in the detection probability models, and there was even less support for an intercept only model of detection and occupancy (i.e. no covariates), which was 133 billion times less likely then the best-supported model.

Using the parameter estimates from the best-supported models, I was able to identify areas predicted to have high values for occupancy and detection probability (Figure 2.4). These maps compare the occupancy patterns of the well-supported models (top panels), and the map of site level detection (bottom panel) illustrates that detection is low for many areas where occurrence probability is highest.

Discussion

This study increases the known localities where holiday darters have been encountered to a total of 38 sites within the Etowah River system. Using the data gathered during the course of this study, I was able to identify several factors influencing occupancy and detection patterns. Downstream link was found to be a good predictor of occupancy patterns for these taxa, and forest cover was found to be a good predictor of the probability of detection within a quadrat conditional on the site being occupied. In other words, holiday darters would be far more likely to be observed in streams that have a low position in the stream network with high forest cover in the watershed upstream of the site. This study has also shown that for much of the distributional range where the two holiday darter taxa are known to occur, site level detection is high ($\mu=0.98$; based on a visit where 30 quadrats are sampled). However, for both taxa there is a gap in the known range between the lower-most collected point within the watershed (Amicalola at

State Route 53 and Etowah River 0.9 miles downstream of Castleberry Bridge Rd) and the next upstream point. For these sites that are low in the stream network position for Amicalola Creek and the Etowah River mainstem, site level detection is much lower than the upstream population (μ=0.60). This pattern corroborates the information available in GMNH sampling records. Two of the sites sampled in this study (Amicalola Creek at Lumpkin CR 92 and Etowah at Dawson CR 2) were within the observed gap of known occupied sites that had been previously sampled by the U.S. Geological Survey, J. Malcolm Pierson (Alabama Power Company) or the University of Georgia. Although each of these sites had been sampled at least twice during the previous studies, holiday darters were not detected. However, during this study the species was detected at both sites. Conversely, I visited the lowest known locality of the Amicalola holiday darter (Amicalola Creek at SR 53), and no detection was made. At this site, holiday darters had been detected on two of the eight historical visits to the site. These results suggest that either capture efficiency is low, abundance is low or that the taxa immigrate/emigrate to and from sites during different seasons or years.

In all well-supported models regarding occupancy patterns, downstream link was included as a positive covariate of occupancy probability. Downstream link carried information about other variables including size, connectivity, production, and greater flow stability (Schlosser 1987, Taylor et al. 2006), and thus may represent these factors with fewer parameters. However, it is hard to tease apart these relationships with occupancy. For example, models that included a parameter accounting for link magnitude (stream size) had considerably less support than models accounting for downstream link. This may be due to the fact that downstream link carried information about stream size and additional information about connectivity or stream network position (and possibly a lot more). Other studies have also found a correlation between downstream link and fish distributions. Osborne and Wiley (1992) found that downstream link explained most of the variance in local species richness in mid-western streams. Wenger et al. (2008) found downstream link to be a predictor for the distribution of two of three other darter species modeled in the Etowah River system. Additionally, other metrics of stream network position have been identified as good predictors of fish assemblages and might help to begin to separate the information carried by downstream link. Smith and Kraft (2005) found that confluence link, the number of confluences downstream of a site before the mainstem, was a significant predictor of stream fish assemblages, but given that the two holiday darter species within the Etowah River system are confined to two headwater systems, this covariate was not used due to the ambiguity of defining a "mainstem" within these systems. Downstream link was also found to be a covariate of detection probability in several of the models in this study, though with considerably less support. Again, I interpret downstream link as carrying information about several ecologically influential factors (including elevation since the two were highly correlated). Wenger et al. (2008) found downstream link to be correlated with detection probability for two of the three percids studied in the Etowah River system as well.

A connection between land use and stream fish assemblages has been well demonstrated within many studies (Jones et al. 1999, Wang et al. 2001, Walters et al. 2003a, Roy et al. 2007). Wenger et al. (2008) report that historic and current land use models were able to predict fish species distributions of interest better than any hydrogeomorphic model used in their study. The data from my study shows that decreases in the amount of the forested area within a catchment will more likely affect the detection of holiday darter species in quadrats rather than occupancy patterns. In this case, it is most likely that detection is a representation of the abundance of holiday darters. Because forest cover was not highly correlated with link magnitude or elevation, I assumed the relationship was not confounded by longitudinal differences in patterns of occupancy and detection. However, the utilization of land use data (NLCD 2001) as a covariate of occupancy or detection also assumes that the distributional patterns observed are the effects of contemporary rather than historical land use. Data for another *Ulocentra* species in the Etowah River system supports the notion that contemporary land use influences the two holiday darter taxa. Wenger et al. (2008) reported that the occupancy patterns of *Etheostoma scotti* were best predicted by the amount of forest within upstream catchments. Additionally, erosion could result from the deforestation of catchments, and Storey (2003) reported that the amount of fine sediment had an effect on the spawning behavior of *E. scotti.*

Several studies have found a strong correlation between stream slope and fish assemblage structure in Piedmont streams. Walters et al. (2003b) found that stream slope and associated variables measured *in situ* predicted assemblage structure more accurately than longitudinal changes in stream-size. Wenger et al. (2008) found that remotely sensed slope measurements could adequately predict species distributions and detection probabilities when used in conjunction with other hydrogeomorphic and land use variables. I also found that link slope was a well-supported predictor of detection within quadrats. However, whereas Wenger et al. (2008) found that map slope (the mean slope of large tributaries within a tributary system) was positively correlated with the detection probability of two percids within the Etowah River system, the results from this study suggest a negative correlation between link slope (the change of elevation divided by the length of the stream link) and the detection of holiday darters in quadrats. This discrepancy could be explained by the fact that the species of interest in Wenger et al. (2008) were known to occur in swift-water habitats. Additionally, the negative relationship observed in this study might also be due to the range of values observed. Many sites sampled within this study were within the headwaters of the Etowah mainstem and Amicalola Creek, and the maximum slope observed was 0.05. In addition, slope measured in this study represented remotely sensed differences in elevation for entire stream segments, whereas Walters et al. (2003a) found a positive relationship between reach slope (i.e., approximately 100m) measured in the field and fish assemblages. The negative effect suggested for holiday darter taxa possibly could be due to cascading changes in elevation seen within the headwaters systems (i.e., Jones Creek, Two Run Creek, Upper Amicalola Creek, etc). These cascades could potentially act as a barrier to colonization (Edds 1993). Furthermore, given that holiday darters spawn in slower habitats (e.g., runs in pools), detection might be a function of the availability of this habitat type, which occurs in lower gradient stream localities. Although there was, on the contrary, relative support for a positive relationship between link slope and occupancy patterns, the credible interval of parameter estimates within these models overlapped zero.

I initially included elevation as a covariate of detection because of possible differences in abundance due to shorter spawning seasons where temperatures rise quickly in the springtime. However, it is hard to separate these effects from the other information that elevation might be carrying. Elevation was found in all the models with substantial support, and 11 of the 20 well-supported models within the candidate set. According to parameter estimates, localities at higher elevations had a greater detection probability than did sites with low elevations. While it is feasible to consider that these results were related to the life history of holiday darters (i.e., temperature induced difference in the abundance of these species), it is equally feasible to consider elevation as a representation of actual differences in capture probabilities.

One of the goals of this study was to test the hypothesis that occupancy and detection patterns were different for the two holiday darter taxa within the Etowah River system. There was substantial support for the hypothesis that the occupancy patterns were indeed different and considerable support for the hypothesis that detection probability was different between the two taxa. However, using the watershed of the site (binary indicator of Amicalola holiday darter) as a covariate of occupancy or detection provided estimates with little accuracy (i.e., credible interval overlapped zero), especially for estimates of detection probability. Freeman et al. (2005) provided a different conservation status for each of the holiday darter taxa within the Etowah, suggesting that the Etowah holiday darter was more imperiled than the Amicalola holiday darter. While I did not intend to assess the level of imperilment that each of these taxa demonstrate, my results suggest that the Etowah holiday darter has a lower probability of occurrence in streams with a downstream link of less than 30, indicating that this species is more restricted to the Etowah mainstem and direct tributaries of the mainstem.

This study suggests that *a priori* selected site level conditions were more predictive of holiday darter detection patterns than *a priori* selected microhabitat variables. However, this does not imply that in-stream parameters are not important in structuring populations of holiday darters. Instead, this outcome most likely suggests that site conditions are more predictive of species abundances (i.e. number of quadrats occupied) or that these variables represent microhabitat relationships (e.g., availability of specific habitat types) with fewer parameters.

Projections of stream segments where occupancy and detection probability are likely to be high will be useful for identifying un-sampled areas that might host populations of these taxa. While this identification was only a snapshot (i.e., it does not take population dynamics into account) of the holiday darter populations within the Etowah River system, these projections help us understand how certain parameters of detection and occupancy influence the populations of these taxa. Although the effect of impoundments on detection and occupancy for the holiday darter taxa was not estimated due to singularities within the data set (i.e., no holiday darters were detected upstream of impoundments), if impoundments or other barriers (e.g., culverts elevated above the streambed, Norman et al. 2009) affect movements by holiday darters, the maps shown in Figure 2.4 provide a basis for estimating potential areas of colonization if barriers were to be removed.

Conclusions

While much effort has been made to describe the way fish assemblages vary spatially according to regional characteristics (e.g., catchment properties), site conditions and in-stream microhabitats, fewer studies have focused on factors shaping the distribution of individual stream fishes. Because the Endangered Species Act is a species-oriented approach to conservation, information on species distributions and mechanisms that affect their occupancy patterns and abundances is of utmost importance. While the holiday darter is not listed as a federally endangered species, it is listed as endangered by the State of Georgia and is considered a species of special concern (GADNR 2008). The methodology outlined in this study and that of Albanese et al. (2007) has several benefits over the traditional MacKenzie et al. (2002) model. By subdividing sites into quadrats and using each quadrat as a survey unit, differences among sites in the abundance of a species were partially accounted for by the detection probability. However, if each site was left undivided, the information used to test for site effects on abundance would have been discarded and multiple visits would have been needed to support occupancy and detection estimation. Furthermore, using this approach to model distributional patterns of species facilitated comparison of specific, biologically interesting hypotheses regarding factors influencing stream occupancy as well as species detection within sites.

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Table 2.1. Locations of sampling sites within the Etowah River system. Easting and Northing refer to UTM 16 coordinates.

Stream Name	Easting	Northing
Jones Creek upstream of confluence with Etowah	765849.235	3829463.819
Etowah River at CR 2	772169.893	3818553.75
Bull Creek at Bull Creek Way *	768477.3873	3832654.611
Nimblewill Creek at Nimblewill Gap Rd.	761215.7512	3829113.934
Nimblewill Creek at CR 128*	763425.7345	3827758.315
Unnamed Tributary to Cochrans Creek at CR 43	756508.3709	3824385.61
Amicalola Creek at CR 192	756480.7354	3815032.28
Unnamed Tributary to Amicalola Creek u.s. of Amicalola Church Rd.	753293.3083	3815764.242
Unnamed Tributary to Little Amicalola Creek at SR 136	752451.0699	3823603.827
Unnamed Tributary to Cochrans Creek at New Hope Rd*	757344.6819	3820914.304
Unnamed Tributary to Amicalola Creek adjacent to SR 53*	755871.3936	3813103.953
Cochrans Creek at SR 183	757121.195	3819239.964
Unnamed Tributary to Amicalola Creek at Falls Trail Rd.	747146.417	3824062.35
Mill Creek at CR 115	765847.0033	3822919.951
Etowah River at CR 75	768453.7514	3828168.327
Wildcat Creek in Dawson Forest WMA	748407.7073	3820855.657
Fall Creek in Dawson Forest WMA	747835.574	3820826.556
Unnamed Tributary to Amicalola Creek d.s. of Liberty Lane	757838.1576	3807255.774
Little Amicalola Creek at CR 25	753909.1304	3821231.506
Amicalola Creek at CR 28	749660.558	3825547.221
Etowah River 0.6 miles d.s. of CR 2	772129.0977	3818202.198
Calhoun Creek upstream of Confluence with Etowah River	770377.0927	3815805.394
Amicalola Creek at SR 53	756179.388	3812956.992
Gad Creek at SR 52	758518.9424	3824911.175
Unnamed Tributary to Little Amicalola Creek at CR 26	751748.0027	3822929.818
Little Amicalola Creek at 136	752020.4231	3823781.193
Little Amicalola Creek d.s. of Johnnytown Rd.	750306.5689	3826737.871
Cochrans Creek d.s. of Dan Fowler Rd.	757079.1761	3827346.421
Etowah River at FS 28-1	766136.2982	3833432.084
Ward Creek CR 28	766473.5832	3835344.263
Edmunston Creek off CR 361	767097.9462	3834329.361
Two Run Creek d.s. CR 187	768187.4233	3834634.958
Moss Creek off FS 28-1	764318.8622	3830901.877
Jones Creek off FS 77A	762727.5586	3832417.524
Etowah River at SR 52	769543.1475	3825373.804
Hurricane Creek at CR 202	766341.2907	3824373.868
Braggs Branch 0.43m u.s. of confluence with Etowah River	772711.7424	3814996.919
Mudd Creek d.s. of CR 75	769495.1925	3827380.998
Hurricane Creek 0.17m u.s. of confluence with Etowah River	769297.0466	3823449.478
Tobacco Pouch Creek d.s. of Tobacco Pouch Lane	768578.0864	3824460.834

*Indicates a site with replicate visits.

Table 2.2. *A priori* hypotheses regarding occupancy and quadrat detection patterns for *Etheostoma brevirostrum* species complex with the Etowah River system.

Hypotheses Regarding Occupancy and Detection (ψ) Probabilities (*p*)	**Covariate**
Larger sites have higher temperatures but more production (prey).	Link Magnitude (+)
Sites with high downstream link are lower within stream networks and have more connectivity for colonization purposes.	Downstream Link (+)
Sites with higher elevation should have cooler springtime temperatures and holiday darters spawn in lower temperatures (10-17°C), however, sites with lower temperatures also have less prey production.	Elevation (+)
Sites with a large amount of disturbance upstream of the site have higher turbidities affecting spawning, higher sediment loads and lower prey production.	Proportion of non-forest in upstream catchment (PNF) (-)
Higher slopes at sites provide coarser sediments, however, lower slopes provide more pool/run habitat for spawning.	Link Slope (?)
Areas of slow velocity provide better habitat for holiday darters.	Velocity (-)
Areas of high depth are indicative of pool habitats where holiday darter are though to occur.	Depth (+)
Coarse sediments provide the best spawning habitat for holiday darters.	Sediment Characteristics

Table 2.3. Model structure, deviance, number of parameters (*K*), relative difference in AIC_C (ΔAIC_C) and model weights (w_i) for well supported occupancy models (ΔAIC_C<10) and an intercept-only model for *Etheostoma* sp. cf. *E. brevirostrum* A & B. Intercepts only (i.e., no covariates) are designated as periods within the model structure.

Model	**Deviance**	***K***	**ΔAIC_C**	**w_i**
Ψ(DLink) *p*(Elev, Slope, PNF)	497.0177	6	0.0000	0.2659
Ψ(Watershed, DLink) *p*(Elev, Slope, PNF)	494.828	7	0.7649	0.1814
Ψ(DLink, Slope) *p*(Elev, Slope ,PNF)	495.1747	7	1.1116	0.1525
Ψ(DLink, PNF) *p*(Elev, Slope, PNF)	497.0115	7	2.9484	0.0609
Ψ(DLink) *p*(DLink, Slope, PNF)	500.0136	6	2.9959	0.0594
Ψ(Watershed, DLink, Slope) *p*(Elev, Slope, PNF)	494.3748	8	3.4568	0.0472
Ψ(Watershed ,DLink) *p*(DLink, Slope, PNF)	497.7508	7	3.6877	0.0421
Ψ(Watershed, DLink, PNF) *p*(Elev, Slope, PNF)	494.7145	8	3.7966	0.0398
Ψ(DLink, Slope) *p*(DLink, Slope, PNF)	498.0953	7	4.0321	0.0354
Ψ(DLink, Slope, PNF) *p*(Elev, Slope, PNF)	495.1747	8	4.2568	0.0316
Ψ(Watershed, DLink) *p*(LMag, Elev, PNF)	499.9965	7	5.9334	0.0137
Ψ(DLink, PNF) *p*(DLink, Slope, PNF)	500.0134	7	5.9503	0.0136
Ψ(Watershed, DLink, Slope) *p*(DLink, Slope, PNF)	497.299	8	6.3811	0.0109
Ψ(Watershed, DLink, PNF) *p*(DLink, Slope, PNF)	497.7165	8	6.7985	0.0089
Ψ(DLink, Slope, PNF) *p*(DLink, Slope, PNF)	498.086	8	7.1680	0.0074
Ψ(Watershed, LMag, Elev) *p*(DLink, Slope, PNF)	499.0533	8	8.1353	0.0046
Ψ(Elev, LMag) *p*(Elev, Slope, PNF)	502.7056	7	8.6425	0.0035
Ψ(Watershed, DLink, PNF) *p*(LMag, Elev, PNF)	499.8725	8	8.9546	0.0030
Ψ(Elev, LMag, Slope) *p*(Elev, Slope, PNF)	500.2536	8	9.3356	0.0025
Ψ(DLink) *p*(DLink, PNF)	509.3836	5	9.5852	0.0022
Ψ(.) *p*(.)	558.537*	2	51.2982	0.0000

*Indicates that -2 Log likelihood was provided instead of deviance

Table 2.4. Summary statistics for continuous covariates measured at 40 collection sites.

Variable	Abbrev.	Group	Mean	SD	Max	Min
Velocity (m/s) at 60% depth in the quadrat	Velocity	Quadrat	0.27	0.22	1.5	-0.03
Depth (ft) of quadrat	Depth	Quadrat	0.71	0.44	3.2	0.06
Substrate of quadrat (3 binary variables)[1]	SedA, SedB, SedC	Quadrat	-	-	-	-
Quadrat from repeat visit (binary)	Resample	Quadrat.	-	-	-	-
Link Magnitude	Lmag	Site	34.51	58.96	219	1
Downstream Link	Dlink	Site	67.8	80.1	241	2
Elevation	Elev	Site	440.62	57.3	577.82	333.56
Link Slope	Slope	Site	0.02	0.01	0.05	0.003
Proportion of non-forest within upstream catchment	PNF	Site	0.07	0.08	0.34	0.001
Site within the Amicalola Creek Watershed (binary)	Watershed	Site	-	-	-	-

[1] The three substrate variables distinguished four substrate types: silt and sand, sand to fine gravel, coarse gravel to small cobble, and bedrock/boulder, with silt and sand as the reference habitat (i.e., 0,0,0).

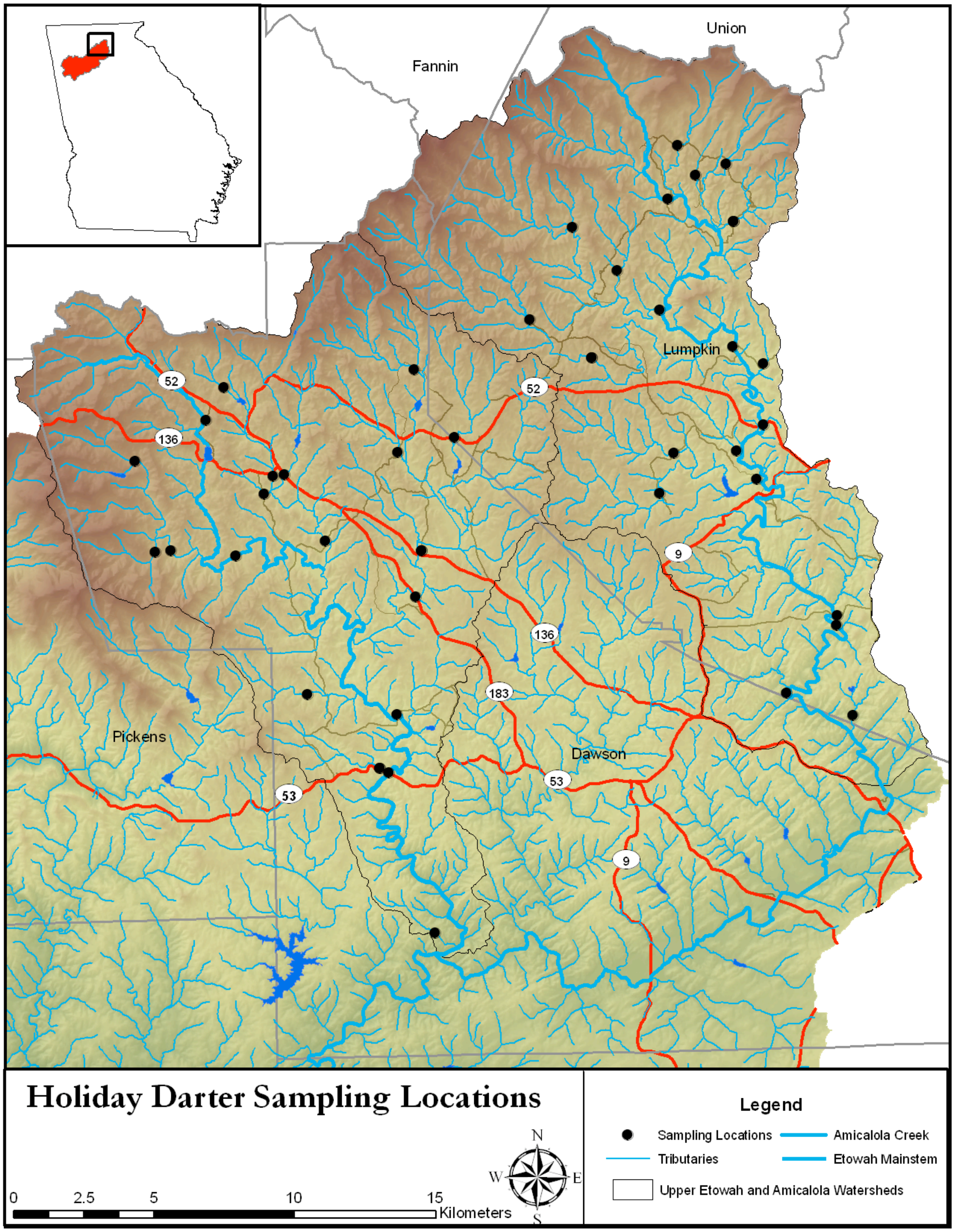

Figure 2.1. Locations (n= 40) sampled for holiday darters within the Etowah River system, GA (inset), June-September 2008.

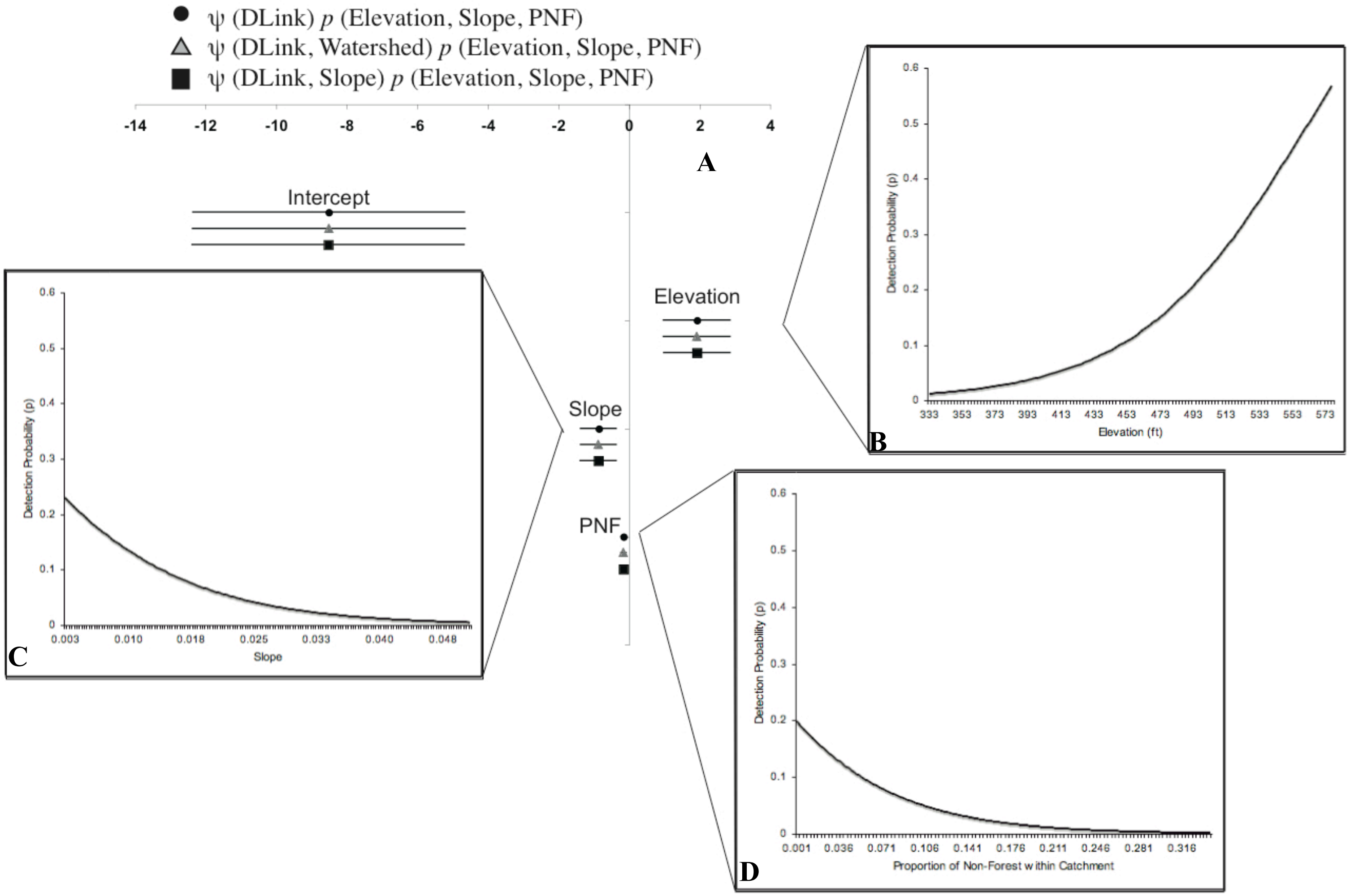

Figure 2.2. Parameter estimates for detection probability of best-supported models ($\Delta AIC_C < 2$) and estimated parameter effects on detection probability. Best-supported models are listed in the legend at the top right and are in order from lowest to highest AIC_C. Parameter estimates (A) are indicated by a shape that corresponds to the model listed in top right. Estimates are shown with 95% credible intervals. Estimated effects on detection probability for each parameter (B-D) are from the best-supported model within the model set (ψ(DLink) p (Elevation, Slope, PNF)) and were calculated using the range of values observed in the study and mean values of additional parameters within the model. See Table B.1 from Appendix B for values of parameter estimates.

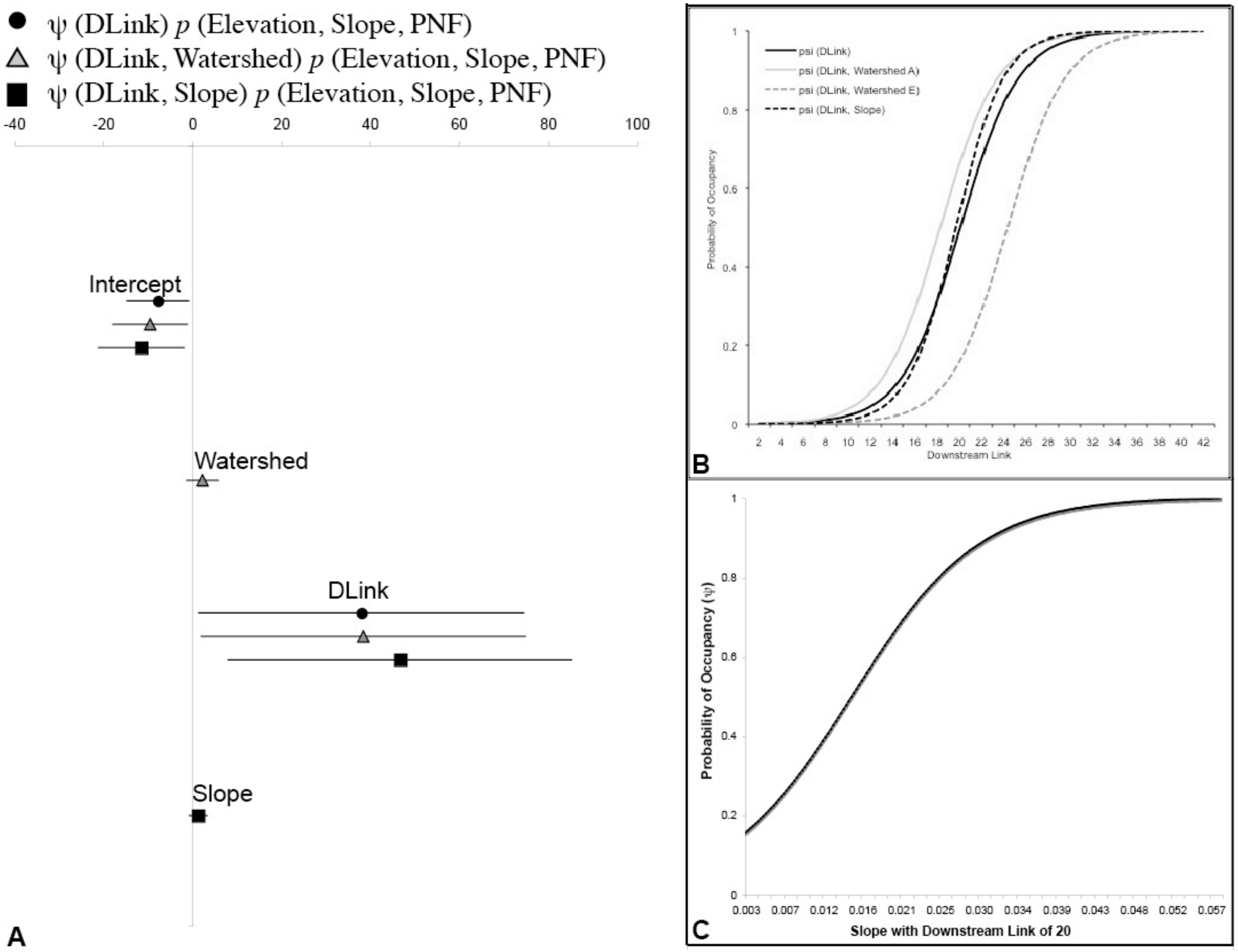

Figure 2.3. Parameter estimates for best-supported models of occupancy ($\Delta AIC_C < 2$) and estimated parameter effects on occupancy. Best-supported models are listed in the legend at the top right and are in order from lowest to highest AIC_C. A) Parameter estimates are indicated by a shape that corresponds to models listed in top right and are shown with 95% credible intervals. B) Top right panel shows the effect of downstream link on occupancy probability for the three different models. The effect of a site being within the Amicalola is shown in solid grey whereas sites in Etowah are show by a dashed grey line. The effect of downstream link (black dashed line) for the model ψ (Dlink, Slope) was calculated using the mean slope observed in the study. C) The bottom right panel shows the effect of slope on occupancy at sites with a downstream link of 20. See Table B. 1 from Appendix B for values of parameter estimates.

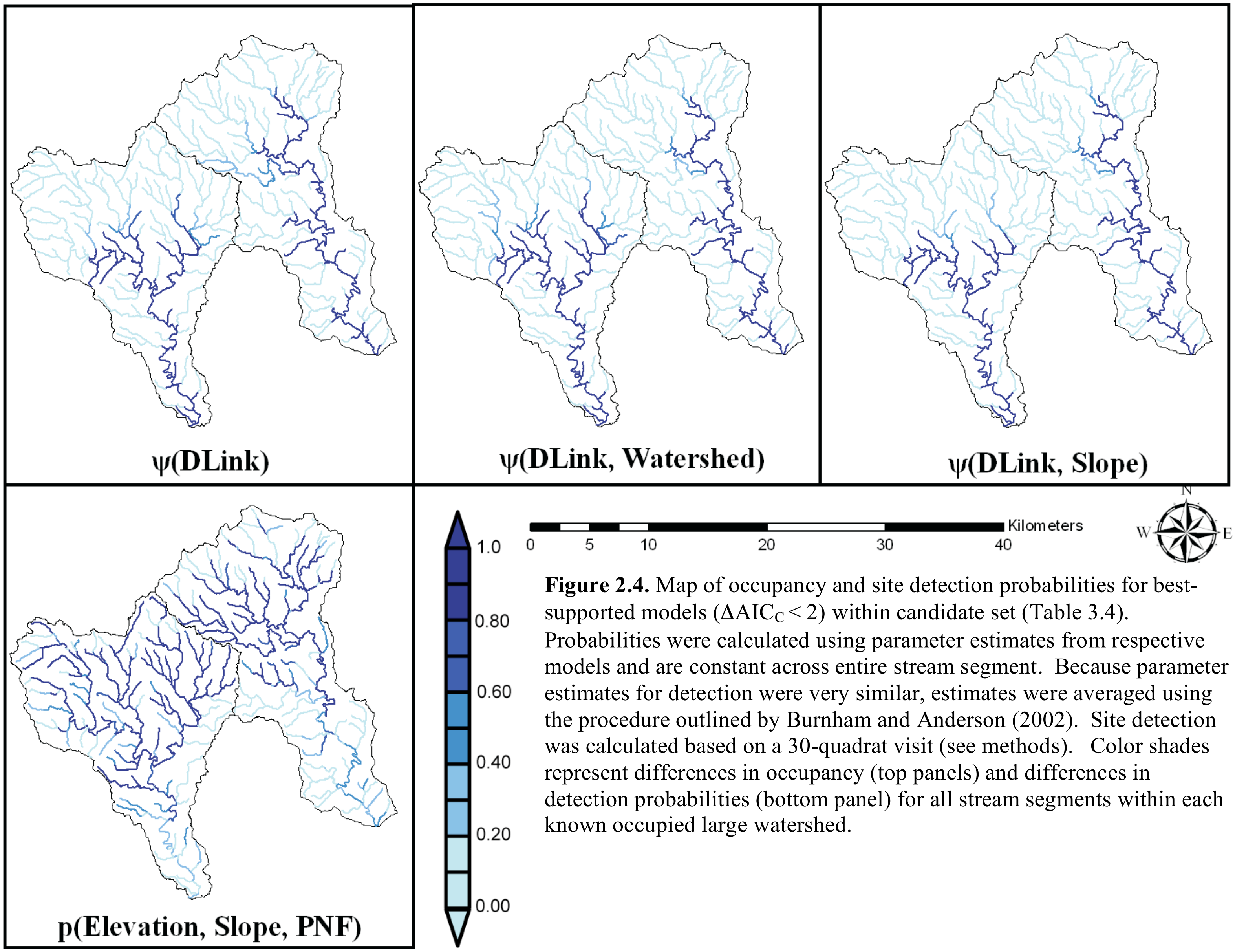

Figure 2.4. Map of occupancy and site detection probabilities for best-supported models ($\Delta AIC_C < 2$) within candidate set (Table 3.4). Probabilities were calculated using parameter estimates from respective models and are constant across entire stream segment. Because parameter estimates for detection were very similar, estimates were averaged using the procedure outlined by Burnham and Anderson (2002). Site detection was calculated based on a 30-quadrat visit (see methods). Color shades represent differences in occupancy (top panels) and differences in detection probabilities (bottom panel) for all stream segments within each known occupied large watershed.

CHAPTER THREE

Reproductive Aspects of Four Imperiled Darter Taxa of the Etowah River System

Introduction

Accurate knowledge of reproductive attributes such as time and duration of spawning, habitat use, reproductive behaviors and temporal migrations enable the identification of extinction prone species and is essential for effective management conservation efforts (Page 1985, Angermeier 1995, Boschung and Mayden 2004). The family Percidae is one of the most imperiled families of North American fishes, with about one third of its species at some degree of risk, yet information on basic life history characteristics, including reproduction, is lacking for many darter species (Boschung and Mayden 2004). Extensive effort has been applied to determining the environmental cues that trigger the start and end of reproductive seasons for percids (Hubbs and Strawn 1957, Winn 1958a, Marsh 1980, Hubbs 1985, Weddle and Burr 1991, Bonner et al. 1998), but not much is known about how reproduction varies within a season.

Occupancy models that account for imperfect detection (MacKenzie et al. 2006) offer an approach to improving estimation of variation in spawning based on field observations. Generally, occupancy models express the probability of species occurrence given that the species might not be detected. In this study, occupancy models have been used to predict the occurrence of the behavioral act of spawning by different percid species, given incomplete detection of the behavior. Within studies on fish reproduction, detection of spawning is complicated by variations in site conditions (e.g., stage, temperature, turbidity, etc.), aggression levels (male/male chasing and fighting), courtship practices (e.g., length, ambiguous behaviors, etc.) and observer error (e.g., disruption of spawning due to movement of the observer). Therefore, it is quite feasible to visit a site and fail to observe a spawning event, even when spawning actually is taking place. By adapting a modeling strategy that accounts for imperfect detection, a spawning season can be delineated with a known level of confidence, discontinuities within the season can be modeled according to conditions (e.g., temperature, turbidity, etc.) and detection can be modeled as a function of local habitat features (e.g., riffle, run, pool, etc.) as well as conditions that alter the proportion of the population that is reproductively active (e.g., water temperature, photoperiod).

In the spring of 2007 a two-year project was initiated to characterize the spawning behavior of four imperiled species of the Etowah River system of northern Georgia: the Amicalola and Etowah holiday darters (*Etheostoma* sp. cf. *E. brevirostrum* A & B), the Etowah darter (*E. etowahae*) and the bridled darter (*Percina kusha*). These species were identified as high priorities for life history studies due to their threatened or endangered status and their limited geographic distributions (see Appendix A). The objective of this study was to examine the behavioral characteristics of spawning pairs of the targeted species of darters within several sites of the Amicalola Creek watershed, as well as two sites near the headwaters of the upper Etowah River mainstem. More specifically, this project intended to assess the reproductive behaviors, spawning habitat use, and duration of spawning season of the four target species, and to compare their attributes to other species within the same subgenera. Additionally, the detectability and probability of spawning occurrences for the Amicalola holiday darter and the Etowah darter were explored in order to further understand these species' reproductive phenologies.

Study Site Descriptions

Based on sampling records from the Georgia Museum of Natural History (GMNH), five easily accessible sites were chosen where multiple target taxa were expected to be present and/or abundant (Table 3.1 & Figure 3.1). All sites were located within the upper Etowah River system in north Georgia. Three sites were chosen within the Amicalola catchment (two on Amicalola Creek and one on Cochrans Creek), and two sites were chosen within the upper Etowah catchment near the headwaters (both on the Etowah mainstem). All sites were characterized by a low to moderate gradient. Sites on Amicalola Creek varied from 8-15 meters in width, and were composed of a variety of substrate types including sand, gravel, cobble, boulder and bedrock. The study site on Cochrans Creek varied from 5-8 meters in width, and the substrate was less heterogeneous, composed mostly of sand, gravel, and cobble, with some riprap near the culvert crossing. The two sites on the Etowah mainstem varied from 15-20 meters in width, and contained a variety of substrate types similar to those of the sites on Amicalola Creek. All study sites ranged in elevation from 396-452 meters with a link magnitude of 14-19 (calculated with a 10 meter resolution digital elevation model and a 1:100,000 national hydrography dataset, respectively). While these sites were typical of the two holiday darter taxa within the Etowah, the sites were higher in elevation and lower in link magnitude than a large portion of the population of both the Etowah darter and the bridled darter (see Figure A.1 from Appendix A).

Methods

Sites were snorkeled by a team of observers (2-3) during the hypothesized spawning season of the target species. In the early spring of 2007, preliminary trips were taken to the study sites to assess the available habitat, install headpins for comparing stage conditions between visits, install hourly temperature loggers (ONSET Optic Stowaway Temp^{TM}), and to note the conditions and locations of each targeted species in order to increase snorkeling efficiency at each site. We visited sites bi-weekly in 2007 and 2008, unless conditions were above base flow. Study reaches varied from 60-100 meters in length, according to the amount of available habitat and the density of the targeted species. Snorkeling began on April 20, 2007 in an attempt to capture the onset of the spawning season for the four target taxa. Observations continued into the summer of 2007 until spawning activity was thought to have come to an end (July 8, 2007). Snorkeling in the second year of study began on March 27, 2008, due to the evidence of an earlier spawning season for *Etheostoma* sp. cf. *E. brevirostrum* A & B than previously hypothesized. As in 2007, surveys were conducted bi-weekly, when possible. Observations continued into the summer of 2008 until spawning activity was thought to have come to an end (July 22, 2008).

Water-quality parameters, stage height, water visibility and weather conditions were recorded prior to each snorkel observation. Conductivity, dissolved oxygen, ph, turbidity and water temperature were measured using a Hydrolab Datasonde® 4a and a Hach Turbidimeter® model 2100P.

Snorkeling Methods: 2007

During sampling visits, the channel length was divided among the number of observers, and each observer worked in an upstream direction for the length of the sample reach. Following the first pass through the reach, observers switched sections of the channel and made a second pass. During each pass, snorkelers recorded the number of individuals of target species and the number of interacting conspecifics. Behaviors such as following or chasing, mounting or any other form of contact, territorial aggression, and other related activities were noted by each observer. During observations of spawning pairs, observers noted the behaviors of both the male and female, the total area used during spawning, the number of mounting occasions, the number of times the pair quivered, the duration of contact, and the approximate time-span of the entire spawning event. A weighted marker was placed at the location of each observed spawning act. Once snorkeling had concluded, we measured the velocity, depth, and bed sediments at each spawning site. Velocity and depth were measured using a Marsh-McBirney Inc. FLO-MATE^{TM} Model 2000 portable flow meter with a top-setting wading rod. Depth was recorded to the nearest tenth of a foot and converted to centimeters for analysis; velocities at 60% depth and adjacent to the substrate of the spawning location were measured in meters per second. Substrate used at the location of the spawn was categorized as silt, sand, gravel, cobble, boulder, or bedrock.

Snorkeling Methods: 2008

To estimate spawning occurrences and variations in the probability of observing spawns according to microhabitat, each site was partitioned into sub-sections (quadrats) of similar habitat and separated by natural channel discontinuities. The number of quadrats varied among sites from 4 to 8 depending on the length of the sample reach (i.e., longer reaches had more quadrats). These quadrats were classified as either a riffle, run, or pool for later analysis. Each quadrat was snorkeled by a single observer during each visit, and all observed individuals of each species were enumerated. Observations of behaviors were sometimes made during a second pass over the sampling area, but during these subsequent passes no enumerations were made. Behavioral observations and habitat variables were recorded similarly to the 2007 season, although in 2008 the intermediate axis of the substrate used during spawning was measured for all species.

Data Analysis

For each of the target species, I summarized the reproductive behaviors observed by date and site, and I used the microhabitat data collected to describe the typical spawning habitat (e.g., swift, mid-channel at depths of 10 to 20 cm). Behaviors observed were plotted against time along with water temperature and daylight to construct possible spawning phenologies. Microhabitat data were then compared between observational years.

Using the behavioral information collected in 2008, I estimated the timing and duration of the spawning season for the Amicalola holiday darter and the Etowah darter. Because spawning may have been occurring at a site during a given visit but may not have been detected by any of the observers, I adopted a modeling strategy that accounted for incomplete detection. The structure of the data collected in the 2008 season allowed me to estimate the probability of spawning being detected, if occurring during an observational period. Observations during each visit to a site were recorded as spawning either being detected (1) or not detected (0) in each of the 4 to 8 quadrats. Each quadrat was treated as a separate survey unit within the visit, and the modeling approach developed by MacKenzie et al. (2002) was used to estimate the probability of detecting a spawning action within a quadrat (p) given that the species was spawning at the site during the visit (ψ). This was similar to the approach taken by Albanese et al. (2007) to estimate species occurrence at a site by estimating detection using individual seine hauls as replicates. A total of 26 visits for the Amicalola holiday darter and 37 visits for the Etowah darter were combined for all sites and dates for analysis. Unfortunately, due to the insufficient number of observed spawns for the Etowah holiday darter and the bridled darter, this approach could not be utilized to estimate the respective time and duration of spawning seasons.

This study addressed two questions: over what period did the species of concern likely spawn, and could the occurrence of spawning be better predicted by water temperature or day length (two factors hypothesized to influence onset and cessation of spawning in darters). To answer these two questions, I wanted to estimate the probability that spawning was actually occurring on a given date even though I failed to observe it. Secondly, I wanted to estimate that probability with the best-supported model given possible environmental influences on both detection and spawning probabilities. Thus, the sequences of detections and non-detections across quadrats for each visit were modeled using combinations of covariates for detection and spawning to represent *a priori* hypotheses concerning factors that influence spawning activity (Table 3.2). Additionally, I included the number of male-female pairs observed during a site visit as a potential covariate on occurrence of spawning. This measure of overall level of male-female interactions during a given visit was itself subject to incomplete detection, but was included as a potentially useful predictor of spawning activity. Although this covariate would not be beneficial in identifying the factors influencing spawning occurrences of the target species, it would help me to delineate the time period over which these species spawn.

The modeling technique used here makes several assumptions. First, spawning was assumed to be constant during the snorkeling visit (i.e., spawning does not commence or cease during the visit to the site). Secondly, the observations made in each quadrat and in separate visits to sites are assumed to be independent of each other. Over-dispersion within the data would be evidence that the assumption of independence among data from separate visits was violated. I assessed evidence of over-dispersion in the data using a goodness of fit test described by Williams et al. (2002). Using one model from each candidate set of models, a dispersion parameter ($\hat{c}$) was calculated as the deviance divided by the degrees of freedom. A parametric bootstrap approach was then used to calculate the mean ($\hat{c}$) for 10,000 bootstrap models. I simulated bootstrap datasets using random numbers between 0 and 1. Each site had one random number for ψ, and n (the number of quadrats) random numbers for the detection probability for each quadrat. If the random ψ was less than ψ_i (estimated from site-specific model parameters) then the site was considered unoccupied, and values for all quadrats were set to 0. Otherwise, a quadrat was given a value of 1 if its random number for p was more than p_i (also estimated by site-specific parameters). The dispersion parameter ($\hat{c}$) calculated from the actual data was then divided by the mean $\hat{c}$ of the 10,000 bootstraps, to assess the relative fit.

Models were constructed using the R (R Development Core Team 2008) package RMark (Laake and Rexstad 2008), which utilizes Program Mark's occupancy-estimation procedure (White and Burnham

1999). Models were compared that expressed the probability of occurrence (ψ) and the probability of detection (p) as functions of each of the covariates hypothesized to influence each. Models were constrained to an intercept and one covariate each for the probability of occupancy and detection to avoid over-fitting the data set.

Models were ranked using Akaike's Information Criterion (AIC) as corrected for small sample size (AICc; Akaike 1973, Hurvich and Tsai 1989, Burnham and Anderson 2002). AIC is calculated such that models that overfit the data are penalized by the number of terms used to estimate the model. In this approach it is not the size of the AIC value that it important, but rather the differences in values (Δ_i) from the best-supported model (Burnham and Anderson 2002). A fitted model that has a relative difference of less than two ($\Delta AIC < 2$) from the best-supported model indicated substantial support for the model, and models with a relative difference of greater than 10 ($\Delta AIC > 10$) essentially had no support and were omitted from further consideration (Burnham and Anderson 2002). Based on the relative difference in fitted models, Akaike weights (w_i) were computed as $w_i = \exp(-1/2\Delta_I)/ \sum \exp(-1/2\Delta_I)$ where the denominator is a sum of $\exp(-1/2\Delta_i)$ for all models in the candidate set (Akaike 1978). A given w_i is the weight of evidence of the fitted model being the actual best model in the set. Evidence was judged by the ratio of Akaike weights (w_1/w_j) where model 1 is the best-supported model and j is the model of consideration (Burnham and Anderson 2002).

Results

Over the course of this study, observations of spawns were made for all target species and at least one spawning act was observed at each of the study sites. A total of 28 visits to sites were made in 2007 and 44 visits were made in 2008 (Appendix C). A total of 49 spawns were documented for the target species: 20 for the Amicalola holiday darter, 2 for the Etowah holiday darter, 21 for the Etowah darter, and 6 for the bridled darter (Table C.1-C.3 from Appendix C).

Spawning Behavior

Spawning behaviors of Amicalola holiday darters and Etowah holiday darters appeared to be similar. For both taxa, the spawning process usually began with a period of courtship, during which the male followed or chased the female. During courtship, it was common to see a second male trying to interrupt courting pairs, but this was not often successful. When being followed, females were often observed pecking at the substrate, which I presumed to be an attempt to clean the substrate before spawning. After the site selection, females positioned themselves, usually vertically, on larger substrate, and were mounted by males. The pair then began to quiver or vibrate intensely, releasing a single egg to attach to the substrate. After a brief period of quivering, pairs departed from the spot with no further acknowledgement of the egg. Individuals were also observed spawning in several different locations, sometimes with different partners and sometimes on different sections of the same piece of substrate.

Spawning pairs of the Etowah darter displayed a complex suite of behaviors before spawning took place. Spawning acts began with a period of courtship, where the male would follow the female in what I presume to be a search of suitable habitat for spawning. During this time, male Etowah darters were frequently seen making contact with females before a suitable location was actually found. This contact ranged from lying next to the female to actually mounting her. Courtship and location selection for this species usually took quite some time, and was often lengthened by male/male acts of aggression. These aggressive actions ranged from chasing to biting, and sometimes, male Etowah darters even showed aggression towards males of different species of percids. Aggressive acts were seen not only between males, but also between females. On several occasions a female was observed chasing another female and attempting to bite her. Additionally, on occasion when the spawning location selection process was lengthy, a second female was seen positioning her abdomen within the substrate near the pair. When this was observed, the female would either chase this second female away, or the male would abandon the female he was presently with and continue on with the second female. When suitable habitat was found (usually coarse sand to fine gravel nested between two large rocks) the female would deposit her abdomen within the substrate by first diving into the sediment with her rostrum and nosing through the substrate burying herself with the surrounding substrate. Once the female was positioned, the male would mount the female and the two would quiver. The quiver was usually intense, often displacing substrate into the water column. In most pairs observed, the male would then dismount the female but remain close by, and the female would rest within the substrate for 2 to 10 minutes. Sometimes the same pair would proceed to a new location and spawn again. One pair was observed spawning three times within the same general location.

Spawning by the bridled darter began with the female being followed by the male. The site selection process appeared to be led by the female, but during selection males were observed apparently competing for the female, with larger males chasing away smaller males. In two pairs, males were observed quickly mounting the female and then retreating before the actual spawn took place, and during this time the female remained motionless. Once a spawning site was selected, the male positioned himself on top of the female and the female buried her posterior abdomen within the substrate, and the two quivered. With most pairs, the female remained within the substrate for about 5 to 10 seconds after the spawn took place. Several pairs were observed spawning at different locations after the initial spawn.

Microhabitat Use

The Amicalola and Etowah holiday darters spawned in runs and pools associated with nearby riffle habitats. Amicalola holiday darters were observed spawning on coarse substrates ranging from gravel to bedrock. One spawning pair was also observed using coarse wood as the substrate (see Table C.1 from Appendix C). Spawning depth for this species ranged from 15.2 cm to 76.2 cm ($\bar{x}$=40.8 cm, *se*=4.1), and depths were not different during the 2007 ($\bar{x}$=43.9 cm, *se*=8.1) and 2008 ($\bar{x}$=38.2 cm, *se*=3.8) seasons. Mean spawning velocity, measured at 60% depth, over the study periods was 0.1 m/s (se=0.04; Figure 3.2). The mean velocity near the spawning substrate, measured only in the 2008 observational period, was 0.03 m/s (se=0.01; see Table C.1 from Appendix C). The two recorded spawns of the Etowah River holiday darter occurred in similar stream locations as for the Amicalola holiday darter, at a depth of approximately 64 cm with a velocity ranging from -0.03 to 0.1 m/s.

The Etowah darter was observed spawning in microhabitats found within swift riffles, most often near the upstream portion of the riffle. *Etheostoma etowahae* was observed spawning primarily in coarse sand to medium gravel juxtaposed between larger substrates of medium gravel to cobble. Depths varied between 7.62 cm to 33.5 cm with a mean of 18.9 cm (se=1.62). The mean velocity, measured at 60%

depth, was 0.48 m/s (se=0.04), but velocities ranged from 0.15 to 0.77 m/s (Figure 3.2). Mean velocity near the substrate, however, was 0.3 m/s (se=0.04) ranging from -0.07 to 0.7 m/s (see Table C.2 from Appendix C). Microhabitat data from the one spawn observed within 2007 was consistent with the microhabitat data collected for the 2008 season.

Pairs of bridled darters were observed spawning in microhabitats found upstream or downstream of swift to moderate riffles. Spawning occurred in sand and gravel, at depths ranging between 24.4 to 57.9 cm, with a mean of 41.2 cm (se=4.85). Velocities, measured at 60% depth, ranged between -0.03 and 0.49 m/s, with a mean of 0.21 m/s (se=0.09; Figure 3.2). Velocities near the substrate ranged from 0.13 to 0.21 m/s, with a mean of 0.17 m/s (se=0.02; see Table C.3 from Appendix C).

Duration and Temperature Gradient of Spawning Period

Throughout the course of the study, a total of 49 spawning acts were documented for the target species between April and August of 2007 and 2008. All twenty observed spawns for the Amicalola holiday darter occurred between April 20 and May 18, 2007 (at 10.6-17.9°C) and between May 2 and May 20, 2008 (at 14.7-17.3°C; Figure 3.3-3.5). The two spawning acts observed for the Etowah holiday darter were made on May 11 and May 24, 2007 (16.9°C and 17.6°C), but no spawns for the Etowah holiday darter were observed in 2008 (Figure 3.6 & 3.7). Twenty-one spawning acts were observed for the Etowah darter, one in 2007 on June 5 (18.7°C) and twenty in 2008 between May 2 and July 7 (at 15.9-22.8°C; Figure 3.4-3.7). Six spawning acts were observed for the bridled darter, five in 2007 between April 20 and June 5 (14.1-18.7°C) and one in 2008 on May 19 (14.4°C; Figure 3.4-3.7). At least one spawn by one of the four target species was observed at each of the snorkel sites in 2007, but no spawns were observed at site number 4 (eto322) in 2008.

Breeding behavior, including male/male aggression, female/female aggression, male/female courtship (e.g. following, chasing, or displaying) or male/female contact (e.g. lying on top or alongside of one another), was observed for each of the target species during the study period (see Table C.4-C.8 from Appendix C). Amicalola holiday darter breeding behavior was observed from April 20-May 18 in 2007 (10.13-17.93°C) and March 27-June 6 in 2008 (10.5-20.3°C; Figure 3.3-3.5). Breeding behavior of the

Etowah holiday darter was observed from April 29-June 14 in 2007 (15.3-17.6°C) and May 7-May 30 in 2008 (13.4-17.6°C; Figure 3.6-3.7). Bridled darter reproductive behavior was observed from April 20-June 5 in 2007 (14.1-18.7°C) and March 27-June 26 in 2008 (12.9-20.7°C; Figure 3.4-3.7). Breeding behavior was observed for the Etowah darter from April 20-July 8 in 2007 (14.1-20.6°C) and March 27-July 22 in 2008 (12.9-24.9°C; Figure 3.4-3.7).

Based on *a priori* hypotheses, one model for both the Amicalola holiday darter and the Etowah darter showed substantially more support ($\Delta AIC_C < 2$) regarding occurrence patterns than other models within the candidate set (Table 3.3). For both species, this model accounted for the number of pairs observed during a visit to a site. This best-supported model for the Amicalola holiday darter was 12.25 times more likely (Table 3.3) than a model that only incorporated an intercept for occurrence probability (ψ), however, for the Etowah darter, the top model was over 2,900 times more likely (Table 3.3) than an intercept-only model. Models that incorporated a temperature parameter for occurrence probability showed considerably less support than the best-supported model for both the Amicalola holiday darter and the Etowah darter (ΔAIC_C 3.69 and 7.98, respectively), and for the Amicalola holiday darter this model was ranked below an intercept model (1.25 times less likely; Table 3.3). Both the best-supported model and the temperature model for the Amicalola holiday darter suggested that spawning was unlikely to occur ($\psi<0.10$) after the first week of June (Figure 3.8). According to the temperature model, probability of spawning decreased to less than 0.10 at 17.77°C. The top model and the temperature model for the Etowah darter suggested that this species spawned from the beginning of May to mid-August, but due to the asymptotic nature of the water temperatures in July and August at these sites, the temperature model suggested that the probability of observing spawns remained high ($\psi>0.10$; Figure 3.9) until temperatures decreased to 15° C or less.

All of the spawning occurrence models suggest that spawning may have been taking place during visits even when it was not detected. Best-supported detection models for spawning acts of both species had a parameter that accounted for the habitat within a quadrat. For Amicalola holiday darters, two qualitative habitat covariates were used to understand spawning detectability. The first was a binary indicator of riffle habitat and non-riffle habitat (pools and runs). It was expected that spawning for this species would take place mostly in runs and pools, and thus riffles habitats would have a lower detection probability. Nevertheless, there was much greater support for a model incorporating differences in detection in pools and non-pools (riffles and runs). According to this model, detection during times when spawning was occurring was 5 times more likely in pool quadrats (*probability of detection*=0.48) than in non-pool quadrats (*probability of detection*=0.10). Other models for Amicalola holiday darter spawning detection showed considerable less support. These models included a parameter that accounted for the temperature or turbidity at the site. According to these models, detection decreased on visits with high turbidity or high temperatures. For Etowah darters, a model that incorporated a habitat parameter that distinguished riffle quadrats and non-riffle quadrats showed overwhelming support. According to this model, spawns were 8.63 times more likely to be observed in riffles (*probability of detection*=0.32) than non-riffle (*probability of detection*=0.04). There was considerable less support for models that incorporated a parameter for turbidity (ΔAIC_C=5.56) and even less for a model that incorporated a temperature (ΔAIC_C=8). These alternative models suggest that detection increased on turbid days and when the water was warmer.

Discussion

The four taxa covered within this study represent two genera, three subgenera and two very divergent reproductive modes. However, all four taxa co-occur, and are similar in that each is imperiled and each begins spawning in springtime. While the results from this study are very similar to those from other studies that involve other taxa within each respective subgenus (see Appendix A for respective subgenus), it has been important to determine how these particular taxa spawn including their behaviors, the location of spawning acts within study sites and the duration of the spawning season. Information about the reproductive attributes of these taxa will help ensure that management practices are conducted with the distribution and life cycles of these species in mind. Because these acts were observed *in situ*, the behaviors and microhabitat preferences can be reported with greater confidence than if observations were made on captive individuals. Moreover, this study demonstrates the importance of modeling detection when estimating the occurrence of spawns. By modeling detection as a function of the microhabitat of a quadrat, I was able to model occurrences with more accuracy. Furthermore, this study shows that spawning occurrences during reproductive seasons may vary among visits within the reproductive season. However, neither temperature nor day length has proven able to estimate the spawning occurrence of the two target species examined.

Spawning Behavior

The behaviors observed in this study include a broad array of interfamily reproductive attributes and are representative of the phylogenetic placement of each taxon. Whereas a connection between phylogeny and imperilment has been identified for other families such as Cyprinidae (Johnston 1999), the connection for percids has not been made. In this study, I have described the behaviors for three styles of reproduction. Each reproductive behavior represents an evolutionary step within the phylogeny of percids and serves as an adaptive survival tactic that provides each taxon with an advantage over precursors. However, each behavior also has its own disadvantages, some of which may be driven by anthropogenic activity (Page 1985).

Burying is the most primitive reproductive behavior within the subfamily Etheostomatinae and is observed in all four genera. Although burying reduces exposure to predation and parasitism, eggs must be buried in habitats of flowing water to provide oxygen. Therefore, alterations of such habitats in the form of stream modifications (i.e., impoundments, channelization) and sedimentation can inhibit the spawning of these species (Page 1985, Warren et al. 1997). In this study, *Percina kusha* acted similarly to other members of the subgenus *Alvordius*, with which it is hypothesized to belong. While the reproductive behaviors of *Percina* are not diverse (i.e., all known are buriers), there is no reason to suggest based on the behaviors observed that *P. kusha* belongs to another subgenus. In his detailed observations of the blackside darter, *P.* (*Alvordius*) *maculata*, Petravicz (1938) described the male mounting the female and the pair vigorously vibrating as milt and eggs were released. Petravicz also noted a green-gold cast and intensified markings of the male before spawning, which is similar to what was observed with *P. kusha*. Johnston et al. (2002) described the reproductive behaviors of *P. kusha* in the Conasauga River. Although courtship practices were similar to those of the Etowah population, Johnston et al. observed the male darter positioning himself alongside the female while the pair vibrated, whereas males within Etowah River system positioned themselves on top of the female. This type of among-population variability has also been observed for other darter species (N. Burkhead, pers. com., 11/8/2007).

As one of the larger subgenera of *Etheostoma*, *Nothonotus* species show a large amount of variation with respect to reproductive behaviors. While many species of *Nothonotus* are known to demonstrate the primitive act of burying and are affected by the consequences from the associated anthropogenic disturbances discussed above, other species within the subgenus are known to exhibit a behavior known as clumping, which is unique to the subgenus. Clumping begins when the male selects a site, usually a cavity under a large rock. The female deposits eggs in the cavity between the interface of the stone and the bed sediments. After egg fertilization, males are known to guard the eggs (Raney and Lachner 1939, Stiles 1972, Page et al. 1982). While the Etowah darter is now known to be a member of the burying clade, this variation within the subgenus suggests that other inter-subgeneric differences in behaviors may also exist. In this study I observed behaviors that were similar to other members of the egg-burying clade within *Nothonotus*. However, the observation of female induced mate switch has not been described for any other species within the clade. Other studies on *Nothonotus* have described female aggression. Mount (1959) noted female aggressive acts among *E.* (*Nothonotus*) *camurum*, during which a female would dart at another female without making contact and darken the bands behind the pectoral fins. This observation suggests that females might play a different role in the courtship practices of spawning pairs than other females of different subgenera. Mount also noted that females were responsible for stimulating males in order to begin the spawning process. Interestingly, Warren et al. (1986) observed two other females burying when a pair of *E.* (*Nothonotus*) *tippecanoe* was observed spawning, and Stiles (1972) observed multiple burials of females around a single male in *E.* (*Nothonotus*) *rufilineatum*. While *E. etowahae* is hypothesized to be closely related to *E.* (*Nothonotus*) *jordani*, Orr and Ramsey (1997) noted neither female aggressive acts nor female stimulation in spawning behaviors.

Whereas species of *Nothonotus* have evolved clumping behaviors over the ancestral burying within Etheostomatinae, other subgenera have paralleled this evolution and attach eggs to substrates (Page 1983). The two holiday darter taxa within this study belong to a subgenus known as *Ulocentra*. The described behavior of species within this subgenus involves the attachment of a single egg to the vertical side of a rock (Bailey and Etnier 1988). This behavior provides the advantage of being able to spawn in areas with a slower velocity because more dissolved oxygen is available above the substrate. However, exposure also decreases survival due to sedimentation and suspended pollutants. Storey (2003) found that *E.* (*Ulocentra*) *scotti*, was less likely to spawn in streams with high sedimentation. This result suggests that alterations to the upstream catchment of sites might have an effect on the spawning behaviors of *Ulocentra* species. Additionally, I have found no behavioral evidence to further the hypotheses that the two holiday darter taxa are different from the holiday darter taxa of Shoal Creek and of the Conasauga River. This result is not surprising because the species of *Ulocentra* show little variation within their reproductive behaviors (see Winn 1958a and Porterfield 1998).

Microhabitat Use

Observing life history characteristics *in situ* provides the advantage of being able to measure accurate microhabitat uses by the species of concern. Although tank and aquaria studies have the advantage of allowing one to manipulate conditions, it's hard to separate habitat use representative of natural behavior from tank artifacts (see Ruzzante [1994] for effects of domestication on fishes). Inevitably, the habitat use of percid species is correlated with species' reproductive behavior, however habitat use by species of the same subgenera does show some variation (Stiles 1972, Page 1983, Porterfield 1998).

Although burying is the primitive state of reproduction within the subfamily Etheostomatinae and is known to be the behavior of all described *Percina* species and several *Etheostoma* species, the range of habitat used for this mode of reproduction includes a large range of substrate coarseness, depth and velocity. Within the subgenus *Alvordius*, known species spawn in pools, raceways and deep riffles. *P.* (*Alvordius*) *roanoka* is known to spawn in deep pools among large rubble and small boulders (Jenkins and Burkhead 1994). *P.* (*Alvordius*) *maculata* is known to spawn in pools and raceways at depths between 30 and 60 cm (Petravicz 1938, Winn 1958a, Winn 1958b). Conversely, *P.* (*Alvordius*) *macrocephala* migrates to shallow gravel shoal areas to spawn. In this study, I observed *P. kusha* spawning in runs upstream or downstream of riffle habitats within coarse sand among larger substrate types. This habitat type is very similar to the habitat described for the Conasauga population of *P. kusha* (Johnston et al. 2002). However, in 2008, I observed a pair of bridled darters spawning in a swifter habitat than known for the Conasauga population. Burying species of *Nothonotus,* have been observed spawning in mid-channel swift shallow habitats (Mount 1959, Bryant 1979, Stiles 1972, Warren et al. 1986). Several spawning accounts within the literature describe spawning substrate that ranges from coarse sand to gravel juxtaposed between larger substrate (Stiles 1972, Bryant 1979, Warren et al. 1986, James and Taber 1986). However, Orr and Ramsey (1997) do not mention juxtaposition between larger rocks for *E.* (*Nothonotus*) *jordani*. All of these studies suggest that these species of *Nothonotus* require swift riffles with coarse substrates for reproductive behaviors.

Habitat selection for *Ulocentra* species reflects the reproductive mode of egg attaching (Page 1985). Snubnose darters generally spawn in areas of little to no velocity on coarse substrate (e.g., cobble, boulder, woody debris; Winn 1958a, Page 1983, Suttkus and Etnier 1991, Bauer et al. 1995, Porterfield 1998, Storey et al. 2006). However, Johnston (1997) observed Conasauga holiday darters spawning in an area of swift velocity (0.54-0.81 m/sec), which is in stark contrast to the observations of Etowah and Amicalola holiday darters made in this study. Winn (1958a) found that the key reproductive stimulus for two species of *Ulocentra* within laboratory aquaria was water depth; breeding did not ensue until the depth was increased to 37 cm. While this observation does not concur with those made in this study, or other studies on *Ulocentra* species both in the field and in aquaria (see Johnston 1997, Porterfield 1998, Storey et al. 2006), it may indicate that depths of spawning locations are important for *Ulocentra* species.

Time and Duration of Spawning Seasons

Correctly identifying the timing and duration of reproductive seasons is of great importance for the conservation of freshwater fishes. Percids generally begin spawning in the early spring and continue into the summer, although several species are known to spawn in other time periods of the year. As with reproductive behaviors and microhabitat uses, this timing varies greatly among members of Etheostomatinae. However, timing and duration is also highly correlated with the geographic latitude of the population of the species. By correctly identifying the phenology of a population, managers can adapt conservation practices that will reduce anthropogenic disturbance during this period of high vulnerability. However, correctly identifying whether or not a species is spawning at a given time and location is not simple. Efforts require repeated observations at sites over the course of a hypothesized spawning season (Etnier and Starnes 1993). Furthermore, a species could be spawning during visits but remain undetected by observers. Therefore, methods that account for incomplete detection when estimating the probability of spawning occurrences are needed to improve the accuracy of identifying spawning phenologies.

Members of *Nothonotus* are mostly late-season spawners with few beginning before mid-May (James and Taber, 1986). Orr and Ramsey (1990) found that peak reproductive activity for *E.* (*Nothonotus*) *jordani* occurred in late April in Opintlocco Creek, Alabama, but females with ripe ova were also found from April 22-June 3 at water temperatures of 18-29.4°C. James and Taber (1986) observed *E.* (*Nothonotus*) *juliae* spawn in water temperatures from 19-23°C, with late May and early June representing the season's peak. Stiles (1972) made reproductive observations on *E.* (*Nothonotus*) *rufilineatum* over the course of two years and found that the species spawned from the end of May until the first of August in the Little River of Tennessee. Interestingly, spawning pairs were only observed in the second year of the study. While Stiles associated this phenomenon with very low flows in the first year of study, this hypothesis does not hold true for the *E. etowahae* (i.e., only one spawn was observed in 2007), as the second season had lower flows than the first. Although spawning behaviors (i.e., following, chasing, contact) were observed in the first year of study for *E. etowahae*, far fewer pairs were observed during the visits in 2007 compared to those in 2008. Nonetheless, while the differences in spawning seasons for *Nothonotus* species are largely due to longitudinal differences in their distributions, these studies suggest that the spawning seasons for other *Nothonotus* might be as long as *E. etowahae* (early May through early August). If the termination of the spawning season is indeed related to the water temperature, the Etowah darter might in fact spawn well into August and even September. However, the best-supported model from this study suggests spawning probably ends in early August. This result suggests that the cessation of spawning is not directly related to temperature for this taxon (Figure 3.9).

Although 20 spawns were observed over the course of the study for the Amicalola holiday darter and 2 spawns were observed for the Etowah holiday darter, the full extent of the spawning season for these species is still not known. Observations in 2008 began before what is believed to be the onset of spawning (3/27/2008), but high flow conditions during April precluded observations by snorkeling. On April 10, 2008, spawning activity was qualitatively assessed by capturing individuals with a seine and checking their body condition. The males were very brightly colored and though some distention was observed in females, they were not releasing eggs in response to pressure. Based on the previous year's first observation of a spawn on April 20, 2007, the onset of the spawning season is believed to be sometime in early to mid-April. This is concurrent with most other *Ulocentra* species (Porterfield 1998), but later than the other two species (*E. coosae* and *E. scotti*) within the Etowah River system. Unfortunately, the modeling technique used in this study was not very helpful in identifying the onset of the spawning season for the Amicalola holiday darter. Because only one observation was made before what is believed to be the onset of the spawning season, the temperature model suggested that there is a negative relationship between the probability of spawning occurrences and water temperature. However, the model does imply that the spawning season ends at about 17.8°C, which agrees with the predicted time of cessation for the best-supported model. Other studies have noted that spawning by members of the subgenus *Ulocentra* cease at a water temperature of 20 to 22°C (Suttkus et al. 1994).

Unfortunately, this study could not delineate the spawning season for *P. kusha* with confidence. Based on the six observations for *P. kusha*, the spawning season probably begins in mid-to-late April and continues through early June. The lack of observations of spawning pairs of this species is most likely a result of low abundance of the species at these sites. Williams et al. (2007) noted that *P. kusha* was a species that naturally occurred in low abundance. While only six observations make up the known spawning season, the estimate given in this study is similar to other species of *Alvordius*. Jenkins and Burkhead (1994) report *P.* (*Alvordius*) *roanoka* spawning during late May to early June, beginning at temperatures of 12°C, and *P.* (*Alvordius*) *maculata* is known to spawn from April to June (Petravicz 1938, Winn 1958b).

Conclusions

The results from this study will allow managers to more confidently outline the spawning seasons of these imperiled taxa. This information is of great importance when making decisions about possible anthropogenic disturbances, such as from construction activities that affect stream habitats. However, additional research is needed to determine vulnerability of other life stages during different seasons. The observations in this study have been corroborated with previous descriptions of behaviors from other reproductive studies on taxa within the same subgenera and may be useful in future phylogenic research on these species. Additionally, the modeling technique employed in this study provides insight into how reproductive behaviors vary across a species' spawning season.

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Table 3.1. Study sites for snorkeling observations of holiday darters, Etowah darter and bridled darter. GMNH=Georgia Museum of Natural history. Easting and Northing refer to UTM 16 coordinates.

Site No.	GMNH Site ID	Stream	Locality	Easting	Northing
1	eto305	Cochrans Creek	County Road 374 (High Hope Road/Old County Road 45), 8.1 air-miles NW of Dawsonville, GA city-center.	757401	3823381
2	eto150	Amicalola Creek	County Road 28 (Faucett Lake Road/Steve Tate Hwy), 9.4 air-miles ENE of Jasper, GA city-center.	750826	3820757
3	eto264	Amicalola Creek	County Road 25 (Afton Road), 9.2 air-miles NW of Dawsonville, GA city-center.	752861	3820854
4	eto322	Etowah River	State Route 52, 4.5 air-miles W of Dahlonega, GA city-center.	769543	3825373
5	eto326	Etowah River	County Road 72 (Hightower Church Road), 6.7 air-miles NW of Dahlonega, GA city-center.	768002	3831824

Table 3.2. *A priori* hypotheses regarding detection and occurrence of spawning for *Etheostoma* sp. cf. *E. brevirostrum* A, Amicalola holiday darter, and *E. etowahae*, Etowah darter.

Hypotheses Regarding the Probability of a Spawning Occurrence	**Covariate**
Species spawn when the water temperature is within a certain range. Spawns begin when the temperature reaches a level of warmth and cease when the temperatures either increase or decrease.	Water Temperature Water Temperature Squared
Species spawning actions are triggered by the amount of available daylight and cease when the daylight increases or decreases.	Day Length Day Length Squared
The number of male-female pairs observed might be an indicator of spawning potential.	Pairs Observed.
Hypotheses Regarding the Probability of Detecting a Spawning Occurrence	
Species spawn in a habitat that is best suited to the preservation of the zygote. Egg attachers spawn in slower habitats (pools) and species that bury spawn in swifter habitat where oxygen remains high within the substrate (riffles).	Pool Habitat Riffle Habitat
Water clarity affects the ability of males and females to visually detect nuptial characteristics (e.g., nuptial colors, distention, etc.) as well as the ability of the observer to see a spawning action.	Turbidity
Visit conditions such as amount of daylight and the water temperature affect the frequency of spawning actions, which in turn affects probability of detecting a spawn	Water Temperature Day Length

Table 3.3. Model structure, number of parameters (*K*), relative difference in AIC_C (ΔAIC_C) and AIC weights (w_i) of well-supported spawning occurrence models ($\Delta AIC_C<10$) and intercept-only model for *Etheostoma* sp. cf. *E. brevirostrum* A, Amicalola holiday darter, and *Etheostoma etowahae*, Etowah darter. Intercepts only (i.e., no covariates) within model structure is represented as a period.

Model	**No. Par**	**ΔAIC_C**	**Weight**
Etheostoma sp. cf. *E. brevirostrum A*			
ψ (Pairs Observed) *p* (Pool Quad)	4	0	0.49
ψ (.) *p* (Turbidity)	3	3.13	0.10
ψ (.) *p* (Temperature)	3	3.41	0.09
ψ (Temperature) *p* (Pool Quad)	4	3.69	0.08
ψ (.) *p* (Pool Quad)	3	3.87	0.07
ψ (Temperature) *p* (.)	3	4.61	0.05
ψ (.) *p* (.)	2	5.18	0.04
ψ (Temperature) *p* (Turbidity)	4	5.57	0.03
ψ (Temperature) *p* (Temperature)	4	6.04	0.02
ψ (Temperature) *p* (Riffle Quad)	4	6.55	0.02
ψ (Temperature) *p* (.)	3	6.80	0.02
Etheostoma etowahae			
ψ (Pairs Observed) *p* (Riffle Quad)	4	0	0.84
ψ (Pairs Observed) *p* (Turbidity)	4	5.56	0.05
ψ (Pairs Observed) *p* (.)	3	7.43	0.02
ψ (Temperature) *p* (Riffle Quad)	4	7.98	0.02
ψ (Pairs Observed) *p* (Temperature)	4	8.00	0.02
ψ (Pairs Observed) *p* (Pairs Observed)	4	8.62	0.01
ψ (Pairs Observed) *p* (Day Length)	4	9.45	0.01
ψ (Day Length) *p* (Riffle Quad)	4	9.68	0.01
ψ (.) *p* (Riffle Quad)	3	9.73	0.01
ψ (.) *p* (.)	2	15.96	0.00

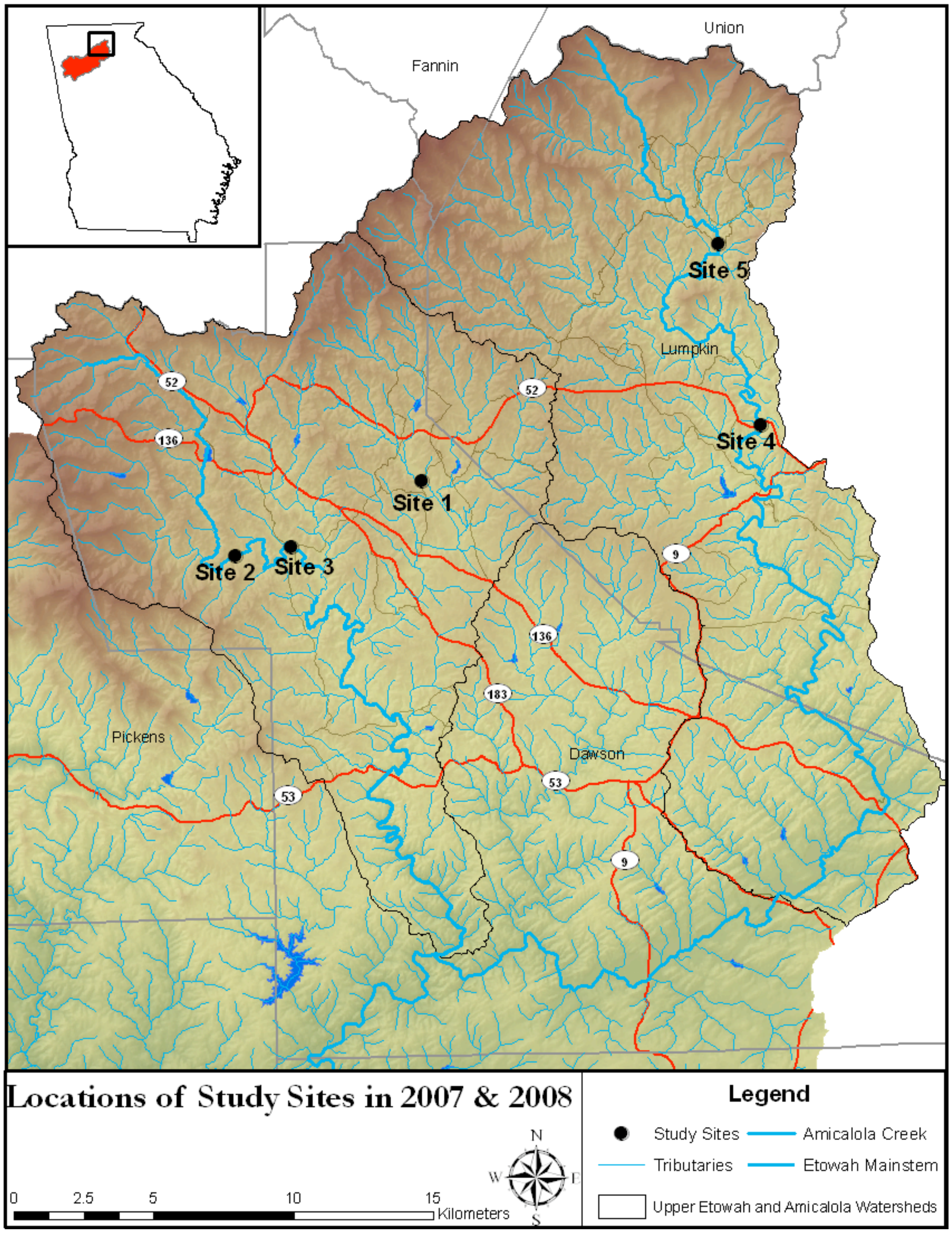

Figure 3.1. Locations of study sites in 2007 & 2008. Black circles indicate sites where the holiday darters, Etowah darter and bridled darter were studied.

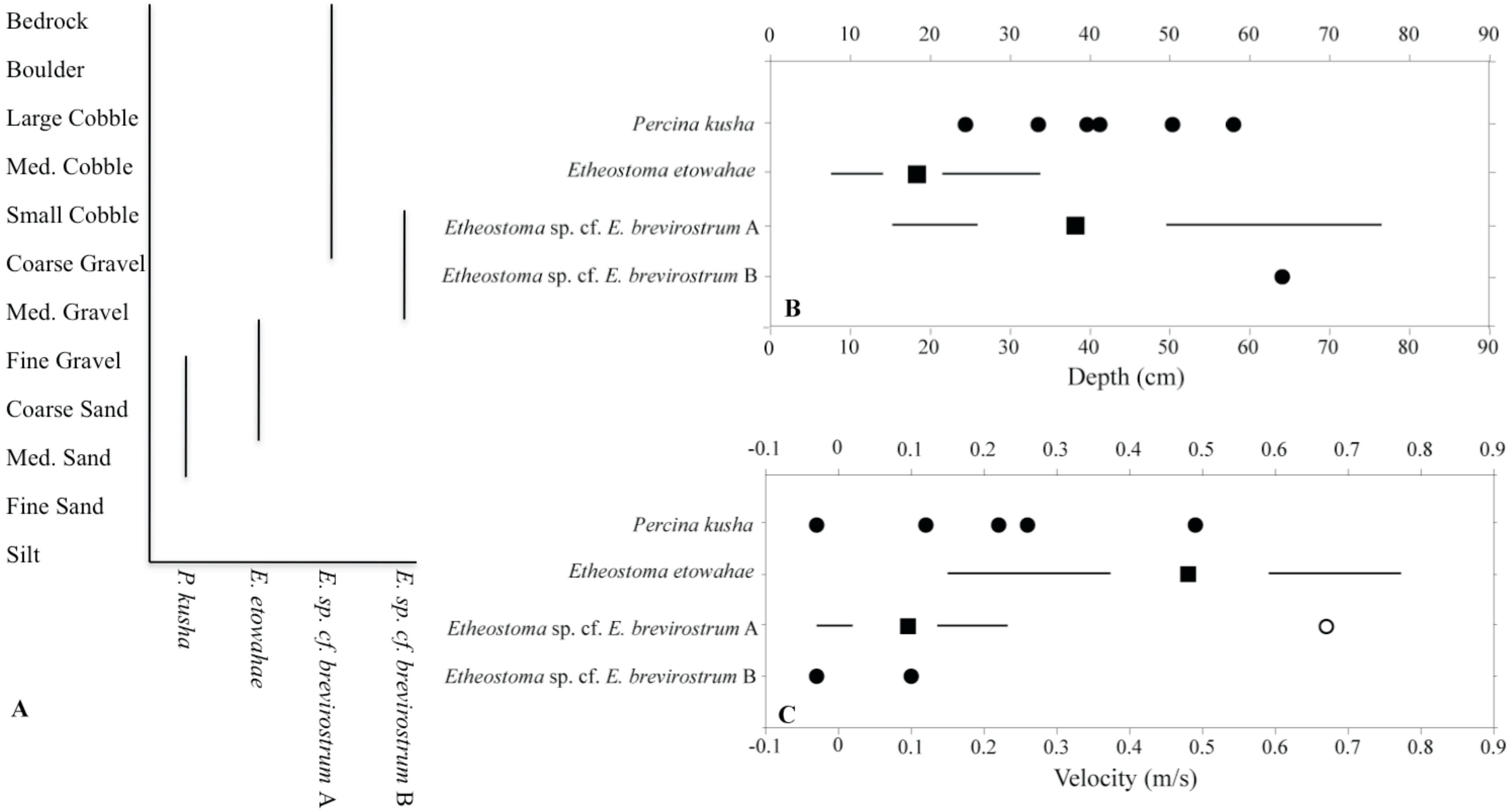

Figure 3.2. Microhabitat parameters of spawning actions for target species. A) Range of bed sediment characteristics used in spawning actions. Bed sediment categories based on adapted Wentworth Scale. B) Distribution of depths used in spawning actions. C) Distribution of velocities used in spawning actions. Velocities measured at 60% depth. Range of values is designated by solid horizontal lines. Inter-quartile range is designated by area between solid black lines. Median values are represented with solid black squares and outliers are designated as hollow circles. Solid black circles are individual values where sample size was small for the target species. See Table C.1-C.3 from Appendix C for specific values.

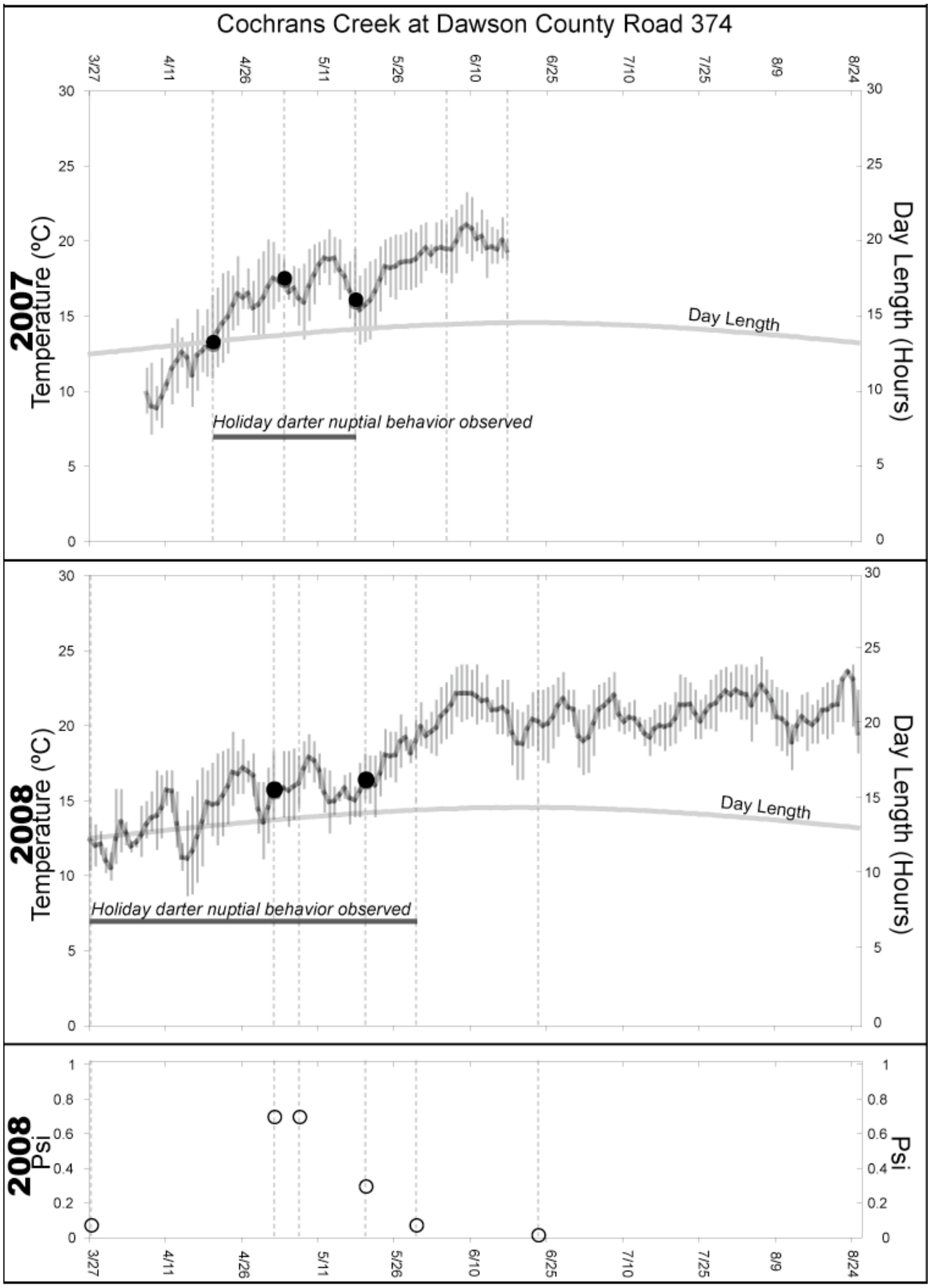

Figure 3.3. Water temperature profile and spawning occurrence probabilities at sample site 1 (eto305) for the sampling periods of 2007 & 2008. Mean daily temperature is represented by a bold line and the range of values observed within a day are illustrated by the vertical solid lines. Day length (i.e., the number of hours with sunlight) is represented with the solid grey line. Visits to each site are portrayed as vertical dashed lines. Observed spawns of *Etheostoma* sp. cf. *E. brevirostrum* A are represented with black circles. Finally, the horizontal bars indicate the range of dates in which nuptial behaviors were observed for *E.* sp. cf. *E. brevirostrum* A. Interruptions in the temperature line indicate failures in temperature recording equipment. The top panel is from 2007, the middle panel is from 2008, and the bottom panel is the probability of spawning occurrences during each site visit for holiday darters in 2008 according to the best-supported model.

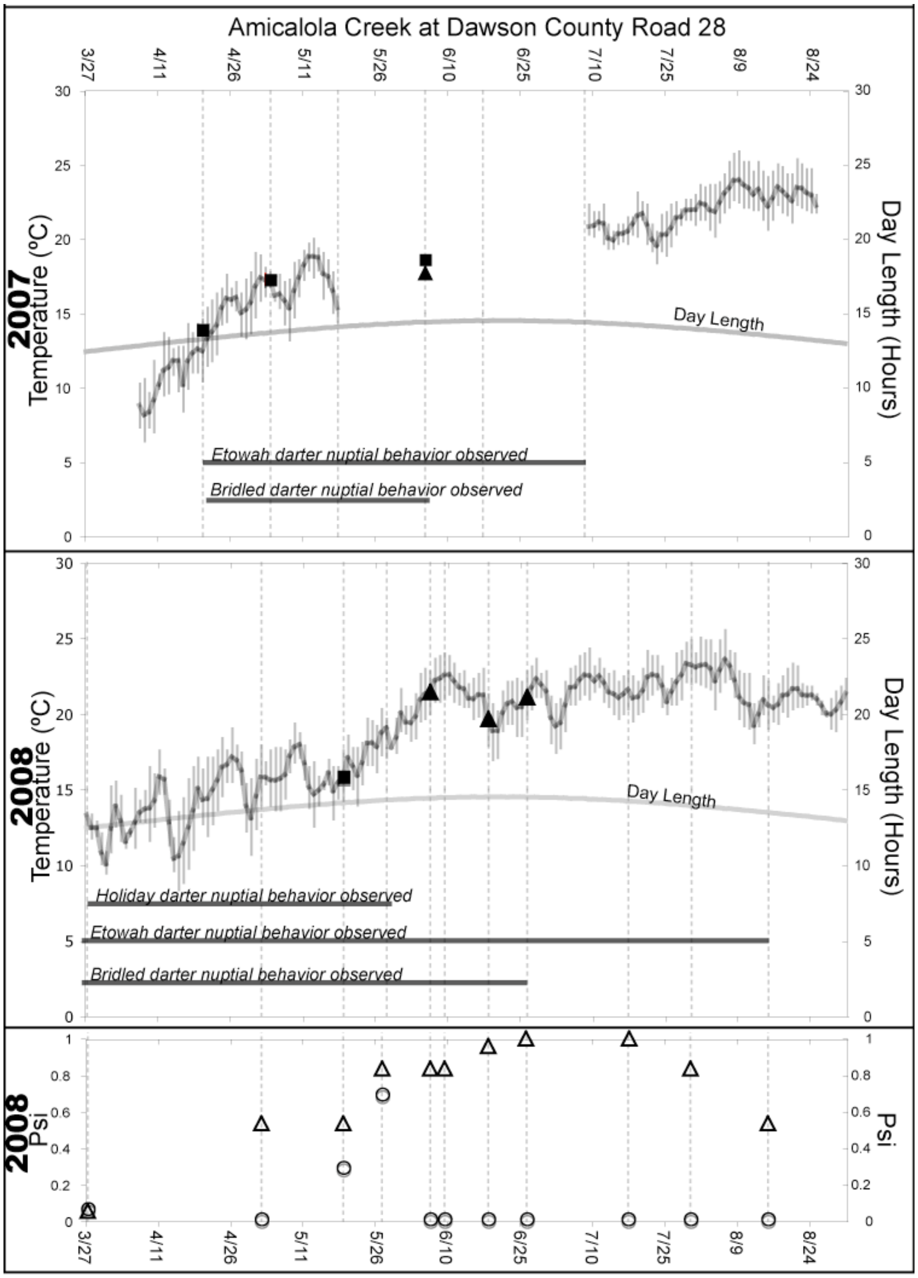

Figure 3.4. Water temperature profile and spawning occurrence probabilities at sample site 2 (eto105) for the sampling periods of 2007 & 2008. Mean daily temperature is represented by a bold line and the range of values observed within a day are illustrated by the vertical solid lines. Day length (i.e., the number of hours with sunlight) is represented with the solid grey line. Visits to each site are portrayed as vertical dashed lines. Observed spawns of *Percina kusha* are represented with black squares. Observed spawns of *Etheostoma* sp. cf. *E. brevirostrum* A and B are represented with black circles. Observed spawns of *E. etowahae* are represented with black triangles. Finally, the horizontal bars indicate the range of dates in which nuptial behaviors were observed for each species. Interruptions in the temperature line indicate failures in temperature recording equipment. Top panels are from 2007, middle panels are from 2008, and the bottom panels are the probability of spawning occurrences during each site visit for holiday darters and Etowah darters in 2008 according to the best-supported models. Shapes correspond to the symbols used to represent the observation of spawns for that species.

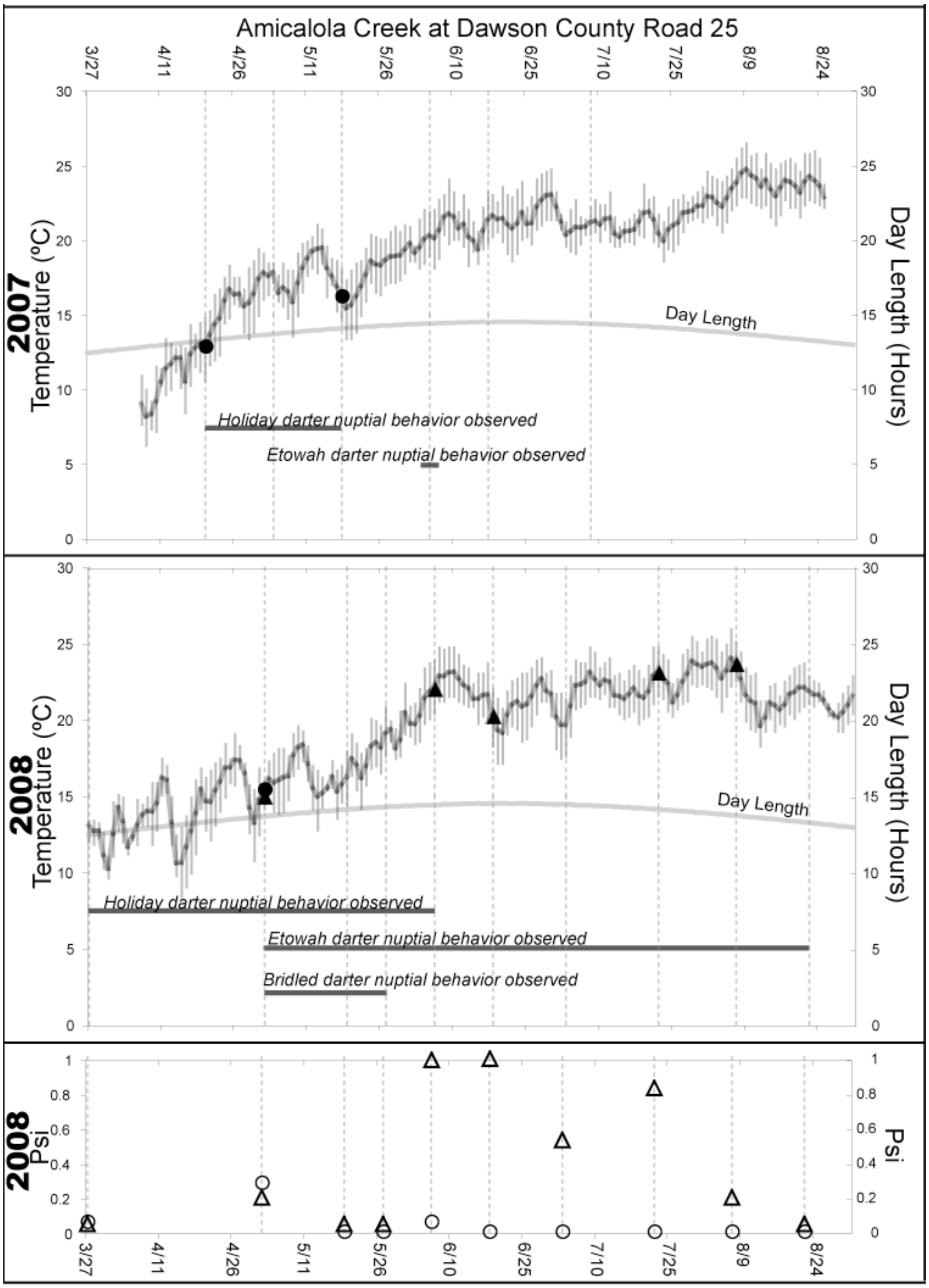

Figure 3.5. Water temperature profile and spawning occurrence probabilities at sample site 3 (eto264) for the sampling periods of 2007 & 2008. See Figure 3.4 for notation.

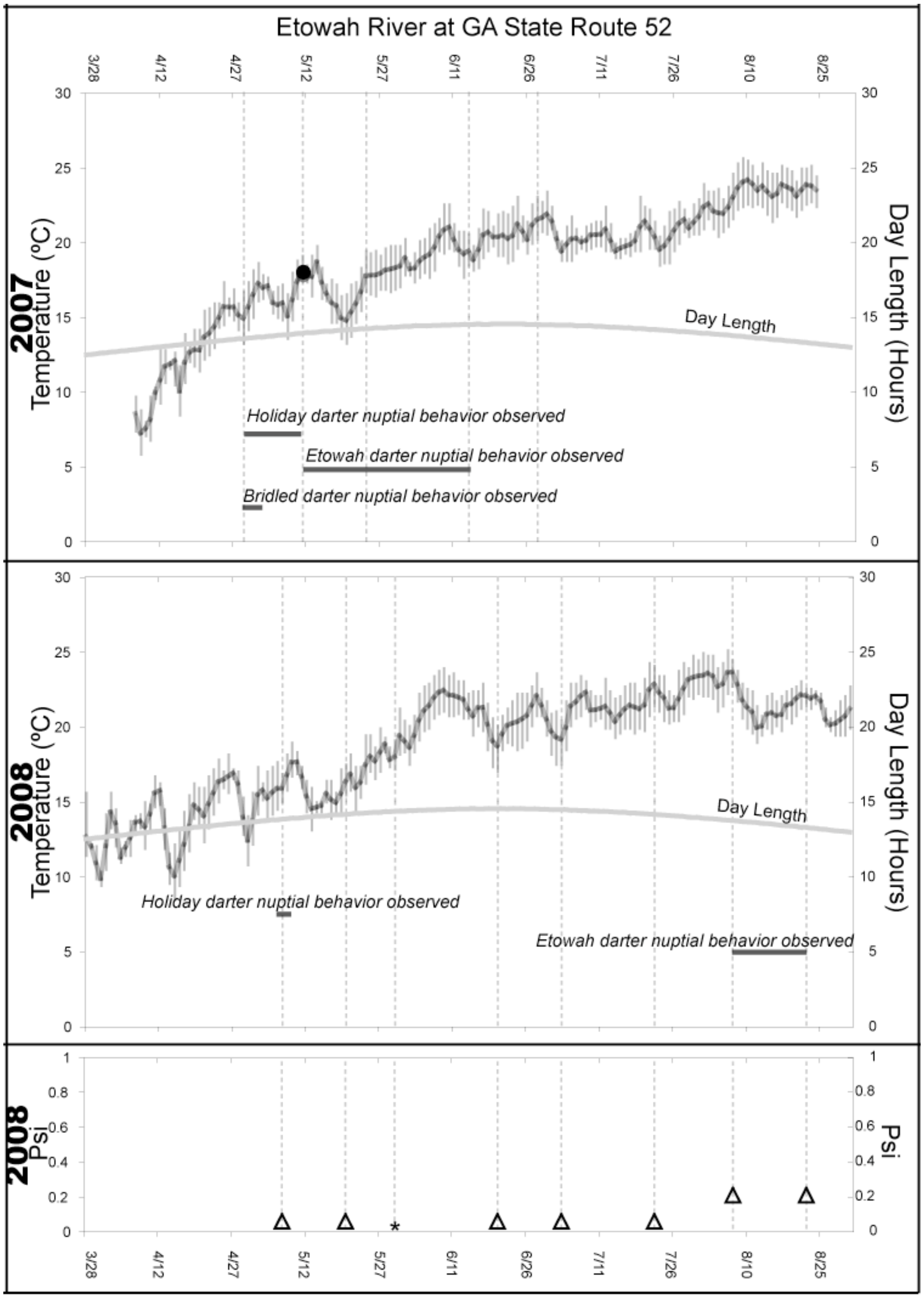

Figure 3.6. Water temperature profile and spawning occurrence probabilities at sample site 4 (eto322) for the sampling periods of 2007 & 2008. See Figure 3.4 for notation.

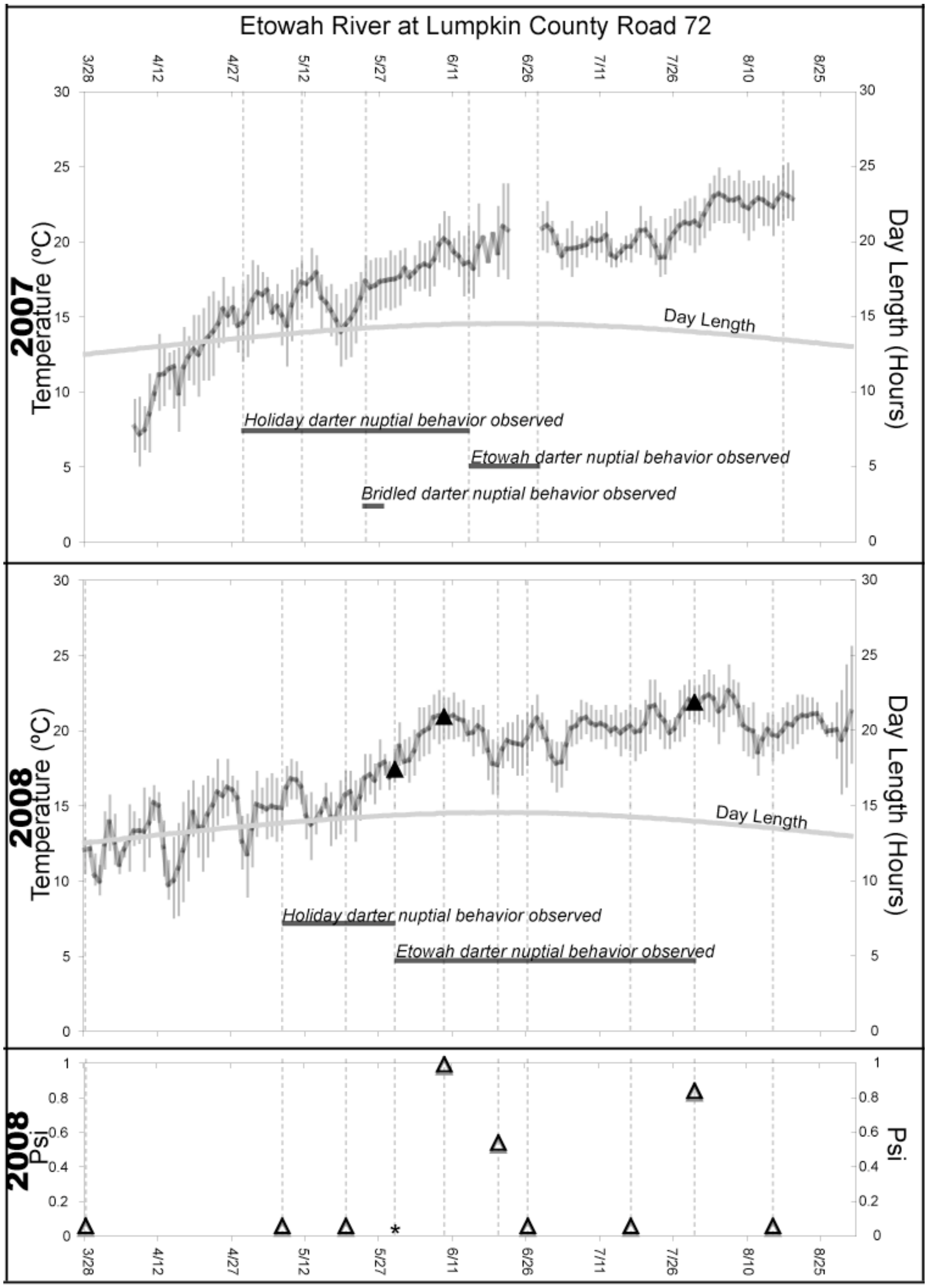

Figure 3.7. Water temperature profile and spawning occurrence probabilities at sample site 5 (eto326) for the sampling periods of 2007 & 2008. See Figure 3.4 for notation.

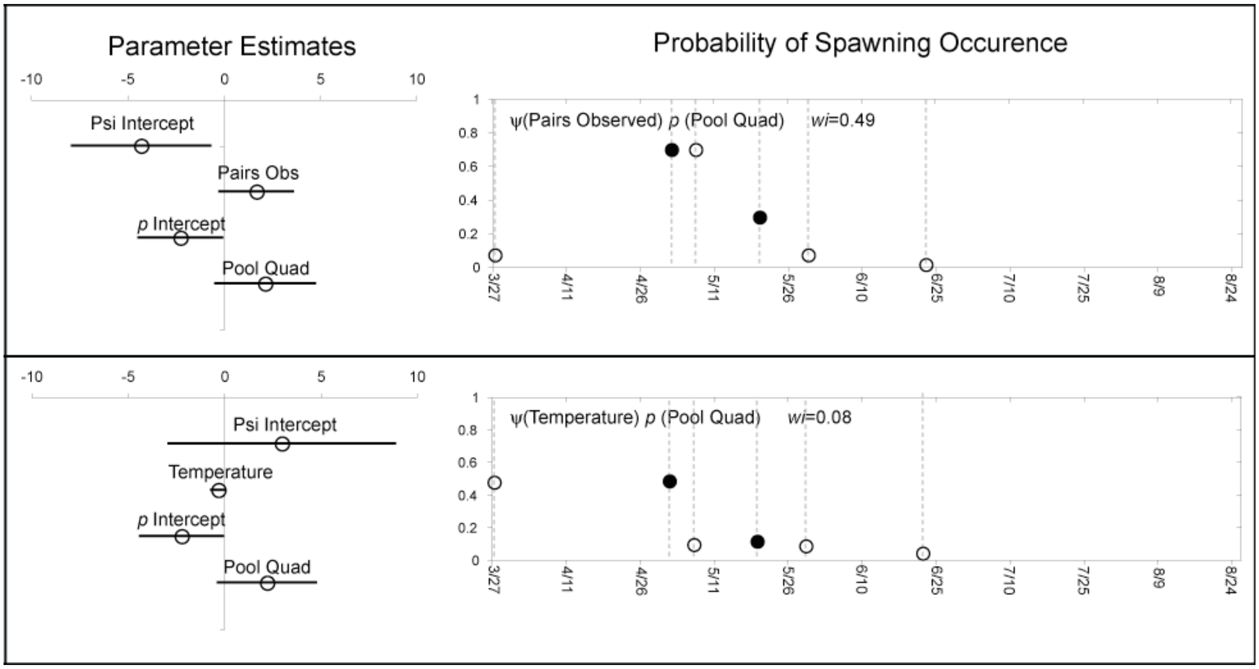

Figure 3.8. Model comparison of best-supported covariate models for occurrence-of-spawning probability (ψ) for *Etheostoma* sp. cf. *E. brevirostrum* A, Amicalola holiday darter. Occurrence probability was calculated using visit conditions for Cochrans Creek at Dawson County Road 374. Circles represent the probability of spawning occurrences during visits for the Amicalola holiday darter, and filled shapes are dates which spawning was observed. See Table C.9 from Appendix C for parameter values with credible intervals

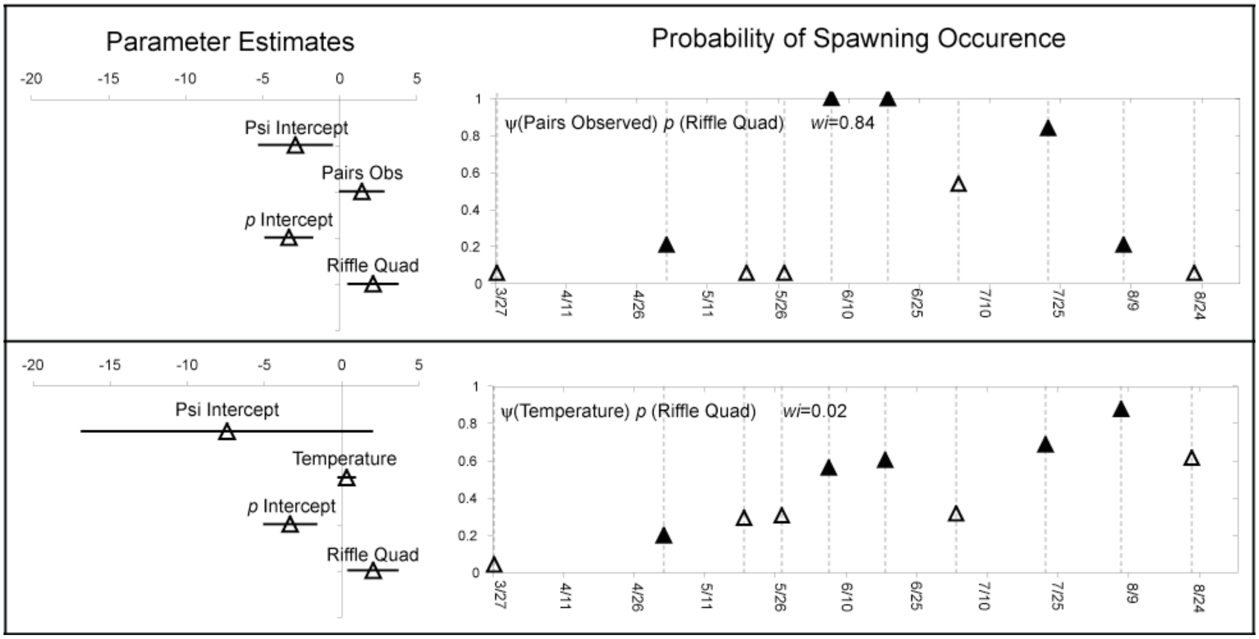

Figure 3.9. Model comparison of best-supported covariate models for occurrence-of-spawning probability (ψ) for *Etheostoma etowahae*, Etowah darter. Occurrence probability was calculated using visit conditions for Amicalola Creek at Dawson County Road 25. Triangles represent the probability of spawning occurrences for the Etowah darter, and filled shapes are dates which spawning was observed. See Table C.10 from Appendix C for parameter values with credible intervals.

CHAPTER FOUR

Management Implications and Conclusions

Based on the sampling efforts discussed in Chapter 2, the number of known localities of the holiday darter has been increased by eight sites. Furthermore, managers now have a basis for predicting where the two holiday darter taxa are likely to occur, and evidence that future loss of forest cover within this distribution may cause the ranges of these two already narrowly distributed taxa to shrink further. The findings of this study are consistent with those of Wenger et al. (2008), showing large decreases in predicted occupancy by several fish species at low levels of effective impervious area (EIA) in the Etowah River system. Undoubtedly, the importance of forest cover for aquatic diversity cannot be stressed enough. Within the southeastern United States, predominantly forested watersheds host most of the biologically diverse streams of the region (Warren et al. 2000). However, only a small percentage of this area (11%) is in public ownership (e.g., national forest, state parks, national parks), and most of that area is at high elevations with relatively low fish diversity (Warren et al. 2000, Wear and Greis 2002). As a result, a large amount of the imperiled fish taxa of the southeastern United States is not protected by federal ownership of the streams they occupy (Warren et al. 2000). The greater proportion of this forested land (71%) is owned by non-industrial private landowners (Warren et al. 2000, Wear and Greis 2002). This points to the strong potential influence that decisions by individual landowners and local governments have on the fate of forest dependent taxa in this highly diverse region.

The two-year study on the reproductive aspects of four imperiled and co-occurring percids (Chapter Three) represents one of the most extensive studies on darter reproduction conducted in the field. Other studies include: Stiles's (1972) examination of the ecology of three *Nothonotus* darters within the Little River of Tennessee and the research by Storey et al. (2006) on the reproductive behaviors of *E. scotti*. For many studies, high turbidity and limited visibility often restrict *in situ* observation of reproduction in stream fishes, especially benthic species. The relatively clear waters of the upper Etowah headwaters, particularly during two low-flow years, have afforded a relatively rare opportunity for field study of reproductive behaviors. One of the most important findings in this research is the updated information about the potential duration of the spawning season for two of the taxa studied (*Etheostoma* sp. cf. *brevirostrum* A and *E. etowahae*). It is the recommendation of this study to reduce the possible anthropogenic impacts on these species during these times of high susceptibility. However, the full duration of the spawning season for *E.* sp. cf. *E. brevirostrum* B and *Percina kusha* is still not known. Since there is no evidence to suggest differences within the reproductive behaviors of the Amicalola and Etowah holiday darter, restrictions of disturbances to spawning can be managed similarly between the populations. Unfortunately, no surrogate information on the full breeding season of *P. kusha* within the Etowah River system exists, therefore mangers must act with caution between April and June (the likely duration of the spawning season for this species).

The roles of temperature and photoperiod in the initiation and cessation of reproductive seasons for the four percid taxa still remain unknown. Several studies have suggested that these variables are important to the reproductive seasons of percids (Marsh 1980, Hubbs 1985); however, their effects might be best observed in aquaria where conditions can be manipulated. Another likely scenario is that these relationships might not be linear, resulting in a possible delay in the response for taxa.

Together, the two studies (Chapter Two and Chapter Three) on the autecological relationships of these taxa provide beneficial information for those who are involved in their management and protection. The last line of defense for any species that possibly faces the threat of extinction is the Endangered Species Act of 1973, as amended (Warren et al. 1997). To effectively implement this defense, two types of information are needed: accounting information (e.g., presence/absence patterns) and ecological information (e.g., habitat requirements; Warren et al. 1997). This thesis provides both types of information for two imperiled taxon (*Etheostoma* sp. cf. *brevirostrum* A and B) listed as state endangered and ecological information for two other taxa (*E. etowahae* and *Percina kusha*). Information on the distributional patterns of the Etowah darter (currently listed as federally endangered), has been made available by Wenger (2006); however, such accounting information is still needed for the bridled darter (currently listed as state endangered).

I would like to close with a few words from Warren et al. (1997) that eloquently state the direction needed for fish conservation within the southeastern United States:

> The last line of defense against extinction of fishes in the Southeast and elsewhere in the United States is the Endangered Species Act of 1973, as amended. It should remain just that, the last line of defense. Clearly, this Act, the strongest environmental law on Earth, cannot begin to meet the herculean task of conserving the Southeast's imperiled fishes, and as a species-by-species safety net, it simply cannot and should not be expected to function alone in conservation of the great southeastern fish fauna. We need shifts in management approaches that avert continued endangerment of fishes. The foundation of such an approach should include a system-led (e.g., drainage unit) rather than species-led focus; explicit biological integrity goals in the context of preventing degradation of high-quality systems and restoring poor-quality systems; commitment to implementing effective land-water management practices rather than implementing bureaucracies; and recognition of land and water resources as integrated parts of the same system.

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APPENDIX A

Information Relevant to Chapter One

The Etowah River System

The Etowah River system (4871 km^2) is a highly diverse aquatic network belonging to the Coosa River system of Georgia, Tennessee and Alabama. This network may have more imperiled fishes and invertebrates (17 spp. and 16 spp. respectively) than any other similarly sized river system in the southeastern United States, and is believed to have been a historical center of aquatic biodiversity within the eastern Mobile River drainage (Burkhead et al. 1997). The Etowah River historically supports 91 native fish species (76 extant), including three federally protected fish species, the Etowah darter (*Etheostoma etowahae*), Cherokee darter (*Etheostoma scotti*) and amber darter (*Percina antesella*), as well as five state-protected fish species, the Coosa chub (*Macrhybopsis* sp. cf. *M. aestivalis*), Coosa madtom *(Noturus sp. cf. N. munitus*), the Amicalola holiday darter *(Etheostoma* sp. cf. *E. brevirostrum* A), the Etowah holiday darter *(Etheostoma* sp. cf. *E. brevirostrum* B), freckled darter (*Percina lenticula*) and bridled darter (*Percina kusha*; Freeman et al. 2005).

The Etowah River system is divided into six physiographic districts composed of a total of 16 large watersheds (USGS 10 digit HUCs). Of the 165 river miles of the main channel, only 85 are free-flowing and unregulated (upstream of Canton, GA and the Allatoona Reservoir; Burkhead et al. 1997). Within this free-flowing section of the system resides the greatest amount of fish diversity, including many of the imperiled species of the watershed. High levels of species imperilment have resulted mostly from extensive loss of habitat and continuity, restricting survival of formally more widespread assemblages to fragmented headwater systems (Burkhead et al. 1997). The greatest percentage of these imperiled species are rheophilic freshwater fishes, and 78% of the imperiled fishes are members of the family Percidae (Burkhead et al. 1997, Freeman et al. 2005).

Information on Target Species

The holiday darter taxa (*Etheostoma* sp. cf. *E. brevirostrum* A and B*)* of the Etowah River system are currently listed as endangered by the State of Georgia Department of Natural Resources (2008). The taxa occupy relatively small high elevation streams (Figure A.1) in the headwater portions of the Etowah River system (Figure A.2). These taxa belong to the subgenus *Ulocentra*, with which two other species (*E. coosae* and *E. scotti*) of the Etowah River system are also affiliated. Species descriptions of members of this subgenus, also known as snubnose darters, rely largely on nuptial male color variation; however, the subgenus is united by few synapomorphies (Porter et al. 2002). Perhaps because *Ulocentra* species evolved through allopatry, several species have been known to hybridize under laboratory conditions (Winn 1958). One possible synapomorphy that has been suggested is the reproductive behavioral character of females laying eggs on vertical rock faces, but reproductive aspects for several species within this subgenus have yet to be studied (Bailey and Etnier 1988). *Ulocentra* species are divided into two species groups, the *E. simoterum* species group and the *E. duryi* species group, the latter being the group to which the *E. brevirostrum* complex belongs (Porter et al. 2002). Life history attributes and habitat patch occupancy information are needed for the holiday darter taxa of the Etowah in order to identify potential stressors and to assess relative vulnerability.

The Etowah darter (*Etheostoma etowahae*) is endemic to the Etowah River system and is currently listed as an endangered species under the federal Endangered Species Act of 1973 due to its restricted range and vulnerability to habitat degradation (Wood and Mayden 1993, Burkhead et al. 1997). The species actually is known from a relatively broad range of elevations and stream sizes (Figure A.1), although most known locales are upstream of Allatoona dam and reservoir (Figure A.3). Described by Wood and Mayden (1993), the Etowah darter is one of two species, along with *E. jordani*, of the Etowah River system belonging to subgenus *Nothonotus* (Burkhead et al. 1997, Ritchea 2006). Designated as sister species, *E. jordani* and *E. etowahae* belong to the same species group (*E. jordani*). The endangered status of *E. etowahae* merits a life history study to inform conservation, and is the only federally listed fish species within the Etowah for which life history information is not yet available.

The bridled darter (*Percina kusha)* is a species recently described by Williams et al. (2007) that is limited to the Conasauga and Etowah River systems. Within the Etowah River system, this species has been collected in locations near the headwaters (Figures A.1, A.4). This species, always found in low numbers, has been characterized as rare by Freeman (1999), vulnerable by Warren et al. (2000) and Freeman et al. (2005), endangered by Williams et al. (2007), and is state-listed as endangered in Georgia (GADNR 2008). The bridled darter is the only species thought to belong to the subgenus *Alvordius* within the Etowah River system, and is a sister species of *P. smithvanizi* (the muscadine darter) of the Tallapoosa River system (Williams et al. 2007). Recent genetic research performed on species within the subgenus *Alvordius* has shown that the subgenus is not monophyletic, and several species including *P. smithvanizi* could not be placed into a monophyletic clade (Near 2002). This could indicate that *P. kusha* was misdiagnosed as part of the subgenus *Alvordius* (Near 2002, Williams et al. 2007). Life history information could be useful in categorizing these species into the proper subgenus. While the seasonal, diel and spawning habitat of *P. kusha* within the Conasauga River were assessed by Johnston et al. (2002), no information on the biology and ecology of the Etowah populations exists to date (Williams et al. 2007).

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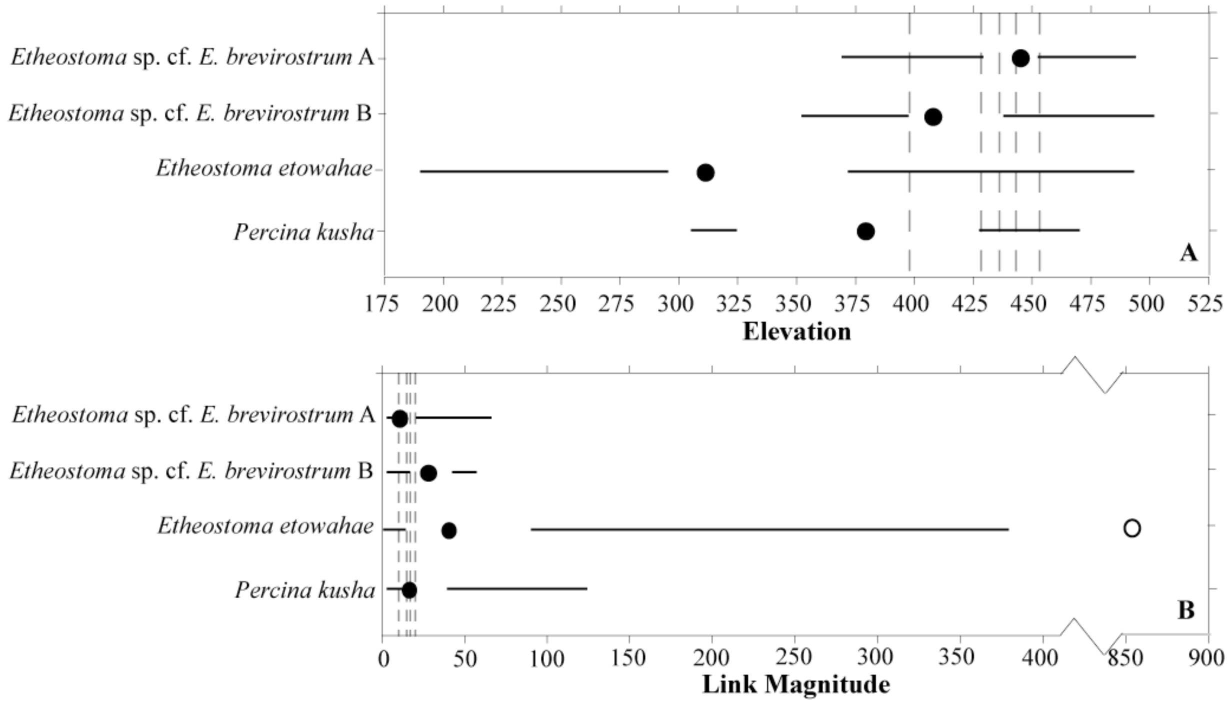

Figure A.1. Elevation (A) and link magnitude (B) of known localities of target species. Range of values is designated by solid horizontal black lines. Inter-quartile range is designated by area between solid black lines. Median values are represented with solid black circles and outliers are designated as hollow circles. Study sites for the reproductive aspects of the target species (Chapter Three) are indicated with vertical dashed lines. Elevation was calculated using a USGS 10 meter resolution digital elevation model and link magnitude is based on a 1:100,000 national hydrography dataset.

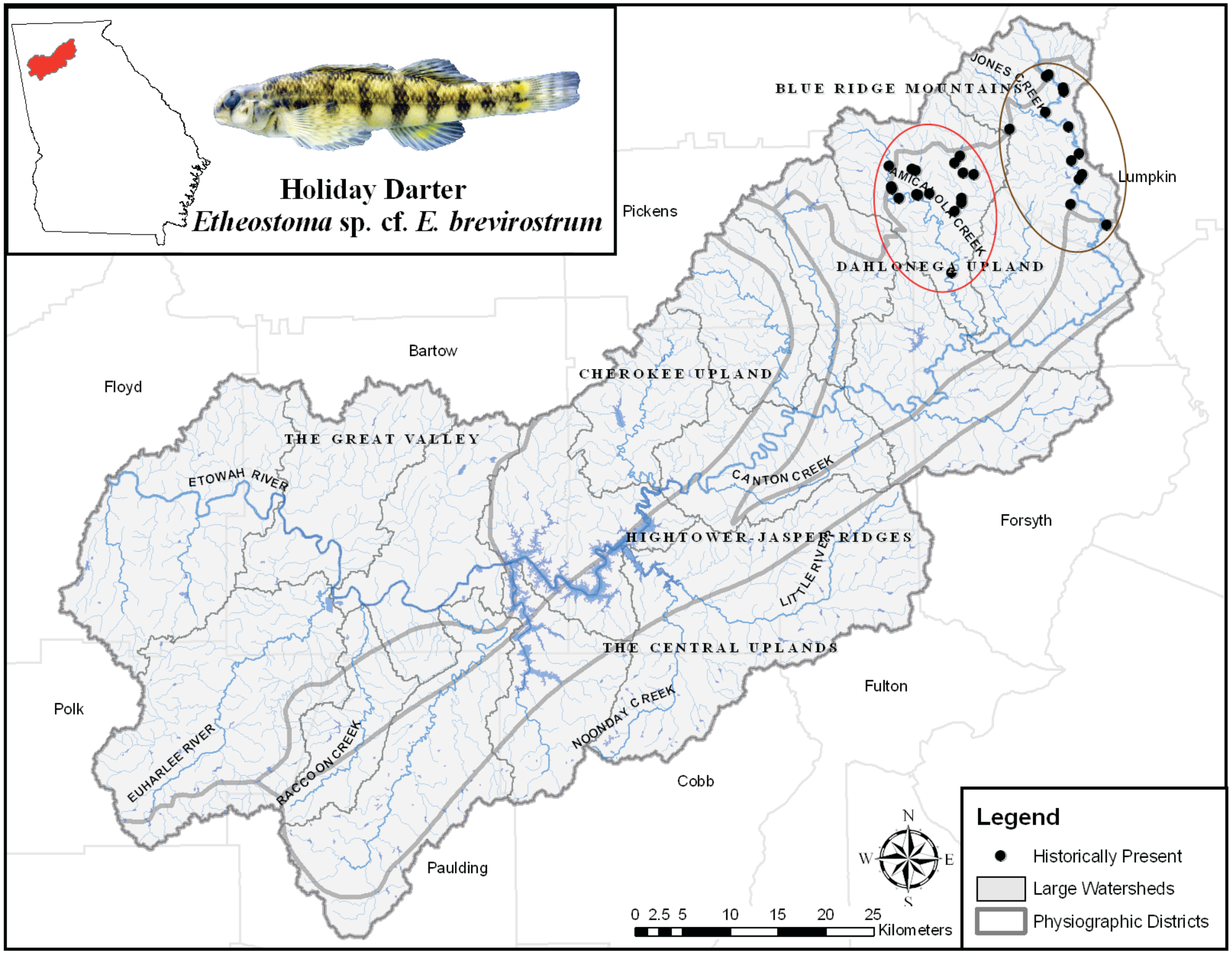

Figure A.2. Known distribution of *Etheostoma* sp. cf. *E. brevirostrum* A and B, Amicalola and Etowah holiday darters. The red circle indicates Amicalola holiday darter population and brown circle indicates the Etowah holiday darter population. Known distribution made available though the Georgia Museum of Natural History. Photograph by Byron J. Freeman.

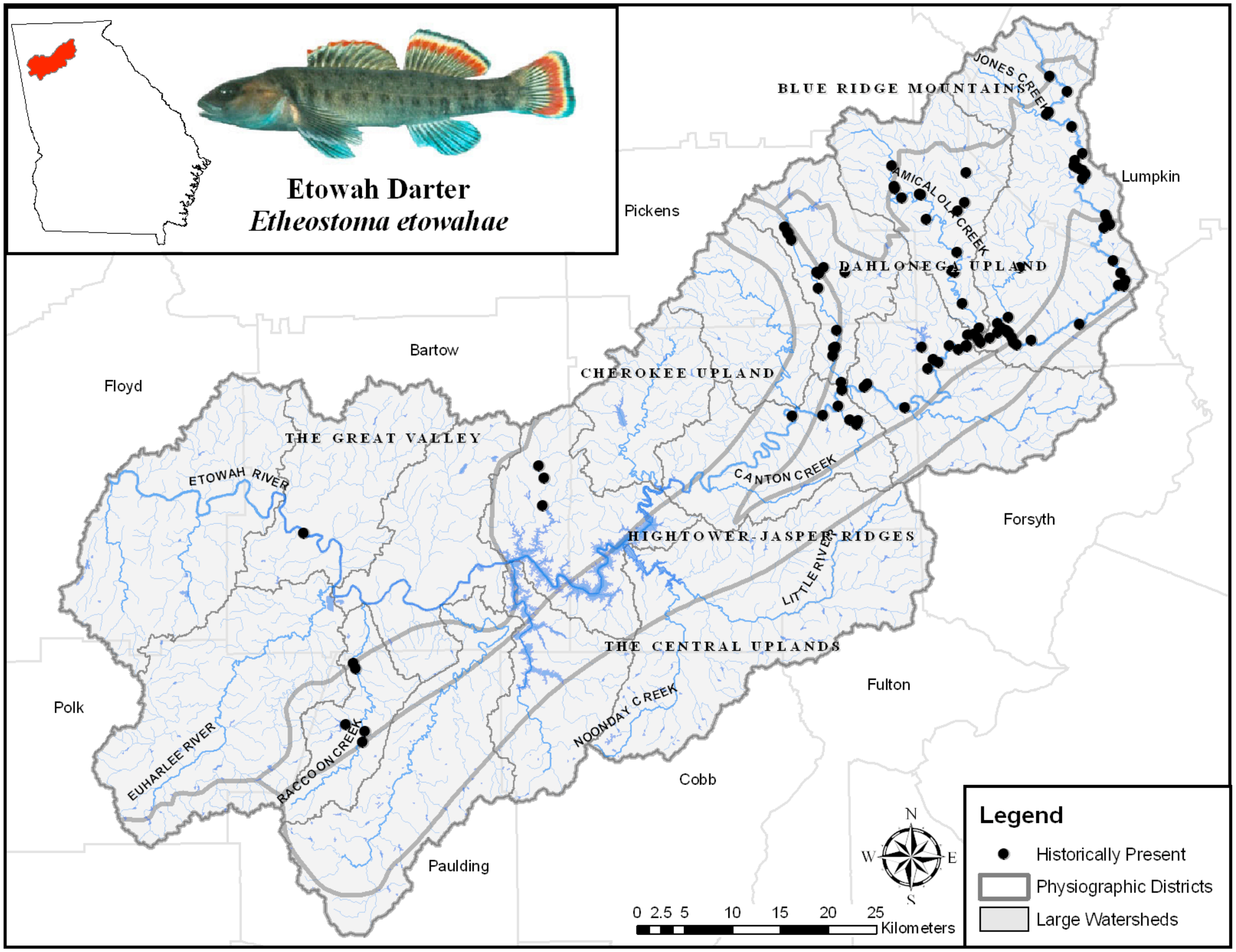

Figure A.3. Known distribution of *Etheostoma etowahae*, Etowah darter. Known distribution made available though the Georgia Museum of Natural History. Photograph by Noel M. Burkhead.

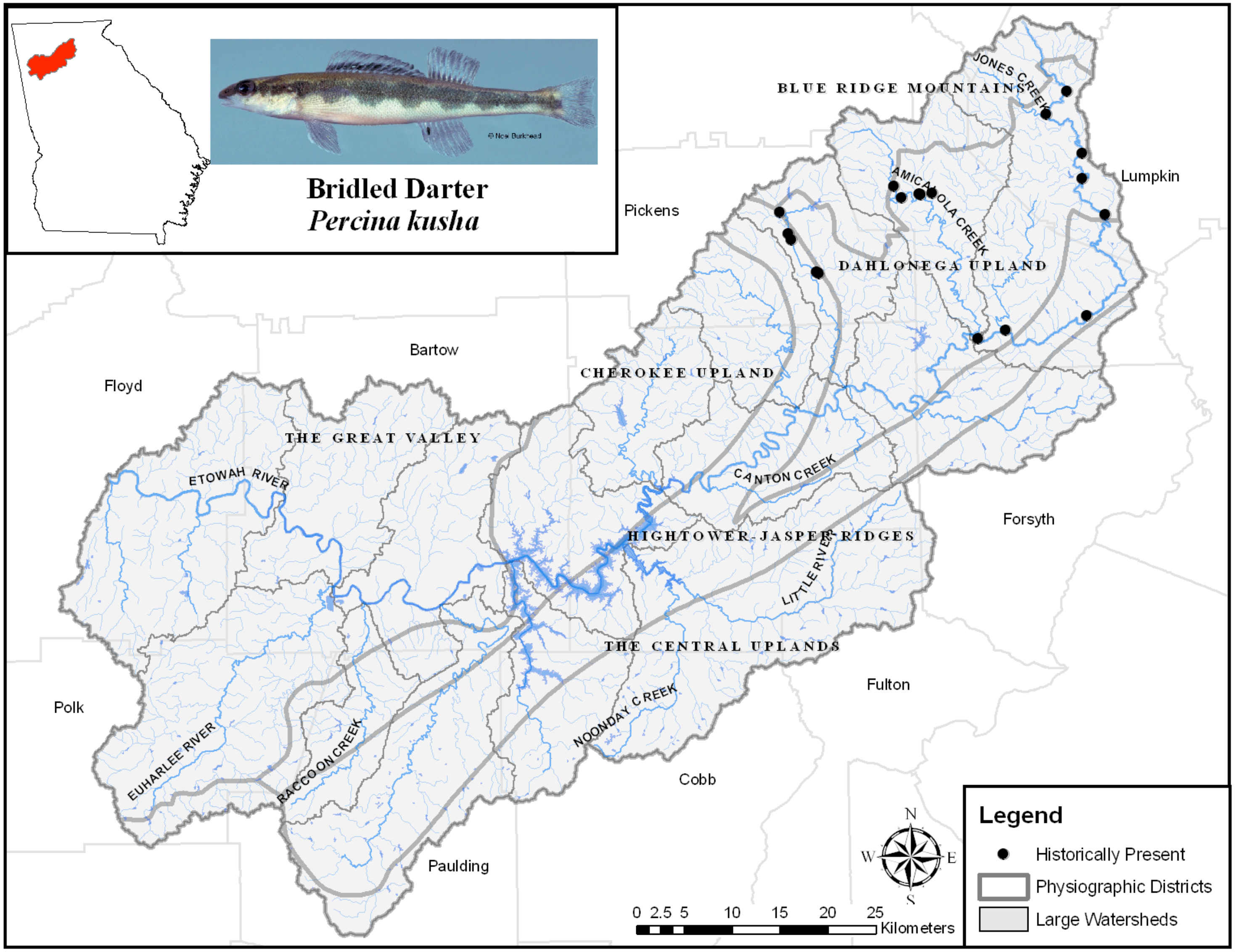

Figure A.4. Known distribution of *Percina kusha*, bridled darter within the Etowah River system (inset). Known distribution made available though the Georgia Museum of Natural History. Photograph by N. M. Burkhead.

Appendix B

Information Relevant to Chapter Two.

Table B.1. Parameter estimates and confidence intervals for best-supported models in candidate set ($\Delta AIC_C < 2$).

		95% Confidence Limits	
Parameter	**Estimate (se)**	**Lower**	**Upper**
Ψ(Downstream Link) *p*(Elevation, Link Slope, PNF)			
Ψ			
Intercept	-7.66 (3.553)	-14.623	-0.697
Downstream Link	38.059 (18.63)	1.545	74.573
p			
Intercept	-8.508 (1.964)	-12.357	-4.660
Elevation	1.93 (0.481)	0.987	2.873
Link Slope	-0.857 (0.256)	-1.360	-0.355
Proportion of Non-Forest in Catchment	-0.15 (0.035)	-0.218	-0.082
Ψ(Downstream Link, Watershed) *p*(Elevation, Link Slope, PNF)			
Ψ			
Intercept	-9.397 (4.274)	-17.774	-1.020
Downstream Link	38.532 (18.572)	2.131	74.933
Amicalola Watershed	2.345 (1.819)	-1.220	5.910
p			
Intercept	-8.497 (1.973)	-12.364	-4.630
Elevation	1.928 (0.483)	0.981	2.875
Link Slope	-0.86 (0.258)	-1.365	-0.355
Proportion of Non-Forest in Catchment	-0.15 (0.035)	-0.219	-0.082
Ψ(Downstream Link, Watershed) *p*(Elevation, Link Slope, PNF)			
Ψ			
Intercept	-11.412 (4.917)	-21.049	-1.776
Downstream Link	46.755 (19.67)	8.195	85.315
Amicalola Watershed	1.364 (1.017)	-0.629	3.357
p			
Intercept	-8.518 (1.966)	-12.372	-4.664
Elevation	1.934 (0.482)	0.990	2.879
Link Slope	-0.868 (0.26)	-1.377	-0.359
Proportion of Non-Forest in Catchment	-0.15 (0.035)	-0.218	-0.082

Table B.2. Site conditions at sampling sites for *Etheostoma* sp. cf. *brevirostrum* A and B. Conductivity (Con) measured in µS, dissolved oxygen (D.O.) measured in ppm, turbidity (Turb) measured in NTU, and ºC refers to water temperature during visit.

Date	Sample Site	Con	D.O.	pH	Turb	ºC
9/24/08	Jones Creek upstream of confluence with Etowah	7	10.6	5.7	3.41	15.2
9/26/08	Etowah River at CR 2	12	10.1	6.4	3.98	15.5
9/24/08	Bull Creek at Bull Creek Way	17	10.4	6.1	2.87	14.1
6/24/08	Nimblewill Creek at Nimblewill Gap Rd.	6	8.6	6.9	4.13	19.6
9/24/08	Nimblewill Creek at CR 128	7	9.8	5.6	3.22	16.9
9/26/08	Unnamed Tributary to Cochrans Creek at CR 43	11	9.5	6.3	7.59	15.2
6/25/08	Amicalola Creek at CR 192	13	8.7	6.9	6.91	20.3
6/25/08	Unnamed Tributary to Amicalola Creek u.s. of Amicalola Church R	14	7.5	6.8	9.05	19.2
7/1/08	Unnamed Tributary to Little Amicalola Creek at SR 136	11	7.8	7.5	4.32	20.9
7/1/08	Unnamed Tributary to Cochrans Creek at New Hope Rd	12	9.1	6.9	12.1	17.4
7/2/08	Unnamed Tributary to Amicalola Creek adjacent to SR 53	14	9.1	7.1	17.1	15.1
7/2/08	Cochrans Creek at SR 183	16	8.6	6.7	12.7	18.4
7/2/08	Unnamed Tributary to Amicalola Creek at Falls Trail Rd.	9	9	6.7	3.52	17.3
7/8/08	Mill Creek at CR 115	13	8.6	6.3	4	20.6
7/8/08	Etowah River at CR 75	-	-	-	5.52	-
7/15/08	Wildcat Creek in Dawson Forest WMA	8.2	9.0	6.8	2.87	18.5
7/15/08	Fall Creek in Dawson Forest WMA	7.9	9.2	6.6	3.14	18.6
7/16/08	Unnamed Tributary to Amicalola Creek d.s. of Liberty Lane	23.4	8.1	5.9	4.46	18.9
7/16/08	Little Amicalola Creek at CR 25	14.7	8.6	6.2	8.14	20.8
7/16/08	Amicalola Creek at CR 28	12.9	8.7	6.4	2.97	21
7/27/08	Etowah River 0.6 miles d.s. of CR 2	13	8.5	7.1	5.82	22.2
7/27/08	Calhoun Creek upstream of Confluence with Etowah River	23	7.5	7.0	7.66	22
7/28/08	Amicalola Creek at SR 53	15	8.4	6.7	5.46	22.3
7/28/08	Gad Creek at SR 52	16	8.6	6.5	4.75	20.2
7/28/08	Unnamed Tributary to Little Amicalola Creek at CR 26	9	7.6	6.1	4.48	19.5
8/11/08	Little Amicalola Creek at 136	15	8.2	6.1	2.24	20
8/11/08	Little Amicalola Creek d.s. of Johnnytown Rd.	9	7.8	6.6	6.17	20.5
8/11/08	Cochrans Creek d.s. of Dan Fowler Rd.	9	8.4	6.4	1.85	19.5
8/12/08	Etowah River at FS 28-1	9	9.1	6.9	2.36	16.4
8/12/08	Ward Creek CR 28	12	7.0	6.7	1.58	17.3
8/12/08	Edmunston Creek off CR 361	11	8.3	6.9	5.21	17.3
9/1/08	Two Run Creek d.s. CR 187	14	8.5	7.0	2.03	20.3
9/1/08	Moss Creek off FS 28-1	8	8.9	6.8	9.15	20.2
9/1/08	Jones Creek off FS 77A	5	8.7	6.2	2.8	19.7
9/2/08	Etowah River at SR 52	11	8.9	6.4	5.2	21
9/2/08	Hurricane Creek at CR 202	8	9.2	6.5	5.92	19.2
9/2/08	Braggs Branch 0.43m u.s. of confluence with Etowah River	23	8.4	6.6	3.38	21.8
9/3/08	Mudd Creek d.s. of CR 75	64	8.3	6.9	4.92	19.3
9/3/08	Hurricane Creek 0.17m u.s. of confluence with Etowah River	14	8.3	6.6	17.1	22.4
9/3/08	Tobacco Pouch Creek d.s. of Tobacco Pouch Lane	16	8.7	6.5	7.33	20.9
6/24/08	Bull Creek at Bull Creek Way	18	9.0	7.2	3.15	16.6
6/24/08	Nimblewill Creek at CR 128	7	7.9	6.7	5.42	21.9
6/25/08	Unnamed Tributary to Cochrans Creek at New Hope Rd	8	8.2	7.3	2.76	15.7
9/26/08	Unnamed Tributary to Amicalola Creek adjacent to SR 53	14	9.9	6.3	3.47	15

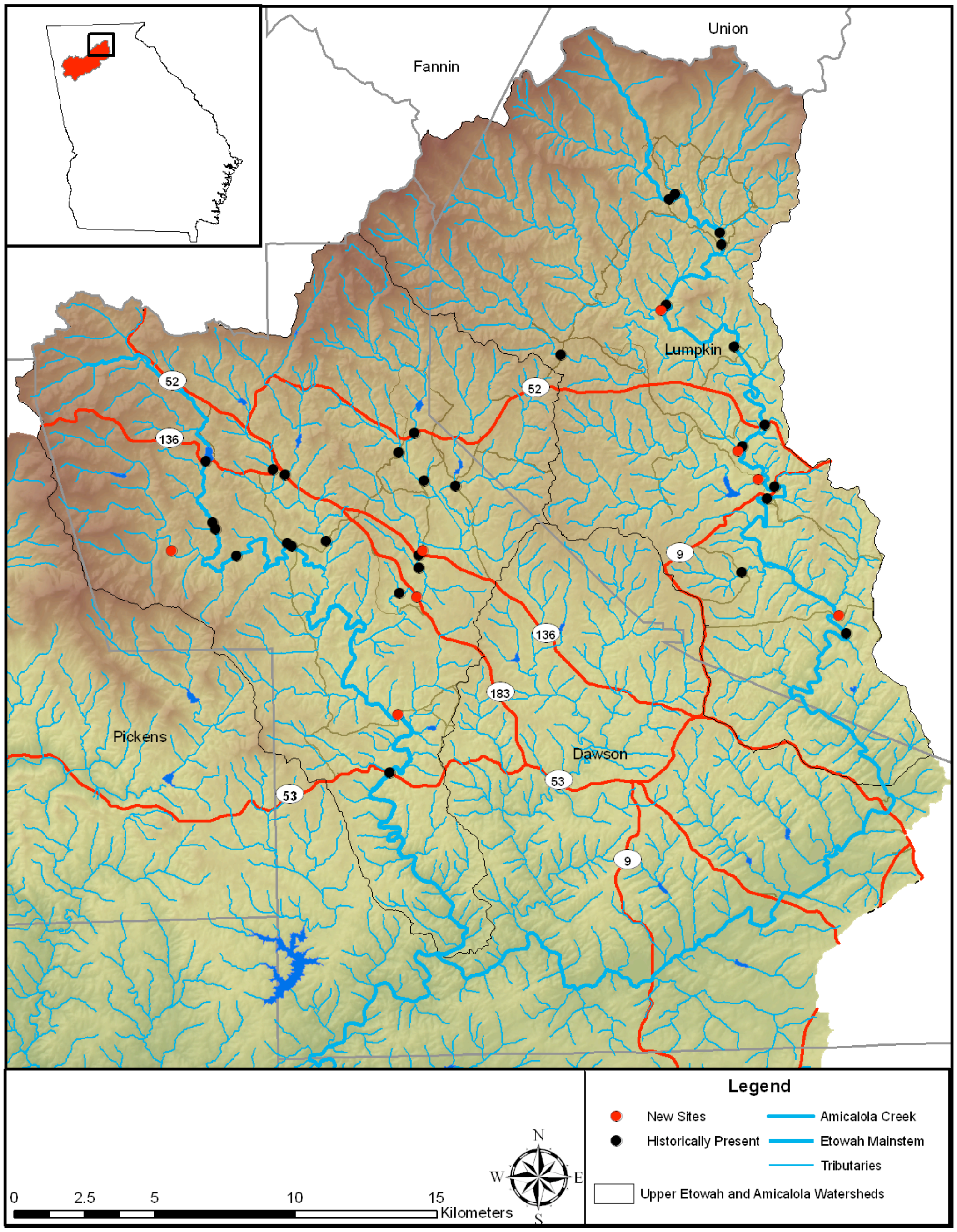

Figure B.1. New and historic known locations of holiday darter within the Etowah River system.

Appendix C

Information Relevant to Chapter Three

Table C.1. Habitat conditions associated with spawning occurrences of *Etheostoma* sp. cf. E. *brevirostrum* A & B (Amicalola and Etowah holiday darters). Substrate for the 2007 study period (April 20-July 17) was qualitatively assessed and placed into a category from the Wentworth scale. In the 2008 study period (March 27-August 22), substrate was measured on the intermediate axis and then place into the category. nr=not recorded. For reproductive behaviors observed and site conditions see Table C.4-Table C.8.

Date	Site	Temperature (C)	Depth (cm)	Velocity (m/s) 60% Depth	Velocity (m/s) Substrate	Substrate
***Etheostoma* sp. cf. *E. brevirostrum* A (Amicalola holiday darter)**						
4/20/07	1 (eto305)	10.58	24.4	nr	nr	Cobble/Gravel*
4/20/07	3 (eto264)	11.78	70.1	nr	nr	Cobble/Gravel*
4/20/07	3 (eto264)	11.78	76.2	nr	nr	Bedrock/Boulder
4/20/07	3 (eto264)	11.78	76.2	nr	nr	Bedrock/Boulder
5/4/07	1 (eto305)	16.86	44.8	0.67	nr	Coarse Wood
5/18/07	1 (eto305)	17.93	15.2	nr	nr	Cobble/Gravel*
5/18/07	3 (eto264)	16.35	36.6	nr	nr	Bedrock/Boulder
5/18/07	3 (eto264)	16.35	25.9	nr	nr	Gravel/Cobble/Boulder*
5/18/07	3 (eto264)	16.35	25.9	nr	nr	Gravel/Cobble/Boulder*
5/2/08	1 (eto305)	14.66	36.6	0.1	0.03	Cobble
5/2/08	1 (eto305)	14.66	45.7	0.11	0.10	Coarse Gravel
5/2/08	1 (eto305)	14.66	57.9	0.07	0.06	Cobble
5/2/08	3 (eto264)	15.93	48.8	0.22	0.11	Very Coarse Gravel
5/2/08	3 (eto264)	15.93	51.8	0.23	0.09	Very Coarse Gravel
5/2/08	3 (eto264)	15.93	39.6	0.09	-0.01	Bedrock/Boulder
5/20/08	1 (eto305)	17.28	33.5	0.01	-0.03	Very Coarse Gravel
5/20/08	1 (eto305)	17.28	39.6	0.11	0.03	Cobble
5/20/08	1 (eto305)	17.28	24.4	-0.03	0.01	Very Coarse Gravel
5/20/08	1 (eto305)	17.28	18.3	0.02	0.01	Cobble
5/20/08	1 (eto305)	17.28	24.4	-0.02	-0.01	Cobble
***Etheostoma* sp. cf. *E. brevirostrum* B (Etowah holiday darter)**						
5/11/07	4 (eto322)	15.27	64	0.1	nr	Cobble*
5/24/07	5 (eto326)	16.85	64	-0.03	-0.1	Gravel/sand*

*Substrate qualitatively assessed.

Table C.2. Habitat conditions associated with spawning occurrences of *Etheostoma etowahae* (Etowah darter). Substrate for the 2007 study period (April 20-July 17) was qualitatively assessed and placed into a category from the Wentworth scale. In the 2008 study period (March 27-August 22), substrate was measured on the intermediate axis and then place into the category. nr=not recorded. For reproductive behaviors observed and site conditions see Table C.4-Table C.8.

Date	Site	Temperature (C)	Depth (cm)	Velocity (m/s) 60% Depth	Velocity (m/s) Substrate	Substrate
6/5/07	2 (eto150)	18.69	21.3	0.28	0.15	Nested medium gravel
5/2/08	3 (eto264)	15.93	15.2	0.35	0.28	Sand coarse sand*
5/30/08	5 (eto326)	17.58	30.5	0.48	0.32	Nested coarse sand to fine gravel*
5/30/08	5 (eto326)	17.58	27.4	0.47	0.21	Nested fine to medium gravel*
5/30/08	5 (eto326)	17.58	27.4	0.53	0.2	Nested fine to medium gravel*
6/6/08	2 (eto150)	22.79	33.5	0.4	0.7	Nested coarse sand*
6/6/08	2 (eto150)	22.79	18.3	0.39	0.28	Nested coarse sand*
6/6/08	2 (eto150)	22.79	21.3	0.55	0.37	Nested coarse sand*
6/6/08	3 (eto264)	20.31	18.3	0.55	0.27	Nested coarse sand to fine gravel*
6/6/08	3 (eto264)	20.31	16.8	0.72	0.56	Nested coarse sand to fine gravel*
6/6/08	3 (eto264)	20.31	12.2	0.59	0.23	Nested coarse sand to fine gravel*
6/6/08	3 (eto264)	20.31	13.7	0.7	0.47	Nested coarse sand to fine gravel*
6/9/08	5 (eto326)	19.65	7.6	0.25	0.03	Nested fine to medium gravel*
6/9/08	5 (eto326)	19.65	8.2	0.4	0.4	Nested fine to medium gravel*
6/18/08	2 (eto150)	17.85	16.8	0.31	0.2	Nested fine to medium gravel
6/18/08	3 (eto264)	20.75	12.2	0.64	0.41	Nested medium gravel
6/18/08	3 (eto264)	20.75	21.3	0.15	0.09	Nested very fine to medium gravel
6/26/08	2 (eto150)	20.69	18.3	0.77	0.26	Nested coarse gravel
7/22/08	3 (eto264)	21.73	27.4	0.75	0.56	Nested fine to medium gravel*
7/30/08	5 (eto326)	20.61	21.3	0.49	-0.07	Nested fine to medium gravel*
8/7/08	3 (eto264)	22.77	7.6	0.37	0.35	Nested fine to medium gravel*

*Substrate qualitatively assessed.

Table C.3. Habitat conditions associated with spawning occurrences of *Percina kusha* (bridled darter). Substrate for the 2007 study period (April 20-July 17) was qualitatively assessed and placed into a category from the Wentworth scale. In the 2008 study period (March 27-August 22), substrate was measured on the intermediate axis and then place into the category. nr=not recorded. For reproductive behaviors observed and site conditions see Table C.4-Table C.8.

Date	**Site**	**Temperature (C)**	**Depth (cm)**	**Velocity (m/s) 60% Depth**	**Velocity (m/s) Substrate**	**Substrate**
4/20/07	2 (eto150)	14.12	41.2	nr	nr	Sand*
5/4/07	2 (eto150)	17.34	50.3	-0.03	nr	Sand/fine gravel*
5/4/07	2 (eto150)	17.34	33.5	0.12	nr	Sand/fine gravel*
6/5/07	2 (eto150)	18.69	24.4	0.26	0.18	Sand/fine gravel*
6/5/07	2 (eto150)	18.69	39.6	0.22	0.13	Sand/fine gravel*
5/19/08	2 (eto150)	14.35	57.9	0.49	0.21	Sand*

*Substrate qualitatively assessed.

Table C.4. Site conditions and spawning behaviors observed on each sampling date at Site 1 (Cochrans Creek at County Road 374; eto305). M/F= male-female courtship (e.g. following, chasing, or displaying), M/M= male-male aggression (chasing, displaying, or defending territory), F/F= female-female aggression (chasing, biting or defending territory) S= spawning occurrence, and NSA= no spawning activity. No. Total= the total number of individuals of the species observed; this was not recorded in the 2007 sampling period, nr= not recorded. No. pairs= the number of male-female pairs observed during the visit. Time refers to the total collective time of all observers. Water Height was measured as the mean subtracted from the benchmark height observed during the length of the study (two years); positive values indicate a water level higher than the mean and negative values indicate a water level lower than the mean.

Site Conditions							**Amicalola Holiday Darter**		
Date	Time (min)	Temp (C)	DO (ppm)	pH	Height (cm)	Turb. (NTU)	No. Total	No. Pairs	Behaviors Observed
4/20/07	120**	10.58	10.13	7.7	6.85	2.73	nr	1	M/F, S
5/4/07	120	16.86	8.57	8.44	0.91	4.06	nr	5	M/M, M/F, S
5/18/07	160	17.93	8.21	7.66	-1.09	3.1	nr	2	M/F, S
6/5/07	90	20.97	nr	nr	-1.09	4.28	nr	0	NSA
6/17/07	240**	19.26	8.56	7.43	nr	2.98	nr	0	NSA
3/27/08	48	10.47	9.96	7.15	0.91	1.95	22	1	M/F
5/2/08	95	14.66	9.37	7.3	-0.09	3.37	49	3	M/M, M/F, C, S
5/7/08	69	17.97	8.65	7.22	-1.09	3	46	3	M/F, C
5/20/08	100	17.28	8.37	7.25	-1.09	2.95	45	2	M/M, M/F, C, S
5/30/08	nr	18.38	8.03	7.18	-0.09	3.07	nr	1	M/F
6/23/08	71	21.02	7.63	7.13	-4.09	3.97	28	0	NSA

**Indicates three observers.

Table C.5. Site conditions and spawning behaviors observed on each sampling date at Site 2 (Amicalola Creek at County Road 28; eto150). See Table C.4 for notations.

Site Conditions						Amicalola Holiday Darter			Etowah Darter			Bridled Darter		
Date	Time (min)	Temp (C)	DO (ppm)	Height (cm)	Turb. (NTU)	No. Total	No. Pairs	Behaviors Observed	No. Total	No. Pairs	Behaviors Observed	No. Total	No. Pairs	Behaviors Observed
4/20/07	225**	14.12	9.53	4.81	1.65	nr	0	NSA	nr	0	M/M	nr	1	M/F,S
5/4/07	120	17.34	8.78	nr	2.35	nr	0	NSA	nr	0	NSA	nr	1	M/M, S
5/18/07	280	14.34	9.35	1.33	2.48	nr	0	NSA	nr	2	M/F, C	nr	0	NSA
6/5/07	320	18.69	8.72	-2.27	2.39	nr	0	NSA	nr	3	M/M, M/F, C, S	nr	2	M/M, M/F, S
6/17/07	300**	22.62	7.92	0.13	2.84	25	1	NSA	nr	1	M/F	5	0	NSA
7/8/07	85*	20.58	7.56	-3.27	2.46	16	0	NSA	nr	4	M/F	1	0	NSA
3/27/08	113	12.92	10.3	nr	1.44	23	1	M/F	10	0	M/M	14	1	M/F, C
5/2/08	138	17.91	8.89	-0.27	2.58	34	0	NSA	6	2	M/F, C	2	0	NSA
5/19/08	191.5	14.35	9.09	7.73	3.35	19	2	M/F, C	32	2	M/F	22	2	M/M, M/F, C, S
5/29/08	nr	17.47	8.08	4.73	3.33	nr	2	M/M, M/F	nr	3	M/F, C	nr	0	NSA
6/6/08	nr	22.79	6.95	0.73	3.61	nr	0	NSA	nr	3	F/F, M/F, C, S	nr	0	NSA
6/9/08	163	24.07	6.49	-0.27	3.56	17	0	NSA	37	3	M/M, F/F, M/F, C	4	0	NSA
6/18/08	277	17.85	6.9	-1.27	3.5	28	0	NSA	43	4	M/F, C, S	9	0	NSA
6/26/08	270	20.69	8.3	-3.27	3.44	40	0	NSA	97	11	M/M, M/F, C, S	16	1	M/M, M/F
7/17/08	351	20.36	8.47	-0.27	4.15	43	0	NSA	107	12	M/M, F/F, M/F, C	15	0	NSA
7/30/08	108*	23.44	6.83	-3.27	2.85	6	0	NSA	45	3	M/M, F/F, M/F, C	7	0	NSA
8/15/08	119	19.35	8.91	-5.27	3.86	25	0	NSA	38	2	M/M, M/F	15	0	NSA

* Indicates a single observer

**Indicates three observers

Table C.6. Site conditions and spawning behaviors observed on each sampling date at Site 3 (Amicalola Creek at County Road 25; eto264). See Table C.4 for notations.

Site Conditions						Amicalola Holiday Darter			Etowah Darter			Bridled Darter		
Date	Time (min)	Temp (C)	DO (ppm)	Height (cm)	Turb. (NTU)	No. Total	No. Pairs	Behaviors Observed	No. Total	No. Pairs	Behaviors Observed	No. Total	No. Pairs	Behaviors Observed
4/20/07	225**	11.78	10.14	5.97	1.98	nr	3	M/M, M/F, S	nr	0	NSA	nr	0	NSA
5/4/07	120	17.1	8.72	nr	2.85	nr	1	M/F	nr	0	NSA	nr	0	NSA
5/18/07	180	16.35	9.35	1.35	2.64	nr	2	M/F, S	nr	0	NSA	nr	0	NSA
6/5/07	230	21.02	nr	-0.65	nr	nr	0	NSA	nr	2	M/M, M/F, C	nr	0	NSA
6/17/07	315**	20.76	8.76	0.55	2.91	25	0	NSA	nr	0	NSA	6	0	NSA
7/8/07	70*	20.95	8.09	-0.65	2.4	16	0	NSA	nr	0	NSA	3	0	NSA
3/27/08	99.5	11.27	10.31	-0.65	1.62	10	1	M/F, C	3	0	NSA	4	0	NSA
5/2/08	139.5	15.93	9.25	2.35	2.69	28	2	M/M, M/F, C, S	7	1	M/M, M/F, C, S	13	1	M/F, C
5/19/08	88	17.31	8.8	5.35	3.09	5	0	NSA	10	0	M/M	17	0	NSA
5/27/08	nr	20.24	7.95	1.35	2.97	nr	0	NSA	nr	0	NSA	nr	2	M/M, M/F, C
6/6/08	199	20.31	7.11	-1.65	3.66	22	1	M/F, C	28	9	M/M, F/F, M/F, C, S	10	0	NSA
6/18/08	181	20.75	nr	-1.65	3.32	9	0	NSA	27	6	M/M, M/F, C, S	12	0	NSA
7/3/08	190	17.6	8.82	-3.65	2.84	47	0	NSA	31	2	M/M, M/F, C	16	0	NSA
7/22/08	243	21.73	8.89	-0.65	3.22	18	0	NSA	40	3	M/F, C, S	12	0	NSA
8/7/08	142	24.88	7.88	-2.65	2.99	2	0	NSA	17	1	M/M, S	3	0	NSA

* Indicates a single observer

**Indicates three observers

Table C.7. Site conditions and spawning behaviors observed on each sampling date at Site 4 (Etowah River at Georgia State Route 52; eto322). See Table C.4 for notations.

Site Conditions						Etowah Holiday Darter			Etowah Darter			Bridled Darter		
Date	Time (min)	Temp (C)	DO (ppm)	Height (cm)	Turb. (NTU)	No. Total	No. Pairs	Behaviors Observed	No. Total	No. Pairs	Behaviors Observed	No. Total	No. Pairs	Behaviors Observed
4/29/07	360**	15.27	10.09	10.47	3	nr	1	M/F	nr	0	NSA	nr	1	M/F
5/11/07	270	17.55	9.08	6.27	4.59	nr	1	S	nr	2	M/F	nr	0	NSA
5/24/07	140	16.72	10.19	2.47	3.89	nr	0	NSA	nr	0	NSA	nr	0	NSA
6/14/07	210	19.79	8.73	nr	4.48	6	0	NSA	nr	2	M/F	2	0	NSA
6/28/07	210	20.27	8.37	8.47	5.42	4	0	NSA	nr	0	NSA	1	0	NSA
5/7/08	100	15.46	9.06	-0.53	3.94	14	1	M/F, C	nr	0	NSA	1	0	NSA
5/20/08	107	15.78	8.82	0.47	4.19	10	0	NSA	10	0	NSA	2	0	NSA
5/30/08	nr	18.84	8.51	nr	5.13	nr	0	NSA	nr	0	NSA	nr	0	NSA
6/20/08	137	19.05	9.3	-6.53	4.74	23	0	NSA	16	0	NSA	8	0	NSA
7/3/08	164	18.81	8.65	-6.53	5.5	24	0	NSA	13	1	NSA	5	0	NSA
7/22/08	160	23.94	8.3	2.47	4.84	31	0	NSA	19	0	NSA	5	0	NSA
8/7/08	227	22.77	8.04	-12.53	4.36	21	0	NSA	29	1	F/F,M/F	9	0	NSA
8/22/08	133	22.16	9.05	-4.53	3.37	17	0	NSA	12	1	M/F	5	0	NSA

* Indicates a single observer

**Indicates three observers

Table C.8. Site conditions and spawning behaviors observed on each sampling date at Site 5 (Etowah River at County Road 72; eto326). See Table C.4 for notations.

		Site Conditions				Etowah Holiday Darter			Etowah Darter			Bridled Darter		
Date	Time (min)	Temp (C)	DO (ppm)	Height (cm)	Turb. (NTU)	No. Total	No. Pairs	Behaviors Observed	No. Total	No. Pairs	Behaviors Observed	No. Total	No. Pairs	Behaviors Observed
4/29/07	315**	12.5	10.5	6.95	2.53	nr	2	M/F, C	nr	0	NSA	nr	0	NSA
5/11/07	90	18.47	8.27	4.15	3.56	nr	2	NSA	nr	0	NSA	nr	0	NSA
5/24/07	190	16.85	10.36	3.95	3.9	nr	1	S	nr	0	NSA	nr	1	M/M, M/F
6/14/07	245	17.38	9.54	0.95	3.82	8	1	M/F	nr	2	M/F	0	0	NSA
6/28/07	240	20.44	9.5	-0.45	4.29	17	0	NSA	nr	1	M/M, M/F, C	2	0	NSA
8/17/07	180	nr	nr	nr	4.31	15	0	NSA	nr	0	NSA	3	0	NSA
3/28/08	82.5	10.41	10.8	8.95	2.545	5	0	NSA	4	0	NSA	0	0	NSA
5/7/08	151	13.44	10.46	3.95	2.91	32	2	M/F, C	12	0	NSA	1	0	NSA
5/20/08	151	14.19	8.93	3.95	3.425	30	3	M/M, M/F, C	19	0	NSA	0	0	NSA
5/30/08	nr	17.58	8.4	2.95	3.48	nr	1	M/F, C	nr	2	M/F, C, S	nr	**0**	NSA
6/9/08	nr	19.65	6.65	1.95	5.45	32	0	NSA	56	5	F/F, M/F, C, S	2	0	NSA
6/20/08	242	15.98	9.56	-2.05	4.78	49	0	NSA	28	2	M/F, C	2	0	NSA
6/26/08	156	17.63	9.05	-3.05	4.065	46	0	NSA	24	0	NSA	5	0	NSA
7/17/08	155	21.52	8.94	-1.05	3.85	46	0	NSA	31	0	NSA	0	0	NSA
7/30/08	100*	20.61	8.6	-3.05	4.39	13	0	NSA	20	3	M/M, M/F, C, S	0	0	NSA
8/15/08	161	20.76	8.86	-28.05	3.055	44	0	NSA	14	0	NSA	4	0	NSA

* Indicates a single observer

**Indicates three observers

Table C.9. Parameter estimates and confidence intervals for well-supported covariate models in candidate set for *Etheostoma* sp. cf. *brevirostrum* A, holiday darter.

		95% Confidence Limits	
Parameter	**Estimate (se)**	**Lower**	**Upper**
Ψ(Pairs Observed) *p*(Pool Habitat) (w_i=0.49)			
Ψ			
Intercept	-4.275 (1.835)	-7.872	-0.679
Pairs	1.704 (0.97)	-0.197	3.604
p			
Intercept	-2.242 (1.107)	-4.412	-0.072
Pool Habitat	2.161 (1.318)	-0.423	4.745
Ψ(Temperature) *p*(Pool Habitat) (w_i=0.08)			
Ψ			
Intercept	3.006 (3)	-2.874	8.886
Temperature	-0.294 (0.186)	-0.657	0.071
p			
Intercept	-2.206 (1.09)	-4.343	-0.070
Pool Habitat	2.238 (1.299)	-0.309	4.785

Table C.10. Parameter estimates and confidence intervals for well-supported covariate models in candidate set for *Etheostoma etowahae*, Etowah darter.

		95% Confidence Limits	
Parameter	**Estimate (se)**	**Lower**	**Upper**
Ψ(Pairs Observed) *p*(Rifle Habitat) (w_i=0.84)			
Ψ			
Intercept	-2.828 (1.197)	-5.175	-0.481
Pairs	1.487 (0.716)	0.084	2.890
p			
Intercept	-3.254 (0.767)	-4.758	-1.750
Riffle Habitat	2.205 (0.812)	0.615	3.796
Ψ(Temperature) *p*(Riffle Habitat) (w_i=0.02)			
Ψ			
Intercept	-7.38 (4.787)	-16.762	2.002
Temperature	0.375 (0.271)	-0.155	0.906
p			
Intercept	-3.277 (0.86)	-4.962	-1.592
Riffle Habitat	2.092 (0.811)	0.502	3.682