A habitat risk assessment and breeding site projection for Slackwater Darter *(Etheostoma boschungi*) in Limestone Creek

by

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Abstract

Catalysts for species decline are difficult to identify and are rarely single. The use of predictive models that incorporate multiple factors have proven useful in recognizing major drivers of species declines in multiple systems. In this study, a geographic information systems model was used to evaluate factors contributing to the decline of the Slackwater Darter throughout its distributional range. The species has suffered a precipitous decline, and it is essential to understand conservation threats and to prioritize habitat for restoration and protection. Variables incorporated into the habitat model included land use/land cover, soil and geographic descriptions, hydrologic variables, and others. Habitat where the species has become extirpated or undetectable by conventional sampling was compared to extant populations to identify factors contributing to population loss. Land cover and farm pond density were identified as potential drivers of extirpation. Finally, a model of suitable habitat was used to predict spawning areas in Limestone Creek, where the species was recently discovered. This model identified eight new spawning sites for Slackwater Darter in the system where breeding sites were unknown, and was 44% accurate in its predictive capabilities; however, positive detections were noticeably clustered and indicate the likely influence of some untested variable. With the information provided by this analysis, a framework for habitat protection can be created to assist with restoration of this species.

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List of Abbreviations

eDNA	environmental DNA
GLM	Generalized Linear Model
NLCD	National Land Cover Dataset
SSURGO	Soil Survey Geographic Database
USFWS	U.S. Fish and Wildlife Service
UTM	Universal Transverse Mercator
UWF	University of West Florida

Introduction

It is important to understand the variables driving the decline of species with declining populations. Many threatened species occupy specific habitats within narrow distributions, making sudden environmental shifts within these regions potentially devastating to populations (Taylor et al. 1994, Cummings et al. 1998). Freshwater species are at heightened risk to such variability due to their dependency on specific watersheds and river systems (Daniels 1989; Cummings et al. 1998; USFWS 2008) and their susceptibility to pressures caused by shifts in hydrology, land cover alteration, and man-made passage barriers (Johnston et al. 2013; Januchowski-Hartley et al. 2013). Habitat loss through channelization (McGregor & Shepard 1995; USFWS 2008) and land use conversion is one of the leading causes of fish extirpation. Hydrologic alterations can have equally dramatic impacts on populations (Platts 1981; Bruland et al. 2003), such as the effects of dams and channelization on diadromous fishes (Limdburg & Waldman 2005) and the decline of *Etheostoma proliare* and *Fundulus dispar* in Illinois (Taylor et al. 1994). With so many potentially compounding variables at play—and often acting concurrently—it can be difficult to pinpoint specific drivers of population decline and subsequently design future management frameworks (Clausen & York 2008; Stendera et al. 2012; Richman et al. 2015).

Spatial analysis models within ArcGIS that incorporate multiple factors have proven invaluable in identifying occupancy patterns of threatened species throughout numerous systems (Stanbury & Starr 1999). Models such as these facilitate the integration of multiple data sources and variables and allow for the inclusion of expert knowledge alongside existing data and simulations (Kaminski et al. 2013; Blanford et al. 2013). Furthermore, spatial analysis tools have the capacity to apply interpolation to fill data gaps, to supply optimization statistics, and to analyse the relationships between environmental data layers to produce habitat suitability models and range expansion maps (Bernal et al. 2015). Multi-factor, GIS-based predictive models are also useful in recognizing major drivers of species decline (Mantyka-Pringle 2014; Eskildsen et al. 2015; Bellamy & Altringham 2015; Fancourt et al. 2015). Such models can be used to diagnose drivers of species decline and can provide managers a landscape-wide view of species distributions and habitat variables (Bellamy & Altringham 2015). A well-crafted GIS interface can assess these relationships and provide habitat predictions and management plans for target populations.

The Slackwater Darter (*Etheostoma boschungi:* Wall and Williams 1974) is a small, federally threatened fish species endemic to tributaries of the Tennessee River. Populations are known within the Cypress Creek and Flint River systems, Alabama and Tennessee, the, Limestone and Swan Creek systems, Alabama, and Shoal Creek and tributaries of the Buffalo River system in Tennessee (Boschung 1976; Fluker et al. 2011). First listed as threatened in 1977, the species has experienced a rapid decline in populations from its initial collection in Lindsey Creek (Lauderdale Co., AL) in 1968 (McGregor & Shepard 1995), and shows little chance of recovery without additional conservation action (Johnston et al. 2013). The Slackwater Darter is a migratory breeder, spending most of its life in slow-flowing, gravel-bottomed creek beds (Wall & Williams 1974) and travelling up to 1 kilometer upstream each winter to spawn (Boschung 1976). Breeding habitat is only seasonally inundated, consisting of flooded seepage areas and fields adjacent to small headwater streams (McGregor & Shepard 1995). Populations are entirely dependent upon connectivity between the two habitats for successful propagation (Boschung 1976, 1979).

As a migratory species, the Slackwater Darter is at heightened risk to passage barriers, such as culverts and dams, which can isolate existing populations, destroy vital habitat, and prevent movement to breeding grounds (Johnston et al. 2013, Pfleger et al. 2016). Nearly all known breeding sites fall on either developed or agricultural land, which further contributes to population extirpation and species-level decline (Utz et al. 2010). Many former Slackwater Darter breeding sites have been converted to farm ponds, thereby destroying vital spawning habitat. Similarly, land use changes that contribute to channel incision through altered hydrology or channel incision may limit access to spawning sites, and transitions to agricultural land use can eliminate natural seepage areas, decreasing infiltration increasing runoff and deepening the water table (Bruland et al. 2003).

Using existing data on known breeding populations (Johnston et al. 2013; Janosik and Johnston 2015), a spatial model can be used to predict areas suitable for Slackwater Darter breeding sites. Although the species has been collected from non-breeding habitat in Limestone Creek in Madison and Limestone Counties, Alabama (J. Powell, 5/25/99, Limestone Creek near Toney, AL; 34.916111 N, 86.748611 W; Janosik & Johnston 2015), breeding sites have not been identified in this basin. Through modeling, it is possible to project the habitat preference data from known breeding populations to Limestone Creek to predict the localities of breeding sites within this system. Environmental DNA (eDNA) can be a remarkably effective tool in determining species presence absence, and can used to validate habitat occupancy with greater accuracy than traditional sampling (Ficetola et al. 2015; McKelvey et al. 2016; Pfleger et al. 2016). The process uses organic material such as scales, feces, and epidermal cells to detect species without any actual contact, and as such can be a useful and non-invasive tool in determining presence of rare and threatened taxa (Janosik and Johnston 2013; Ficetola *et al.* 2015).

Recent studies suggest a potential correlation between hydrologic alteration and Slackwater Darter decline (Johnston et al. 2013). Globally, anthropogenic activities have greatly affected stream connectivity, channel structure, and land use. When societal need dictates the construction of obstacles such as dams and culverts, aquatic systems undergo population shifts and ecological disruption (Johnston et al. 2013; Tummers et al. 2016).

Dams and road crossings occur in alarmingly high numbers across a global scale, affecting upstream and downstream stream longitudinal connectivity by inhibiting aquatic migration patterns and retaining flow of materials and organisms (Januchowski-Hartley et al. 2013; Chelgren & Dunham 2015). Even the smallest impediment or environmental shift can function to disrupt Slackwater Darter migrations or life history trajectories. Such a heavy reliance by the species on open, saturated land and hydrologic connectivity means that site identification as well as planning and execution of management goals has become even more important than it was in the past to protect and improve population numbers

This study has three primary objectives: (1) To characterize existing Slackwater Darter habitat based on a series of environmental variables and create corresponding habitat maps for each drainage within the species' distribution (2) Use the information on the relationship between darter population success and habitat parameters to prioritize specific populations for conservation, and (3) To use information on extant populations to predict breeding sites within Limestone Creek and validate the analytical capabilities of the model.

Materials and Methods

All mapping and spatial analysis performed within this study utilized ESRI software ArcGIS 10.3. Presence/absence data used in all models was collected by Johnston et al. (2013) and Janosik and Johnston (2015). All historic collection localities are listed in Appendix I. All models created within the course of this study are designed to encompass the entirely of the Slackwater Darter's known range, as well as Limestone Creek where only a few distinct populations are known. Base layers for the habitat model were downloaded from the online databases listed in Appendix II and projected to the UTM conformal projection Zone 16 to georeference data for subsequent analysis.

Habitat model creation

This study applied a combination of empirical data, expert judgement, and spatial simulations to select the habitat variables considered in the habitat models. In order to create a viable breeding site projection for Limestone Creek, it was first necessary to isolate relevant variables from the five drainages where Slackwater Darter distribution is much more well-known. Six environmental factors were analysed: water table depth, hydric soil classification, soil drainage class, geomorphic description, distance from stream, and land use/land cover (hereafter just "land use"). Each of the first four variables was extracted as an individual layer from the SSURGO dataset (Web Soil Survey) and established as an independent file. The initial SSURGO water table data only covered select regions within each watershed, so focal statistics were applied to interpolate data for the missing areas by creating a matrix of local values and calculating missing values through a "moving average" approach (Zhang et al. 2007). Values were assigned to all gaps by applying the Kriging tool within the Spatial Analyst toolbox; this statistical package averages the values of rectangles drawn around each data pixel to fill in blank data values (Oliver & Webster 2007). A series of simulations was run to determine the minimum distance of breeding sites from stream channels that included all known Slackwater Darter populations. This distance was used to create a buffer around the streams in the target watersheds to form the boundary for projected habitat while accounting for estimated seasonal bank overflow. Land cover data was imported from the 2011 National Land Cover Dataset (NLCD).

Once each data layer was established, independent levels of each of the six environmental variables were analysed in relation to the data points to determine which variables' conditions correlated with existing populations. Categories within each variable—such as land use—was ranked based on number of Slackwater Darter populations on land containing that specific variable. Data layers were reclassified through the Raster Calculator in the Spatial Analyst toolbox to create new pixel-based data layers ranking conditions based on Slackwater Darter occupancy. All rankings were on a 1-5 scale, with a score of 5 representing highest probable occurrence, and thus optimal habitat conditions. A series of preliminary simulations was conducted to identify the relative importance of each environmental variable to Slackwater Darter habitat within the five prioritized drainages. These values were then applied in a Weighted Overlay analysis, which added the six scaled, weighted data layers together to produce a final model demonstrating the greatest relationship between viable habitat and extant Slackwater Darter populations. Data values within the final models were split into five categories by the Jenks natural breaks method of optimization (Jenks 1967). In this instance, habitat designations were organized into five classifications, with a higher score representing land best-suited for Slackwater Darter occupation.

Habitat risk assessment

To evaluate which factors are driving current depopulation, a backwards, stepwise Generalized Linear Model (GLM) was applied in R to create a best-fit model for the presence-absence data. This model tested an array of variables for significance: proximity to farm ponds, proximity to road crossings, farm pond density, road crossing density, water table depth, land use, land use change from 1992-2001, and topographical slope. Data for the regression were extracted from the habitat model in Arc to a single table linking each population to the associated variable. Variables were removed from the regression model in order of lowest significance until only those significantly affecting Slackwater Darter presence remained. Additional independent logistic regressions were run comparing each of the top three environmental variables to Slackwater Darter presence. The land use/land cover variable was further broken down into independent components to analyse the relationship between each land use/land cover type and Slackwater Darter presence.

Additionally, the relationships between the primary habitat variables and Slackwater Darter site locality were further explored in a non-metric multidimensional scaling (NMDS) model. Each documented Slackwater Darter population was projected onto an ordination frame; habitat variables were overlaid onto the ordination plot as vectors.

Once identified, the primary influential habitat variables were incorporated into a new model ranking current populations in order of conservation priority. Three initial data layers were created for each drainage: Slackwater Darter habitat from the initial model; land cover sorted into agricultural, developed, forested, and other; and a ring of buffers around each known Slackwater Darter site. These values were all reclassified based on associated impact on Slackwater Darter persistence. Habitat was ranked 1-5, with 5 being optimal to Slackwater Darter occupancy. Land use categories were ranked in order of least to greatest risk (1-5), with agricultural and developed land being highest priority for conservation concerns. Two sets of buffer rings were created around each site to model the proximity of farm ponds and road crossings to known habitat sites. Distances were calculated from each site to the nearest farm pond and road crossing proximity analyses on all Slackwater Darter populations. Buffer rings were ranked using Jenks Natural Breaks goodness of variance fit to condense the distance into five categories ranked 1-5: categories closer to the sites received a higher priority rating (5).

These four ranked data sets were then added together using the Raster Calculator in ArcGIS to produce a single, watershed-specific output. Pixels were scored 4-16; higher-ranked pixels were indicative of increased threat of extirpation. A final model was created ranking extant sites in order of conservation concern.

Limestone Creek habitat assessment and model evaluation

The same modelling procedures outline above were applied to produce a habitat model and prediction of at-risk habitat for Limestone Creek. These models were used as the foundation for selection of 22 sites throughout the drainage as potential breeding sites. All but one of the sites were new and based solely on the predictive model. eDNA samples were collected from these sites during the February spawning season in 2017. Collection protocol followed that outlined by Ficetola et al. (2008) and incorporated application of a sodium acetate buffer to the water samples. Collections for each site were taken by filling three 50 mL Falcon tubes containing 1.5 mL of pre-prepared sodium acetate buffer solution with 15 mL of surface water and 33.5 mL of 95% ethanol. Samples were kept on ice and stored in a freezer until processing. All eDNA samples were processed at the University of West Florida in Pensacola, FL following the standardized procedure outlined by Ficetola et al. (2008) using QUIAGEN DNeasy © extraction kits. Slackwater Darter presence was assessed via polymerase chain reactions (PCR) for all extracted samples using two primers from cytochrome *b* gene of the mitochondrial DNA (L: GTGACTTGAAAAACCACCGTTC; H: CAACGATCTCCGGTTTACAACAX). Primers were initially designed by Janosik (Janosik & Johnston 2015) using GenBank (222.ncbi.nlm.nih.gov) and to not amplify species outside *Etheostoma*. Multiple PCR reactions were run for each site, and results were visualized under UV light on a 1% agarose gel stained with ethidium bromide. Data for all sequences were aligned and screened using BLASTn (GenBank). eDNA results were then assessed in relation to site locality and compared to the Limestone Creek habitat projection to determine the predictive capabilities of the predictive model.

Results

Habitat models for each drainage known to contain Slackwater Darter populations indicated areas of suitable habitat within each watershed (Figures 1-6). The number and percentage of known Slackwater Darter sites was calculated per each variable to justify inclusion of each factor in the model (Tables 1-4). Sites were located on three primary land use types: developed, agricultural, and forested (Table 1). Of the 96 sites sampled, over 75% occurred on either forested, agricultural, or developed land. The greatest number of total populations (extant and extirpated) occurred on deciduous forest; however, the greatest amount of extant populations occurred on hay/pasture land. More extirpated populations were located on deciduous forest than any other land use category. Soil hydric rating was split nearly evenly between hydric and non-hydric soils among all sites, and only Cypress Creek demonstrated an overwhelming majority of populations occupying sites with hydric soils (Table 2). Water table depth followed a more predictable trend, with most sites occurring within the shallowest or next-shallowest range (Table 3). Populations were dispersed across a range of soil drainage classes, but most were concentrated on moderately well-drained to poorly-drained soils (Table 4).

The area and percent total of each habitat class was calculated for each watershed upon completion of the habitat model (Table 5). In area alone, the Flint River system had the greatest amount of suitable Slackwater Darter habitat: over 400 km^2 were ranked as highest or next-highest suitability. Dividing suitable land by total area gave normalized land area statistics for each model, which function as a better representation of the relative suitability of each drainage comparable to existing populations. The Flint River, Cypress Creek, and Swan Creek systems had the greatest percentage of viable habitat per unit land at 53%, 52%, and 51%, respectively.

Proximity to farm ponds and land use had the greatest biological impact on Slackwater Darter population persistence (Table 6). While none of the p-values are statistically significant at the 0.05 level, land use/land cover was significant in multiple iterations of the of the model, and the p-values for both land use and farm pond density were quite close to the 0.05 p-value significance limit in the final regression and are significant at a 0.1 level (Table 6). The habitat vectors on the ordination plot of Slackwater Darter populations demonstrated a strong relationship between distance to road crossings and Slackwater Darter population location. Land use and farm pond density were similarly shown to have a relatively strong effect on different aspects of site locality. They were also the only two variables clustered together, supporting the probable correlation between land use, farm pond density, and Slackwater Darter success.

Land use, farm pond density, and road crossing density were then analysed individually (Tables 7, 8). When run as the only independent variable in a logistic regression, farm pond density and land cover both demonstrated a significant relationship to Slackwater Darter presence. Slackwater Darter populations are 2.05 times as likely (0.54-1.99, 95% C.L.) to be present at sites with greater densities of surrounding farm ponds than at sites where farm ponds are not highly clustered (ß=0.716, p=0.023). Slackwater Darter presence is 1.02 times as likely (1.01-1.03, 95% C.L.) to be affected by land use/land cover (ß=0.021, p=0.029). The significance of the land cover variable was further explored by breaking it down into separate components. The major land use categories were compared to identify any significant relationships between most categories and their respective effects on Slackwater Darter presence (Table 8). When considered independently, the relationship between open developed land (roads and impervious surface) and Slackwater Darter presence/absence was significant at the 0.05 level (p=0.046). Similarly, the relationship between deciduous forest and Slackwater Darter presence/absence was also significant at the

0.05 level (p=0.0097). However, no significant relationship was observed between hay/pasture land and persistence.

The risk assessment models for known Slackwater Darter populations demonstrated over 320 km^2 across of land to be considered “at risk” throughout the known distribution (Figures 8-13; Table 9). Cypress Creek had by far the greatest number of high-priority sites, followed by the Flint River (Figure 14). Table 10 identifies active sites ranking within the top 25% of habitat risk and their associated land use category. Nearly all the most-threatened Slackwater Darter populations occur in the Flint River and Cypress Creek watersheds and are dominated by hay/pasture land. The most threatened population identified by the model is the Chief Creek site near Barnett Road in the Buffalo River system; this site occurred on deciduous forest, but was directly adjacent to both open developed and hay/pasture land. Likewise, the Flint River system’s most threatened population is located on open developed land in between two large agricultural properties and occurs along a tributary of Brier Fork near State Line Road. The most threatened populations in the Swan Creek (tributary along Huber Road), Cypress Creek (Dodd site), and Limestone Creek (near Elkwood Section Road) systems all occur on hay/pasture land. The Shoal Creek system did not have an extant site within the top 25% of threatened habitat.

The eDNA results for Limestone Creek demonstrated 8 positive samples from the 22 surveyed (Table 11; Figure 15). 18 of the 22 sites occurred on either “most suitable” land or the next-most suitable category. With one exception, the positive detection sites were clustered in the northern portion of the drainage, and all the positive detections occurred on land predicted to be either most-suitable or of the next-highest suitability ranking (Figure 16). While some of the sites with negative results also occur in predicted “suitable” habitat, others do not, or at least occur near noticeable gaps in viable habitat. Nearly all the populations detected occur on pasture or cropland (Figures 17, 18). Interestingly, farm pond density is greater in the northern part of the drainage where most of the active populations are concentrated (Figure 19).

Discussion

Land use/land cover and farm pond density were identified as potential factors in Slackwater Darter extirpation. Secondarily, road crossings, which represent passage barriers, were also identified as potential catalysts for population declines. When combined with models of suitable habitat, these factors generate a prioritization of at-risk populations.

The habitat model identified Slackwater Darter populations as occurring in areas with shallow water tables and poorly-drained soils, which typify geomorphic landforms that support the formation of wetland conditions. While it was expected that most sites would occur on developed or agricultural land, it was also anticipated that many of these sites would occur on hydric soils; however, this was not the case. Instead, a comparison between watersheds demonstrated a nearly even split between hydric and non-hydric conditions. As two of the pivotal factors affecting breeding site selection are vegetation and site hydrology (Boschung 1976, 1979) and both are supported by hydric conditions, this was unusual; only the Cypress Creek system demonstrated an overwhelming dominance of hydric conditions. Though known Slackwater Darter breeding habitat does occur in wetland areas, these sites are not explicitly restricted to wetlands. Rather, breeding sites are more consistently found in regions prone to periodic flooding (McGregor & Shepard 1995; Hartup 2005). There is also a possibility that agricultural land use has altered the physical soil characteristics in these regions promoting a shift away from identified hydric conditions (Allan 2004; Germer et al. 2010).

As demonstrated by the habitat suitability models for each known watershed, much of the projected “best-suited” habitat occurs in the Flint River, Cypress Creek, and Swan Creek watersheds. These systems contain the greatest number of previously known populations and retain the habitat conditions necessary to support breeding populations. Interestingly, much of this suitable habitat is located on or directly adjacent to agricultural or developed lands. The flooded seeps, fields, and springs preferred for breeding often overlap these sites (Boschung 1976, 1979), so the habitat model for the Slackwater Darter incorporated this relationship to allow for the prediction of breeding sites within heavily impacted areas. The resulting habitat models therefore illustrate a strong connection between suitable habitat and agricultural land.

Multiple studies note the negative impact of both urbanization and agricultural development on aquatic systems and associated species (Platts 1981; Wang et al. 1997; Steinman et al. 2003; Allan 2004; Utz et al. 2010). Urbanization has a highly destructive and disproportionately large impact on stream and aquatic ecosystems (Allan 2004). Similarly, the onset of modern agricultural demands has facilitated stream channelization and wetland draining to increase available farmland (Wang et al. 1997). Stream degradation is exacerbated through removal of upstream riparian buffers and reduction in groundwater recharge (Scanlon et al. 2005), as well as shifts in soil characteristics such as penetration resistance, hydraulic conductivity, and water table accessibility (Germer et al. 2010). While most studies focus on the impacts of row crops rather than pasture (Steinman et al. 2003), strong evidence also supports the negative impacts of pasture-based agriculture on aquatic fish species. Cattle can reduce natural grassy buffers, increase nutrient input, and trample and compact soil (Steinman et al. 2003); their environmental modifications can inhibit infiltration of surface water to deep soils and lower the natural water table (Platts 1981).

Agricultural fields provide excellent habitat if left alone, but constant plowing and ditching disrupts Slackwater Darter breeding efforts (Johnston et al. 2013). Farm ponds have had pronounced impact on Slackwater Darter populations, as noted by close correlation between their occurrence and Slackwater Darter presence (Tables 6, 7). More often than not, they are constructed from the very wetlands on which many Slackwater Darters use for breeding. Field alteration often redirects natural seepage water into farm ponds and irrigation. Reservoirs contribute to downstream sedimentation, nutrient loading, and erosion (Lowrance et al. 2002; Halstead et al. 2014). Numerous historical breeding sites have been destroyed by farm pond construction (Johnston, pers. obs.), and the subsequent ditching, draining, and trampling of additional wetland regions in these areas creates oxidized soil conditions, continuous surface flow, and a decrease in water table levels (Bruland et al. 2003).

Road crossing density was the third most significant habitat variable considered in the analysis. Passage barriers are known to disrupt migration in many species (Januchowski-Hartley et al. 2013; Tummers et al. 2016). In the case of the Slackwater Darter, perched culverts that do not connect to the stream channel can be considered a passage barrier and serve to inhibit movements between primary channels and breeding seeps by limiting water table accessibility. In most circumstances where passage barriers are an issue, management plans suggest complete removal or adjustment of these barriers; however, such actions are also expensive and may be structurally unsound for vehicles (Januchowski-Hartley et al. 2013; Chelgren and Dunham 2015). Instead, barriers in Slackwater Darter habitat can be avoided or minimized through creation of alternate migration paths and localized stream restoration to facilitate reliable flooding and flow (Januchowski-Hartley et al. 2013; Tummers et al. 2013).

Much of the 300-plus km^2 of “at risk” land is clustered around existing Slackwater Darter populations in the Cypress Creek and Flint River systems, which parallels the results of the habitat model. The sites where the Slackwater Darter is consistently found are also the very sites currently facing extirpation. At one time, the Cypress Creek system was considered the stronghold of the Slackwater Darter (Boschung 1976) but now collection of a live specimen from this system is all but impossible (Johnston et al. 2013). Prime breeding habitat overlaps with agricultural fields that plow, dredge, and convert wetland areas to row crops or pasture (Steinman et al. 2003). Slackwater Darter habitat is often lost or destroyed long before it can be identified. Improved knowledge of primary drivers of Slackwater Darter decline and identification of those sites facing the most immediate risk from these factors offers an opportunity to address populations on a case-by-case basis.

The relatively equal amount of viable breeding and at-risk habitat within the Limestone Creek system suggests an existing relationship between suitable habitat and threatened habitat; most persisting Slackwater Darter populations are located on or alongside anthropogenically-altered land, and occur on or adjacent to pasture or cropland. The majority of the northern portion of the Limestone Creek watershed is designated as potential breeding habitat; however, most of this land is also agricultural pastures and fields, and is has a high occurrence of farm ponds and road crossings. The eDNA data collected during the study proves that representatives of the species persist in the area despite this, and indeed shows that this region has the highest concentration of occurrences within this drainage. The lack of detections in the central and southern portion of the watershed are interesting, as viable habitat occurs throughout the drainage. Farm ponds, cropland, and pasture land are similarly distributed, which indicates that there are likely additional, unconsidered parameters influencing breeding site locality.

The Limestone Creek model did not successfully select sites that all resulted in positive eDNA detections; only 8 out of 22 sites demonstrated positive eDNA collections. However, eDNA is not without its own set of drawbacks, and without verifying the results through on-site assessments we cannot say for sure how successful those eight new sites are as breeding locations, or whether or not they even support full populations. A positive detection only indicates that a single live specimen exists at the site in question (Jerde 2013);

it says nothing about abundance or population dynamics. Even when conditions are perfect there is always the possibility that the sample fails to detect the target organism or yields false positives; usually, running multiple samples and amplifications can account for this, but there is always the possibility of experimental error (Ficetola et al. 2014). False positives can occur on rare occasions when DNA persists after the undetected extirpation of the target species, or with contamination of the water sample; similarly, the risk of false negatives exists as a result of inadequate sampling methodology, degraded DNA, or failure to collect target DNA even when present (Darling & Mahon 2011). All of the sites in this study with positive detections occurred on land designated as ideal breeding habitat, and while most of the sites where no DNA was detected also occur on similarly-categorized land, the possibility always remains that individuals are there but remain undetected, or that populations once existed in these regions but have since become extirpated. they do require the input of additional parameters to explore the interesting relationship between latitude and breeding site locality.

With one exception occurring on developed land, all the newly-detected Slackwater Darter populations are on either crop or pastureland. There is also a high likelihood that these sites are surrounded by farm ponds on all sides. The discovery of these new populations further supports the observed relationship between Slackwater Darter breeding habitat and agricultural development, and serves as further support for the need to reclaim and restore of as many of these areas as possible for Slackwater Darter protection and future population viability.

Management objectives

As with any conservation endeavour, management efforts and distribution of resources are not simply a matter of ecological factors and objectives but stakeholder interests (Robinson 2016). The predictive models developed herein serve as a roadmap for further, site-specific restoration, and provide a physical representation of potentially viable Slackwater Darter habitat in a previously-unsampled drainage. From our data, it is evident that each of the six drainages contains populations of great conservation priority. The top existing sites of conservation concern are the Chief Creek population near Barnett Road in the Buffalo River system, populations in Swan Creek along Huber and Elkton Roads, the Dodd site in Cypress Creek, and a series of populations along Brier Fork in the Flint River system. Many resources note the negative ecological effects of row crop and pasture-based agriculture—land use categories shared by all of these sites—on natural systems (Platts 1981; Wang et al. 1997; Utz et al. 2010). Improving prior converted Slackwater Darter habitat should include stakeholder engagement on the importance of restoring critical habitat.

Furthermore, all the newly-discovered Slackwater Darter populations in Limestone Creek occur on either pasture or cropland, and are surrounded on all sides by farm ponds. If these eight populations have any chance of persisting, they must be studied in greater depth and protected through land use conversion and a return to natural hydrologic conditions. The environmental conditions offered by pastureland are ideal for Slackwater Darter populations, but the associated pressures from cattle and crop activity are not. Land use must shift from active agriculture to fallow fields on land supporting *and* surrounding these populations. In each of these target population areas, new farm pond construction must cease, and existing ponds and land restored to pre-existing hydrologic conditions. Bruland et al. (2003) demonstrate the resounding success of reestablishment of wetland hydrology in agricultural sites, and wetland hydrology is truly at the heart of Slackwater Darter success.

In order to accomplish these goals, emphasis must be placed on the education of private landowners—over 70% of forested land in Alabama, and an even higher percentage of open land, is privately owned (Glover & Jones 2001). Furthermore, over 40% of US farmland is owned by “absentee owners”, whose distance from the sites and lack of involvement with sites of concern can affect the target audience for conservation (Petrzelka et al. 2013). Despite limited budget and manpower, the Alabama Forestry and Wildlife Extension program which offers landowners countless educational opportunities for maintenance sustainable forestry and land-management principles (Glover & Jones 2001). By interacting with landowners on a one-on-one and group-oriented scale, and working to compromise and educate rather than demand, meeting conservation for the Slackwater Darter and other species of concern becomes a much more tangible objective. If the appropriate steps are taken, the effects of wetland conversion to agriculture can be reversed to restore hydrologic conditions capable of sustaining breeding Slackwater Darter populations.

This study is only the beginning of the work necessary to restore the Slackwater Darter populations to any semblance of their previous numbers. With these models, we now have a preliminary understanding of the primary drivers of the notable decline that has been taking place over the course of the last forty years. We also have pinpointed specific populations in dire need of conservation attention and specific management regimes, as well as identified eight new breeding sites within the Limestone Creek system. Now that the groundwork has been laid, there is opportunity for future studies to explore additional influential habitat variables—such as those contributing to the interesting cluster of Slackwater Darter in the northern third of the drainage—as well as the opportunity for managers to begin creating site-specific conservation goals and leading ground teams to extensively sample the newly identified sites within the Limestone Creek system. From there, steps may be taken to move forward in the successful protection and restoration of this imperilled species.

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Table 1. Distribution of land use/land cover categories across known Slackwater Darter populations. Total number of sites per category is indicated in the “All” column; the following percentage shows the distribution of sites across each land use type. Total number of extant and extirpated populations per land use type are indicated by the “Present” and “Absent” columns, respectively, with the associated percentages following each category.

Land Use	**All**	**%**	**Extant**	**%**	**Extirpated**	**%**
Cultivated crops	7	7%	4	13%	3	5%
Deciduous	26	27%	6	20%	20	30%
Developed, low	2	2%	1	3%	1	2%
Developed, open	14	15%	3	10%	11	17%
Hay/pasture	25	26%	10	33%	15	23%
Herbaceous	1	1%	1	3%	0	0%
Mixed forest	1	1%	0	0%	1	2%
Shrub/scrub	13	14%	2	7%	11	17%
Woody wetlands	7	7%	3	10%	4	6%

Table 2. Distribution of Slackwater Darter sites in relation to hydric soil classifications.

Cypress Creek

	Sites	Percent Total
Hydric	38	76%
Non-hydric	12	24%

Buffalo River

	Sites	Percent Total
Hydric	0	0
Non-hydric	2	100%

Swan Creek

	Sites	Percent Total
Hydric	7	39%
Non-hydric	8	44%

Shoal Creek

	Sites	Percent Total
Hydric	1	20%
Non-hydric	4	80%

Flint River

	Sites	Percent Total
Hydric	7	44%
Non-hydric	9	56%

Table 3. Distribution of Slackwater Darter sites across the range of minimum water table depths. Category 1 is indicative of the deepest range of water tables, while Category 5 indicates the shallowest water tables.

Cypress Creek

Category	Sites	Percent of Total
1	4	8%
2	2	4%
3	14	28%
4	13	26%
5	17	34%

Buffalo River

Category	Sites	Percent of Total
1	0	0
2	0	0
3	1	50%
4	1	50%
5	0	0

Swan Creek

Category	Sites	Percent of Total
1	0	0
2	0	0
3	0	0
4	5	28%
5	13	72%

Shoal Creek

Category	Sites	Percent of Total
1	0	0
2	0	0
3	0	0
4	12	75%
5	4	25%

Flint River

Category	Sites	Percent of Total
1	0	0
2	0	0
3	1	20%
4	2	40%
5	2	40%

Table 4. Distribution of Slackwater Darter sites across the drainage class habitat variable within the five target watersheds. Number of sites and associated percent of total sites are adjacent to each drainage class category.

Cypress Creek

Class	Sites	Percent of Total
Somewhat excessively drained	0	0
Well drained	16	19%
Moderately well drained	22	19%
Somewhat poorly drained	0	6%
Poorly drained	12	56%

Buffalo River

Class	Sites	Percent of Total
Excessively drained	0	0
Somewhat excessively drained	1	50%
Well drained	0	0
Moderately well drained	1	50%
Somewhat poorly drained	0	0
Poorly drained	0	0

Swan Creek

Class	Sites	Percent of Total
Excessively drained	0	0
Well drained	2	11%
Moderately well drained	1	6%
Somewhat poorly drained	8	44%
Poorly drained	7	39%

Shoal Creek

Class	Sites	Percent of Total
Excessively drained	0	0
Well drained	1	20%
Moderately well drained	2	40%
Somewhat poorly drained	1	20%
Poorly drained	1	20%

Flint River

Class	Sites	Percent of Total
Somewhat excessively drained	0	0
Well Drained	3	19%
Moderately well drained	3	19%
Somewhat poorly drained	1	6%
Poorly drained	9	56%

Table 5. Area and percent total of all habitat suitability categories within each drainage. Habitat categories are ranked 1-5 in order of least to most suitable for each watershed. The associated relative percent total for each system is also included.

		Cypress Creek	**Buffalo River**	**Flint River**	**Shoal Creek**	**Swan Creek**	**Limestone Creek**
	Model rank	Area in acres	Area in acres	Area in acres	Area in acres	Area in acres	Area in acres
Least suitable	1		6.85	9.89		120.47	34.86
	2	2,813.19	8,589.98	9,194.85	12,122.07	11,928.85	7,141.44
Suitable	3	61,922.1	111,740.7	83,195.94	17,1215.3	58,810.01	43,599.86
	4	62,242.97	67,109.27	87,741.33	44,975.26	52,779.48	33,494.53
Most suitable	5	6,191.63	4,852.29	15,868.90	4,747.426	20,389.59	6,318.00
	Total area	133,169.89	192,299.09	196,010.91	233,060.06	144,028.40	90,588.69
	Model rank	Percent total	Percent total	Percent total	Percent total	Percent total	Percent total
Least suitable	1		0%	0%		0%	0%
	2	2%	4%	5%	5%	8%	8%
Suitable	3	46%	58%	42%	73%	41%	48%
	4	47%	35%	45%	19%	37%	37%
Most suitable	5	5%	3%	8%	2%	14%	7%

Table 6. Statistical p-values generated by a stepwise GLM. The variable with the least amount of statistical significance was removed during each subsequent iteration of the logistic regression until only those variables most likely to influence the dependent variable remained.

Variable	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7	Step 8	Step 9
Land cover	0.0501	**0.0488**	**0.0487**	0.0628	0.0628	0.0795	**0.0246**	**0.0341**	0.0584
Farm pond density	0.116	0.0864	0.0858	0.1098	0.125	0.1165	0.067165	0.0892	0.0548
Road crossing density	0.1486	0.1485	0.1488	0.1365	0.108	0.0988	0.1292	0.1631	
Water table depth	0.3214	0.3213	0.3002	0.1425	0.1304	0.14332	0.1772		
Slope	0.1279	0.1277	0.1316	0.143	0.1323	0.146			
Habitat	0.2356	0.2357	0.2447	0.2822	0.3248				
Proximity to road crossings	0.2729	0.2731	0.275	0.3339					
Drainage class	0.452	0.4503	0.4639						
Land use change	0.7731	0.7738							
Proximity to farm pond	0.9773								

Table 7. Statistical p-values generated by logistic regressions analysing the three most statistically-significant habitat variables indicated by the stepwise GLM.

	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
Road crossing density	0.197	0.144	1.369	0.171
Farm pond density	0.716	0.324	2.208	0.027*
Land cover	0.021	0.010	2.177	0.029*

Table 8. Statistical p-values generated by logistic regressions analyzing the relationship between land use categories. In the first set of regressions, the effect of each land use type on presence/absence was compared to that of developed land. The second set of regressions compares all other land use categories to pasture, and the third uses deciduous forest as the reference category.

Developed reference

	Estimate	Std. Error	z value	Pr(>|z|)
Developed, open	-1.299	0.651	-1.995	0.046 *
Developed, low	1.299	1.557	0.834	0.404
Deciduous	0.095	0.801	0.119	0.905
Herbaceous	-15.267	2399.545	-0.006	0.995
Mixed Forest	-0.405	1.008	-0.402	0.687
Shrub/scrub	17.865	2399.545	0.007	0.994
Hay/pasture	0.894	0.769	1.163	0.245
Cultivated crops	1.587	1.004	1.581	0.114
Woody wetlands	1.012	1.004	1.008	0.314

Pasture reference

	Estimate	Std. Error	z value	Pr(>|z|)
Hay/pasture	-0.406	0.408	-0.993	0.321
Developed, low	0.406	1.472	0.275	0.783
Developed, open	-0.894	0.769	-1.163	0.245
Deciduous	-0.799	0.619	-1.290	0.197
Herbaceous	-16.161	2399.545	-0.007	0.995
Mixed forest	-1.299	0.870	-1.493	0.135
Shrub/scrub	16.972	2399.545	0.007	0.994
Cultivated crops	0.693	0.866	0.800	0.423
Woody wetlands	0.118	0.866	0.136	0.892

Deciduous reference

	Estimate	Std. Error	z value	Pr(>|z|)
Deciduous	-1.204	0.465	-2.587	0.0097*
Developed, low	1.204	1.489	0.809	0.419
Developed, open	-0.095	0.801	-0.119	0.905
Herbaceous	-15.362	2399.545	-0.006	0.995
Mixed forest	-0.501	0.899	-0.557	0.577
Shrub/scrub	17.770	2399.545	0.007	0.994
Hay/pasture	0.799	0.619	1.290	0.197
Cultivated crops	1.492	0.894	1.668	0.095
Woody wetlands	0.916	0.894	1.024	0.306

Table 9. Distribution of at-risk land for Slackwater Darter across known distribution. Threatened habitat is ranked 1-5 in order of least to greatest threat, with the corresponding area of each category listed for all drainages.

		Cypress Creek	**Buffalo River**	**Flint River**	**Shoal Creek**	**Swan Creek**	**Limestone Creek**
	Model rank	Area in acres	Area in acres	Area in acres	Area in acres	Area in acres	Area in acres
Least risk	1	8,027.38	97.82	512.08	301.15	580.24	2,430.90
	2	10,271.45	951.79	585.36	1,595.91	2,913.92	13,437.69
Moderate risk	3	17,255.66	937.46	4,784.16	3,023.52	2,841.37	17,666.85
	4	18,726.14	1,286.7	10,418.47	1,173.92	5,209.46	21,634.98
Greatest risk	5	2789.35	242.5	1,679.63	278.67	1487.6	23,427.03
	Total area	57,069.98	3,516.27	17,979.70	6,373.17	13,032.59	78,597.46

Table 10. Extant populations ranked within the top 25% of determined risk. Risk factor is identified by the pixel score assigned to each locality by the risk assessment model. Sites are listed in order of greatest to least determined risk. The most threatened population for each watershed is marked with an asterisk. Pixel scores ranged 4-16 throughout the species distribution.

Site name	Risk	Land use	Lat	Long	Drainage
Chief Creek, Barnett Road*	16	Deciduous	87.4194	35.35988	Buffalo
Trib, Swan Creek, Huber Road*	16	Hay/pasture	-86.95419	34.86981	Swan
Swan Creek, Elkton Road	14	Hay/pasture	-86.95181	34.83174	Swan
Dodd Site*	13	Hay/pasture	-87.772	35.0592	Cypress
Trib, Brier Fork, State Line Road*	13	Developed, open	-86.67798	34.99195	Flint
Trib, Cypress Creek, Natchez Trace Pkwy	12	Deciduous	-87.82314	35.016	Cypress
Trib, Middle Cypress Creek	12	Hay/pasture	-87.77153	35.0592	Cypress
Trib, Brier Fork, Elkwood Section Road	12	Deciduous	-86.6707	34.9623	Flint
Trib, Brier Fork, Scott Road	12	Cultivated crops	-86.67798	34.99894	Flint
Brier Fork, Fowler Road	12	Hay/pasture	-86.6553	35.0154	Flint
Greenbrier Branch, Co. Rd. 85	12	Deciduous	-87.7828	34.9616	Cypress
Trib, Middle Cypress Creek	12	Hay/pasture	-87.77153	35.06161	Cypress
Limestone Creek, Elkwood Section Road*	12	Hay/pasture	-86.71379	34.977521	Limestone
Trib, Brier Fork, Scott Orchard Road	12	Developed, open	-86.6767	34.86981	Flint

Table 11. eDNA results for Limestone Creek. Sites with positive DNA detections are indicated by a 1.

SITE #	COORDINATES	LOCATION	BRANCH	PRESENCE
1	35.01598916, -86.76190115	Jones Road	Taft	1
2	35.01931271, -86.80651903	Minnie Brown Road	Johnson	0
3	35.00300559, -86.90133566	Maddox Road	Smith	1
4	34.99205560, -86.77122222	State Line Road	Davis	1
5	34.970944, -86.70797222	Golightly Spring Road	Trib, Limestone	1
6	34.9776944, -86.73075	Elkwood Section Road	Trib, Limestone	1
7	34.9715556, -86.7378333	Jo Mar Road	Trib, Limestone	1
8	34.9626111, -86.76452777	Pulaski Pike	Tyrone	0
9	34.9771667, -86.80866666	Cedar Hill Road	Little Limestone	1
10	34.9057222, -86.73136111	Old Railroad Bed Road	Trib, Limestone	1
11	34.8963889, -86.82158333	Highway 251	Fall	0
12	35.0069722, -86.8252777	Henry Bayless Road	Sherrill	0
13	35.0156111, -86.70652777	Pepper Road	Jones	0
14	34.9333056, -86.7445555	Ready Section Road	Dry Creek	0
15	34.9627222, -86.80886111	Pulaski Pike/Pinedale	Little Limestone	0
16	34.9465278, -86.80127777	Old Railroad Bed Road	Little Limestone	0
17	34.9481667, -86.78941667	Coggins Road	Little Limestone	0
18	34.8965, -86.75038889	Toney School Road	Buffalo	0
19	34.8840833, -86.8840833	Wall Triana Highway	Buffalo	0
20	34.8758056, -86.81369444	Jennings Chapel Road	Trib, Limestone	0
21	34.8585, -86.7847222	County Line Road	Love Ditch	0
23	34.8927, -86.8927	Limestone Road	Limestone	0

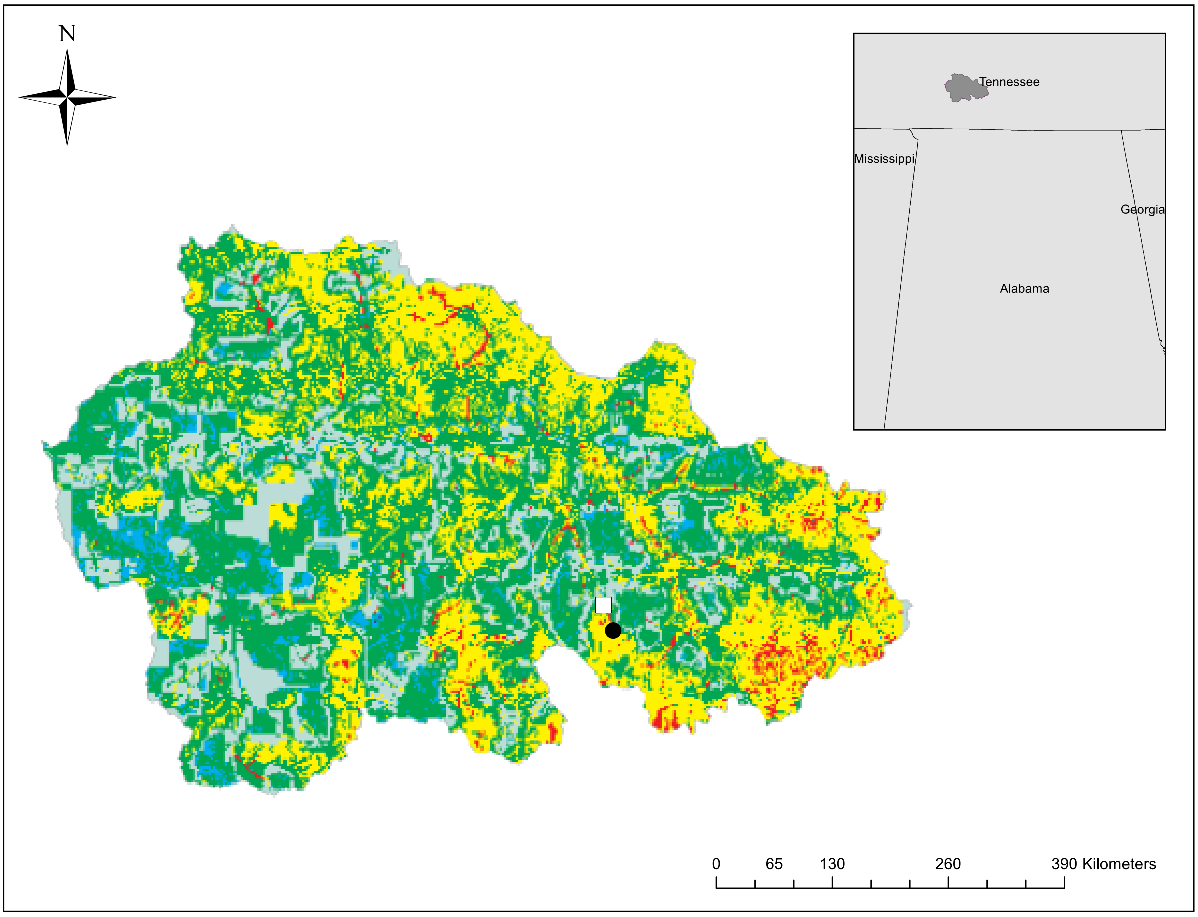

Figure 1. Habitat suitability map for Slackwater Darter in the Buffalo River watershed in Tennessee. Habitat designated by red and yellow is best suited to Slackwater Darter occupancy. Extant populations are denoted by black circles. Extirpated populations are designated by a white square.

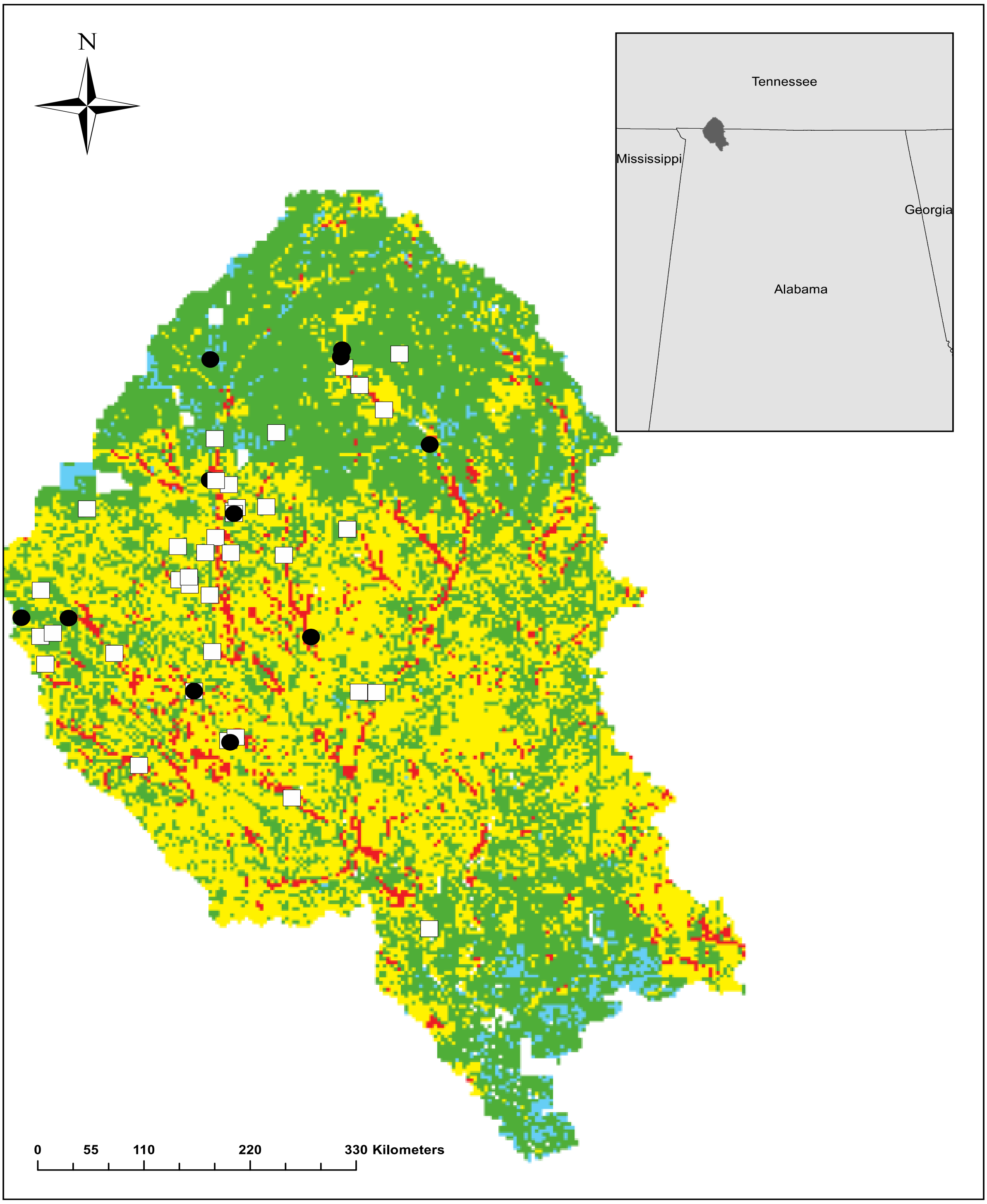

Figure 2. Habitat suitability map for Slackwater Darter in the Cypress Creek watershed in Tennessee and Alabama. Habitat designated by red and yellow is best suited to Slackwater Darter occupancy. Extant populations are denoted by black circles. Extirpated populations are designated by a white square.

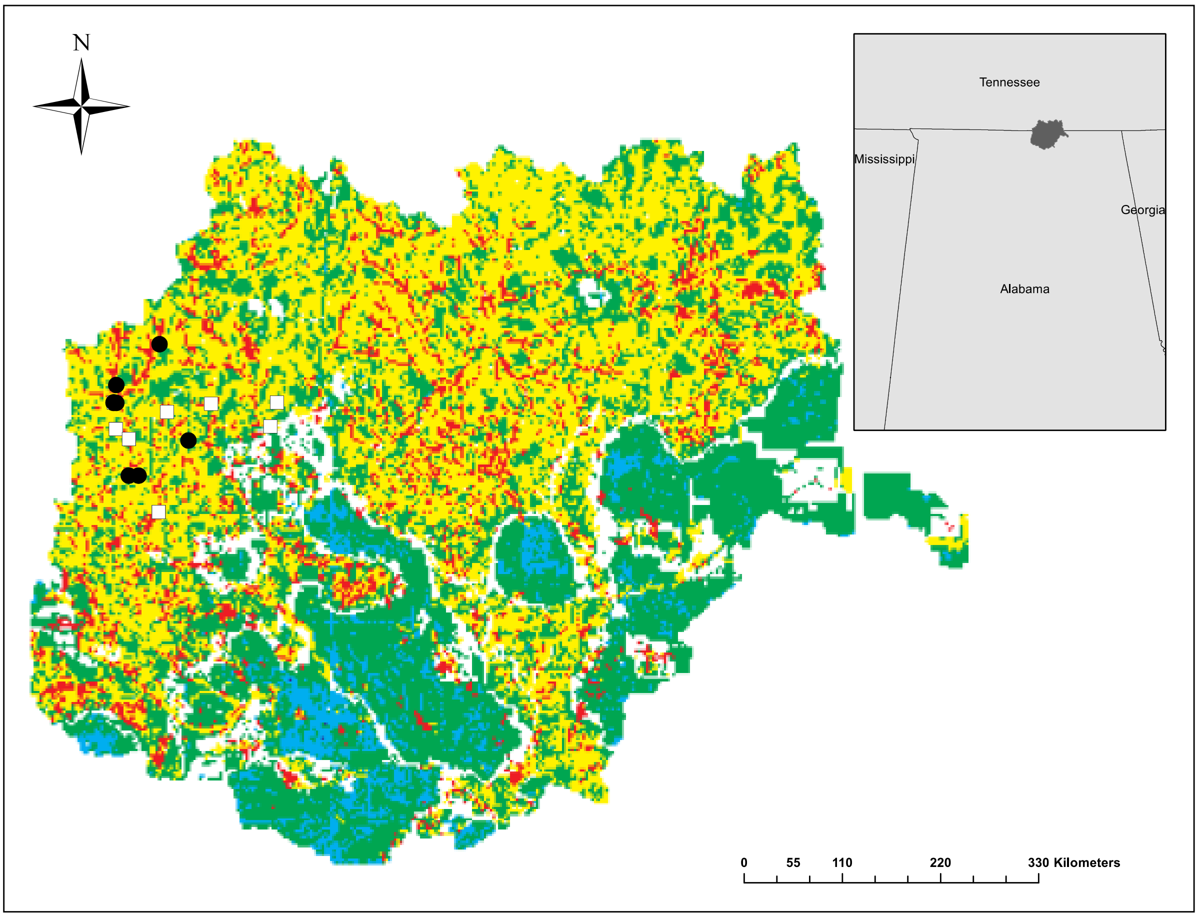

Figure 3. Habitat suitability map for Slackwater Darter in the Flint River system in Alabama and Tennessee. Habitat designated by red and yellow is best suited to Slackwater Darter occupancy. Extant populations are denoted by black circles. Extirpated populations are designated by a white square.

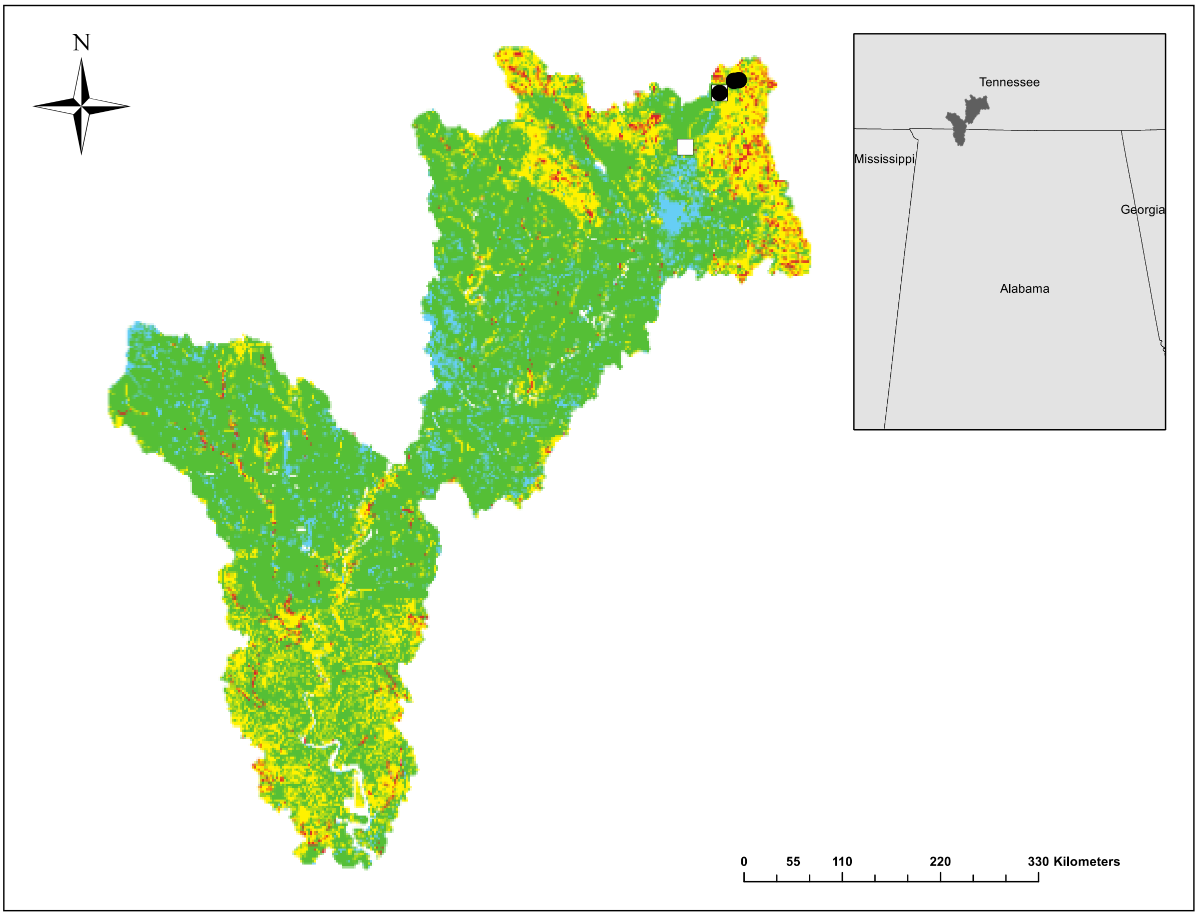

Figure 4. Habitat suitability map for Slackwater Darter in the Shoal Creek system in Alabama and Tennessee. Habitat designated by red and yellow is best suited to Slackwater Darter occupancy. Extant populations are denoted by black circles. Extirpated populations are designated by a white square.

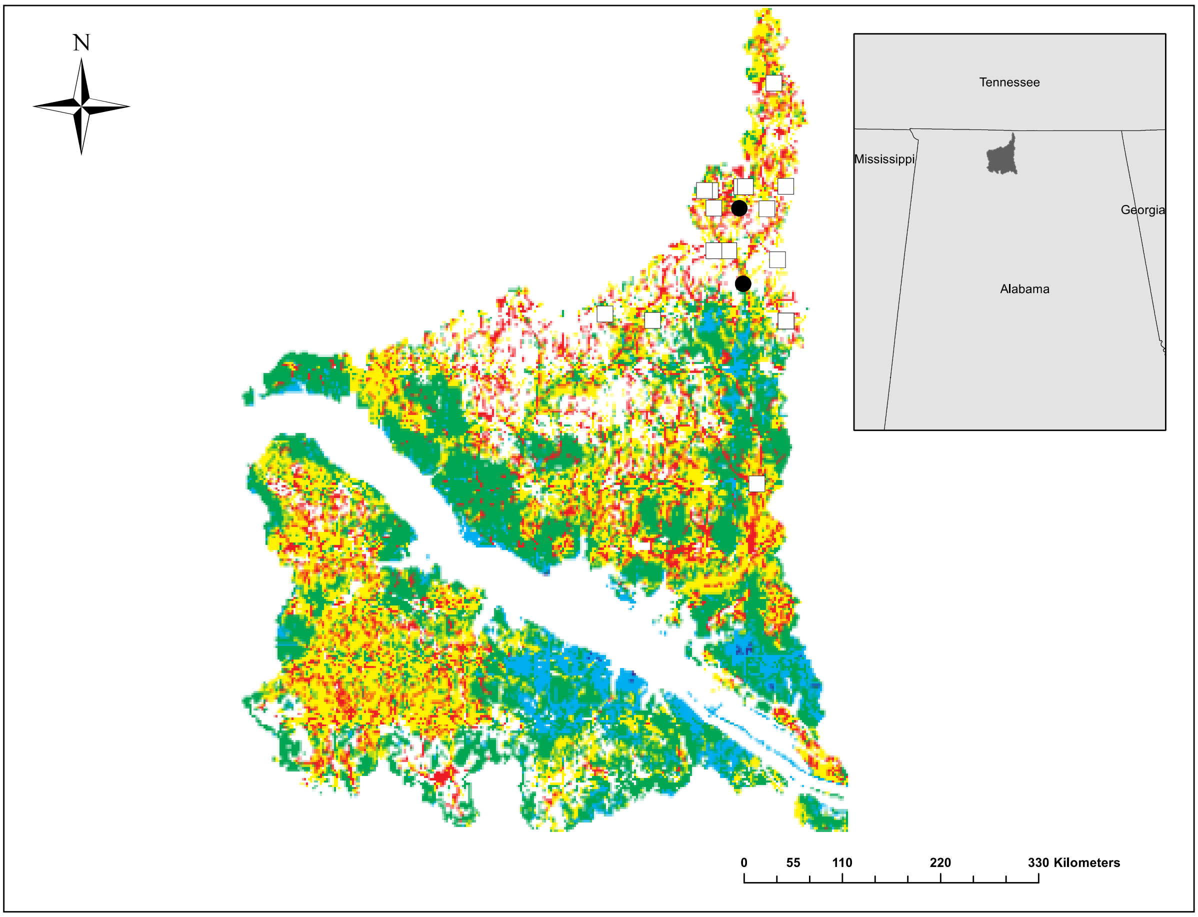

Figure 5. Habitat suitability map for Slackwater Darter in the Swan Creek system in Alabama. Habitat designated by red and yellow is best suited to Slackwater Darter occupancy. Extant populations are denoted by black circles. Extirpated populations are designated by a white square.

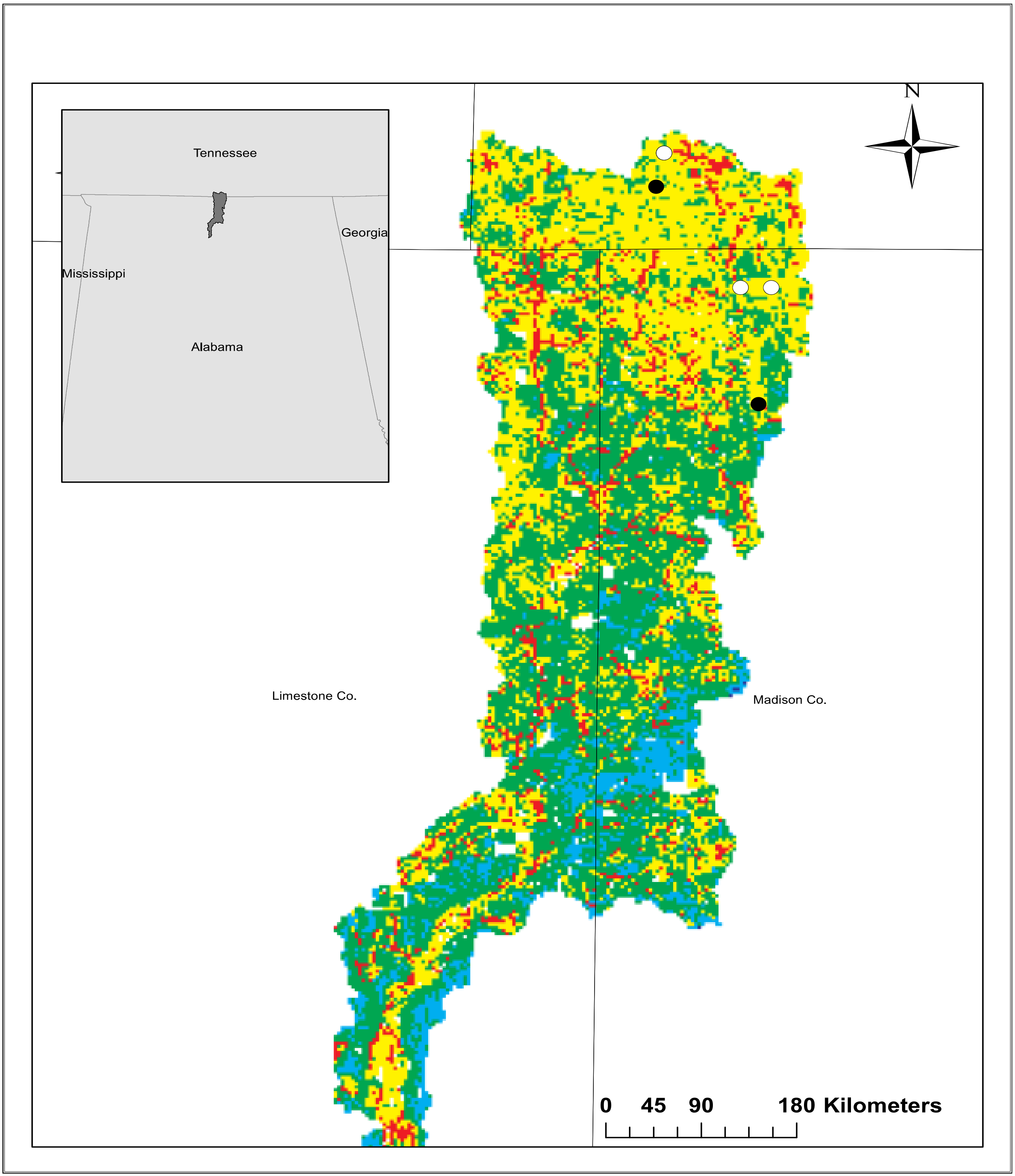

Figure 6. Habitat suitability map for Slackwater Darter in the throughout Limestone Creek in Alabama and Tennessee. Habitat designated by red and yellow is best suited to Slackwater Darter occupancy. Extant populations are denoted by black circles. Extirpated populations are designated by a white square.

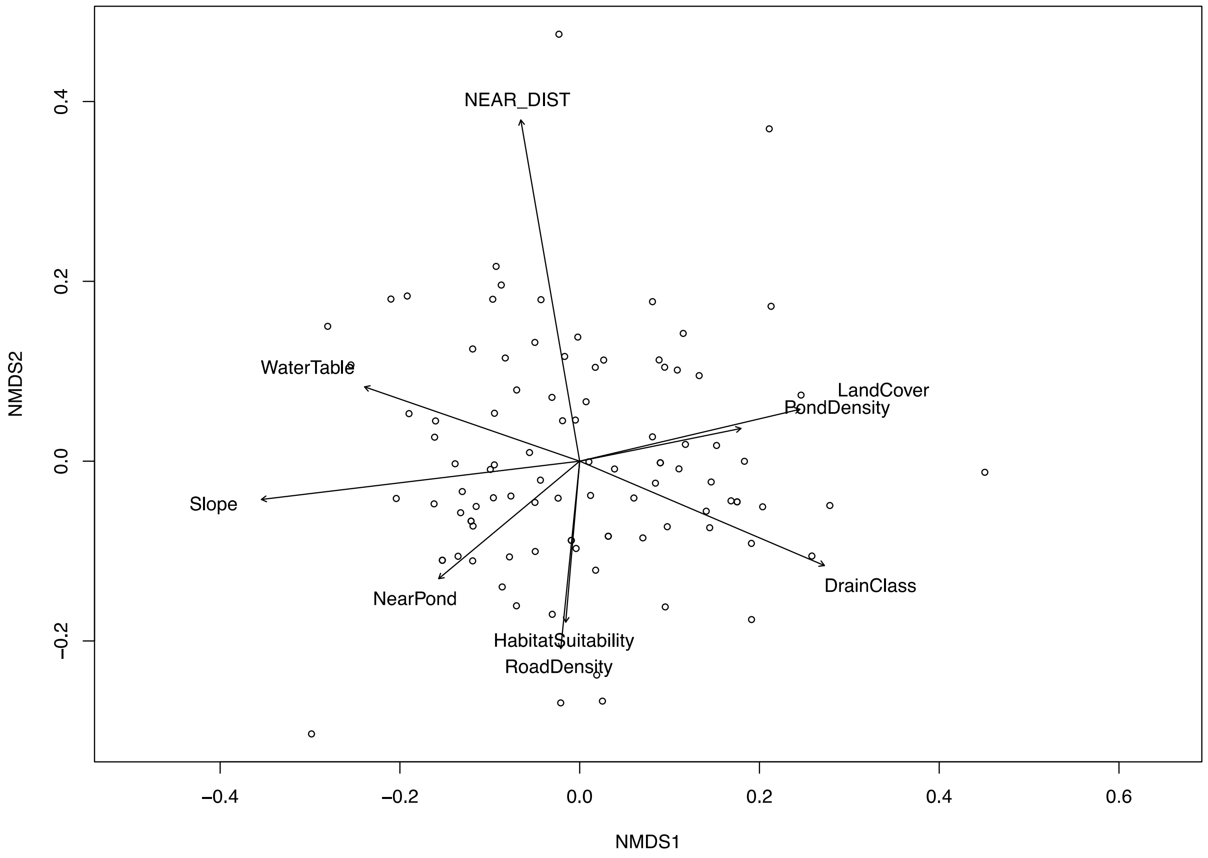

Figure 7. Non-metric multidimensional scaling model of all Slackwater Darter populations overlaid with corresponding habitat vectors. Length of the vector line indicates the strength of the associated variable on Slackwater Darter population location. Clusters of environmental variables indicate relationships between the effects of those variables and Slackwater Darter populations.

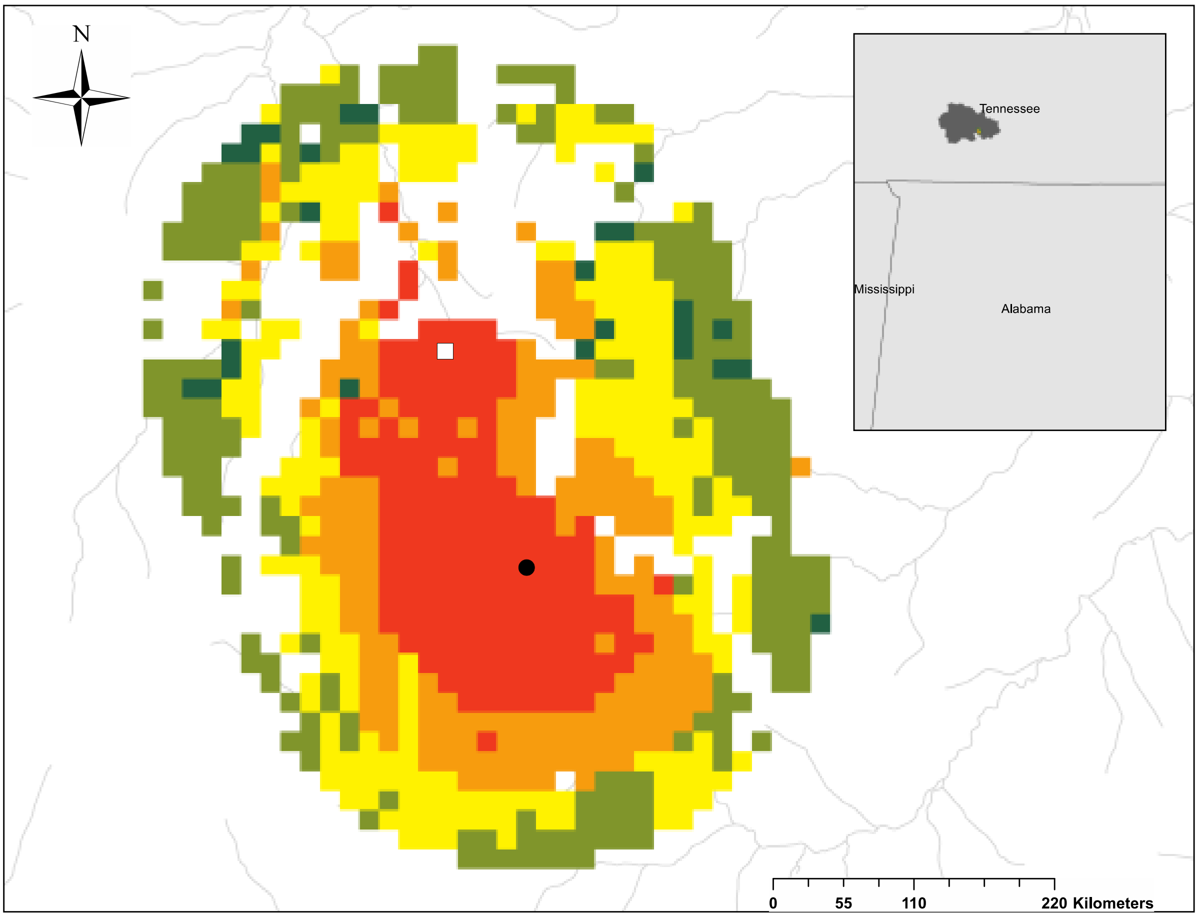

Figure 8. Habitat risk assessment for the Buffalo River system in Tennessee. Extant populations are designated by a black circle. Extirpated populations are denoted by a white square. Habitat is ranked green to red in order of least to most threatened.

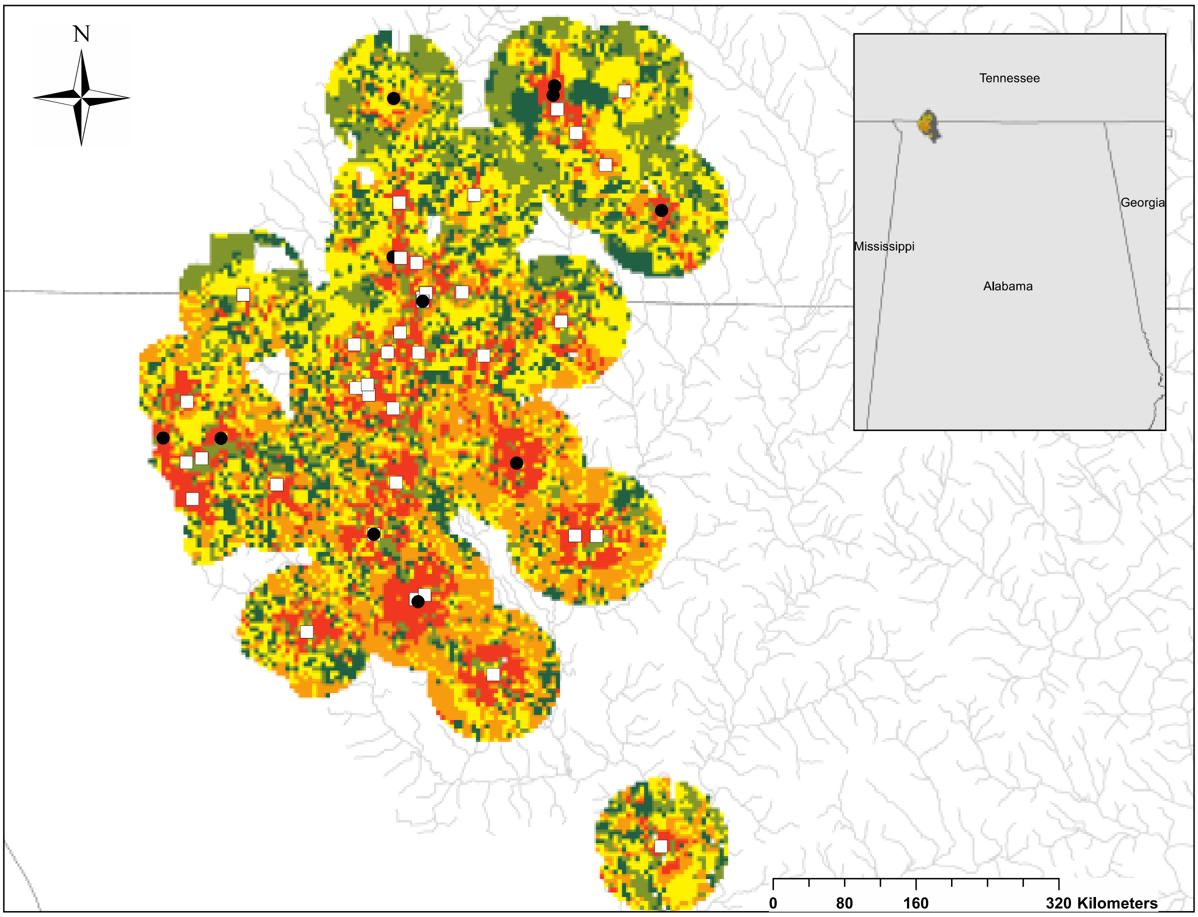

Figure 9. Habitat risk assessment for the Cypress Creek system in Tennessee. Extant populations are designated by a black circle. Extirpated populations are denoted by a white square. Habitat is ranked green to red in order of least to most threatened.

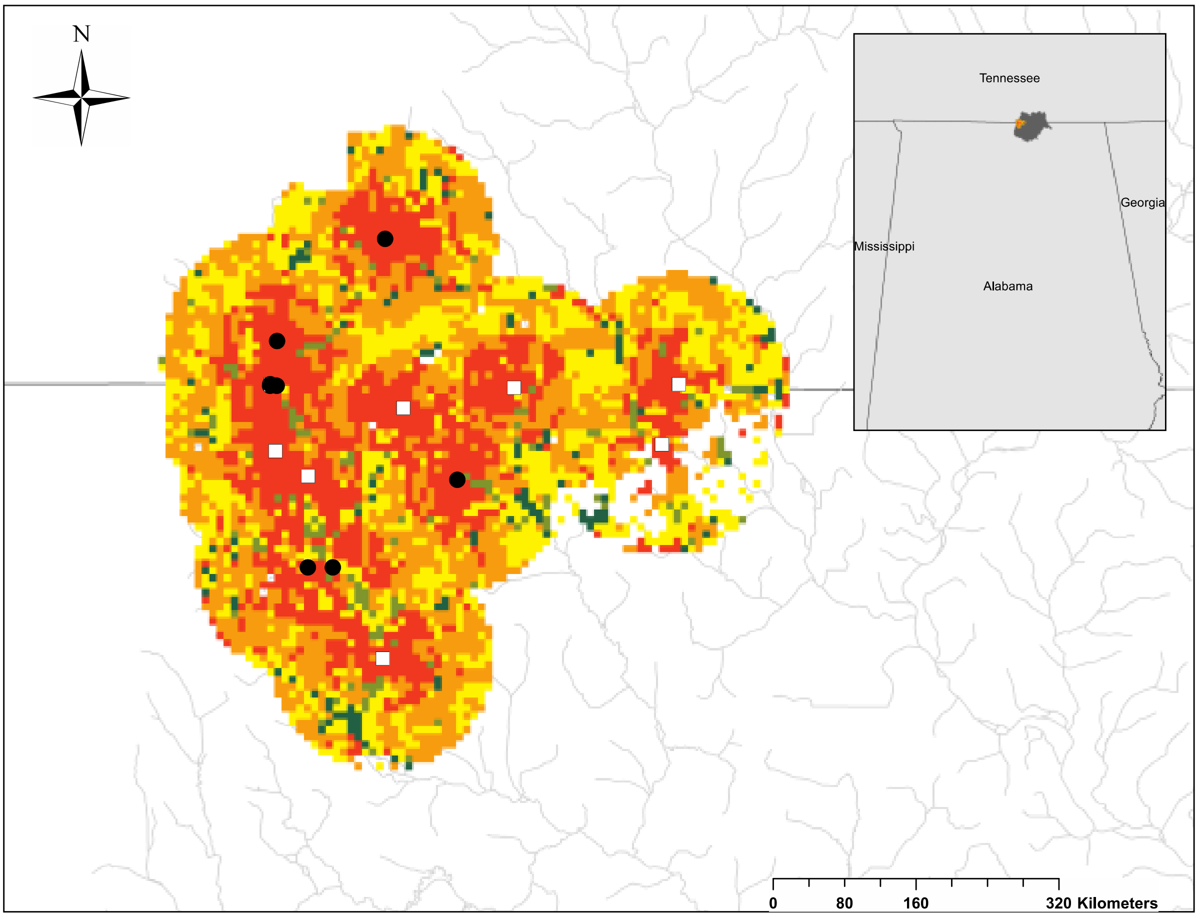

Figure 10. Habitat risk assessment for the Flint River system in Alabama and Tennessee. Extant populations are designated by a black circle. Extirpated populations are denoted by a white square. Habitat is ranked green to red in order of least to most threatened.

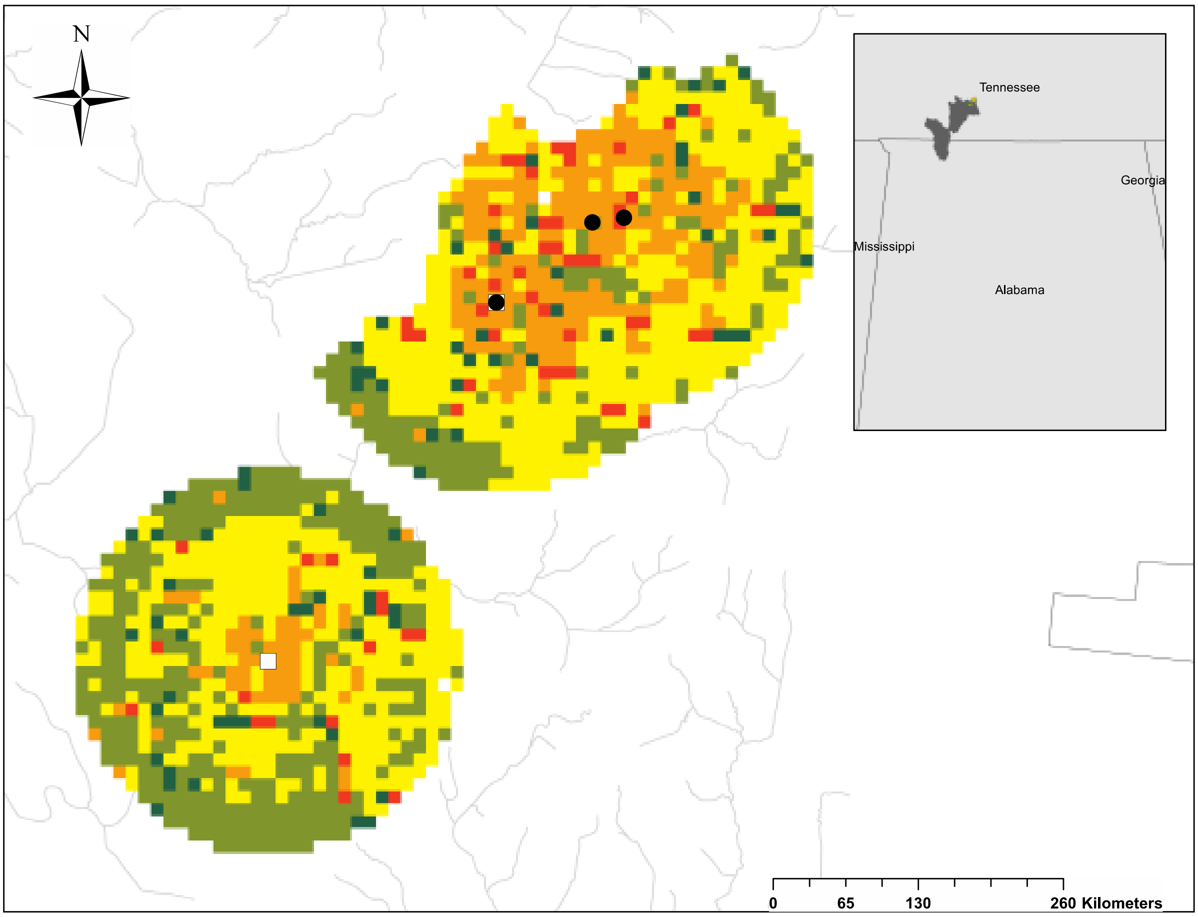

Figure 11. Habitat risk assessment for the Shoal Creek system in Alabama and Tennessee. Extant populations are designated by a black circle. Extirpated populations are denoted by a white square. Habitat is ranked green to red in order of least to most threatened.

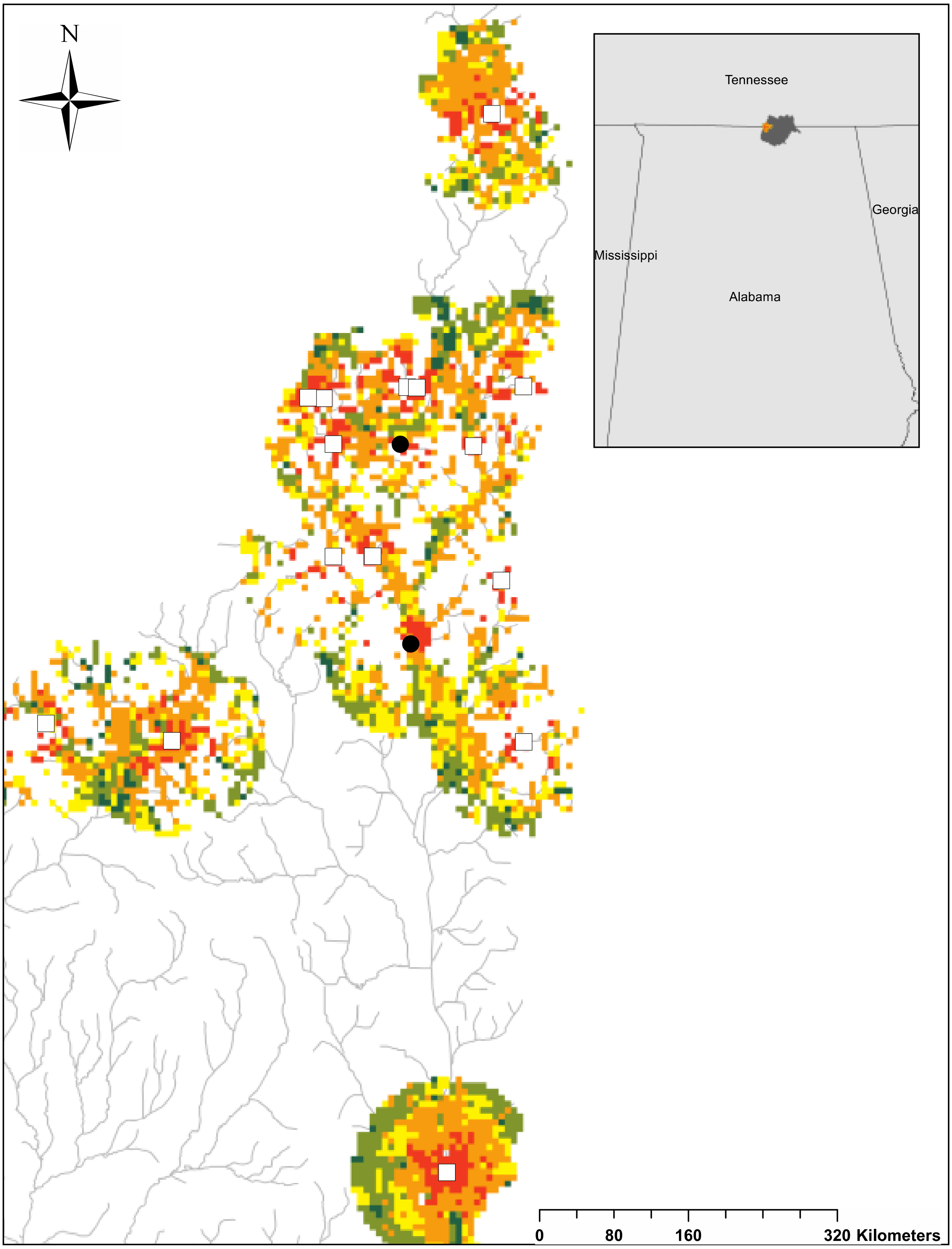

Figure 12. Habitat risk assessment for the Swan Creek system in Alabama and Tennessee. Extant populations are designated by a black circle. Extirpated populations are denoted by a white square. Habitat is ranked green to red in order of least to most threatened.

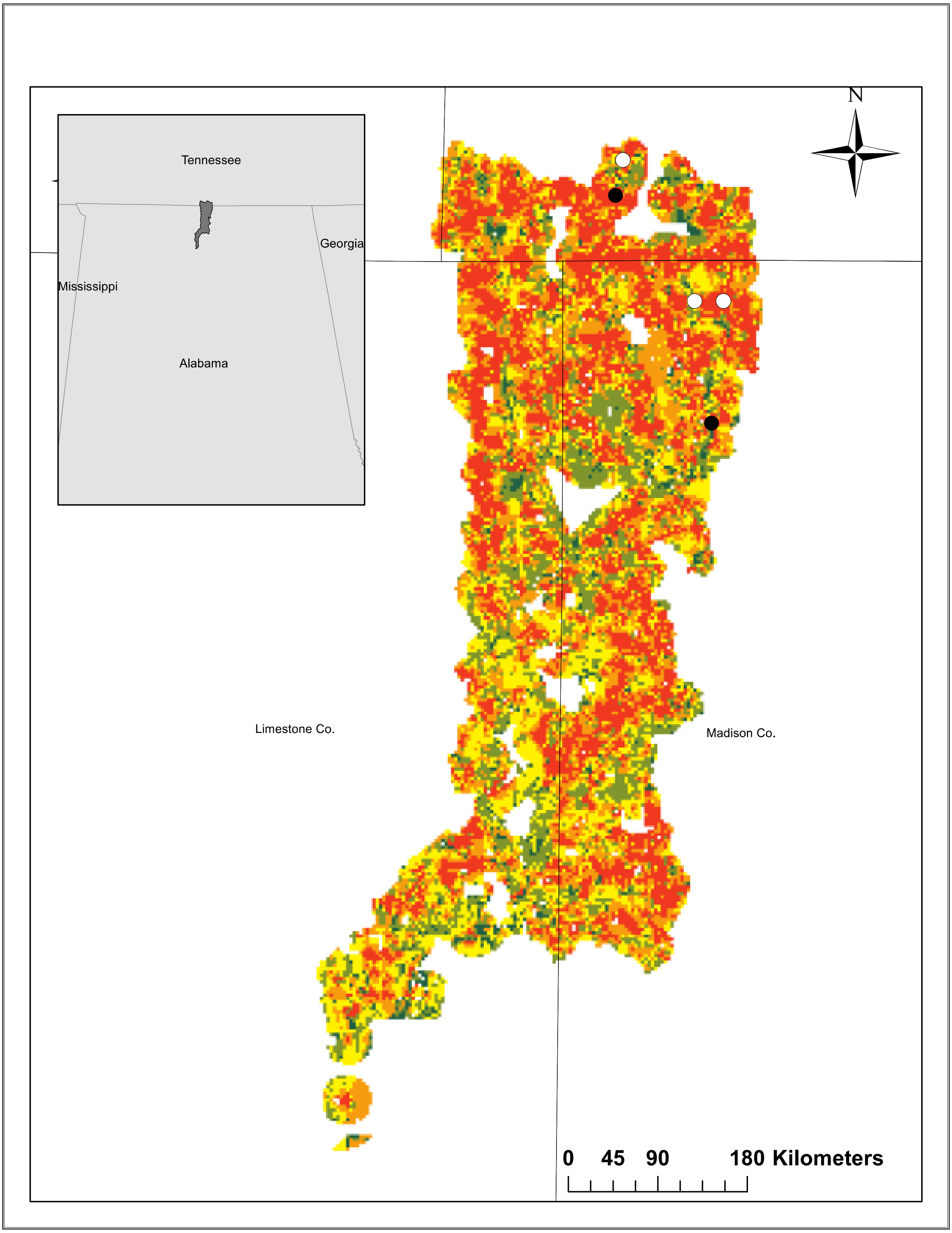

Figure 13. Habitat risk assessment for the Limestone Creek system in Alabama and Tennessee. Extant populations are designated by a black circle. Extirpated populations are denoted by a white square. Habitat is ranked green to red in order of least to most threatened.

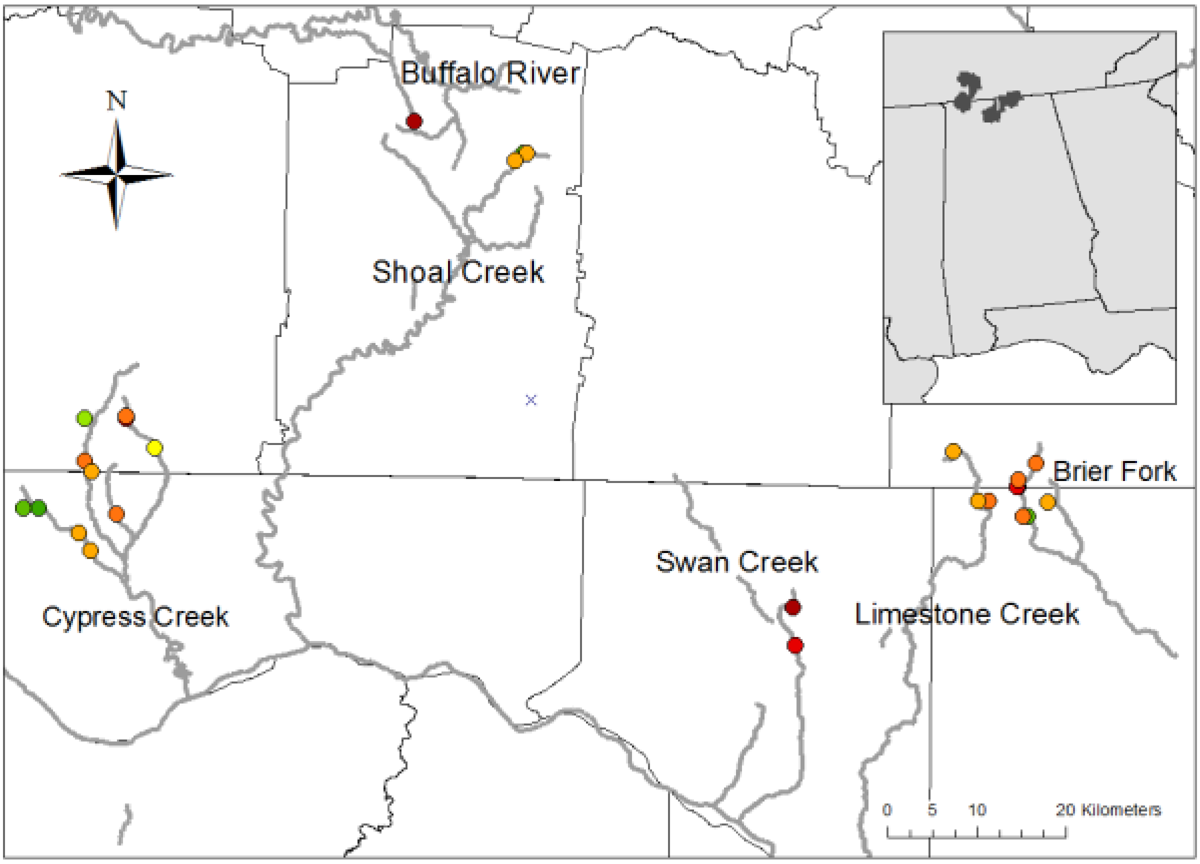

Figure 14. Slackwater Darter populations displayed according to relative calculated risk. Extirpated sites are denoted by hollow circles. Extant sites are ranked by color according to the risk value assigned by the threat assessment model. Populations designated by red or orange circles are most critically threatened. Populations designated by yellow circles are less-critically threatened but still of great conservation concern.

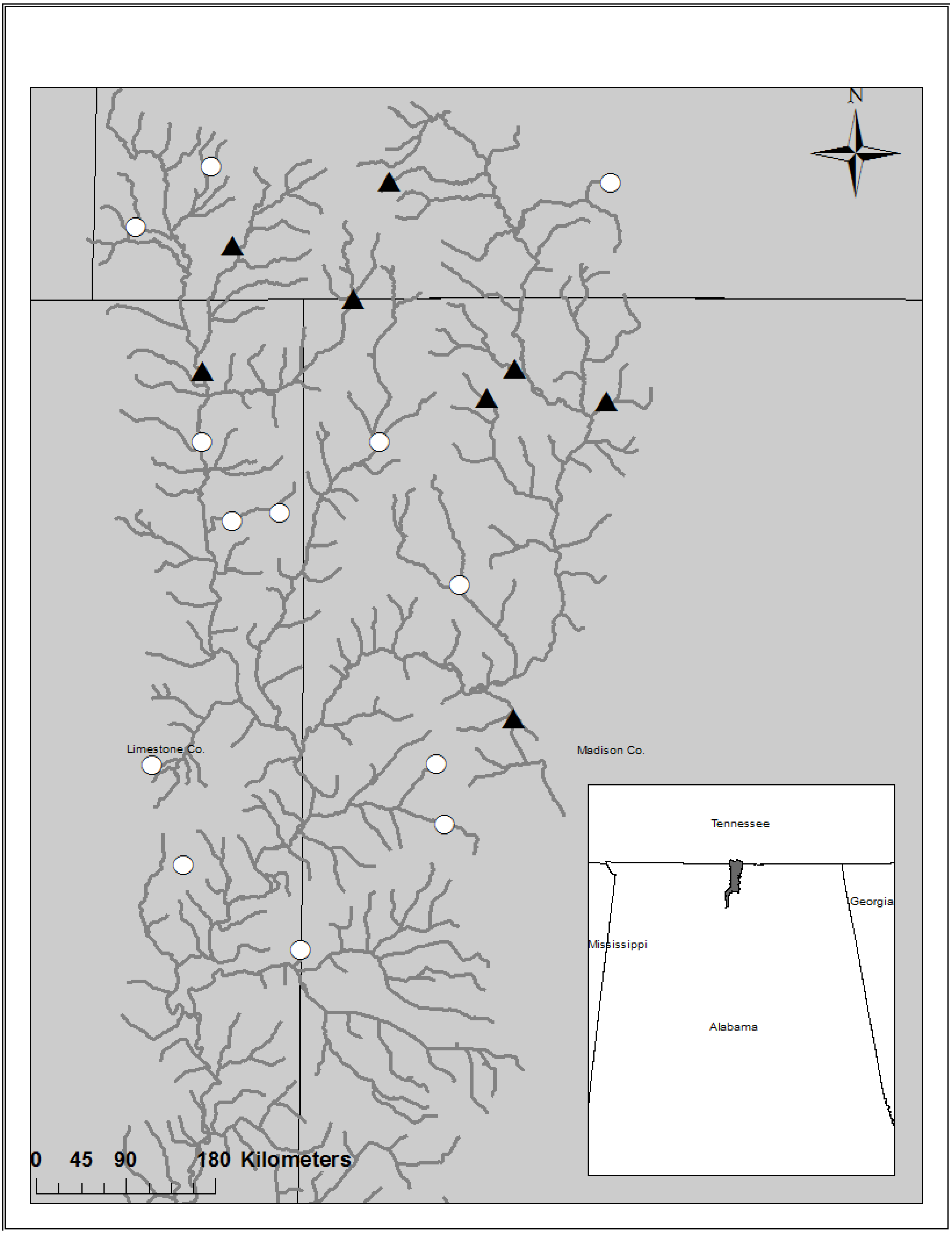

Figure 15. Map of eDNA sample sites in Limestone Creek. Site selection was determined by the breeding site projection model for the watershed (Figure 6). Positive detections are indicated by black triangles; negative detections are white circles.

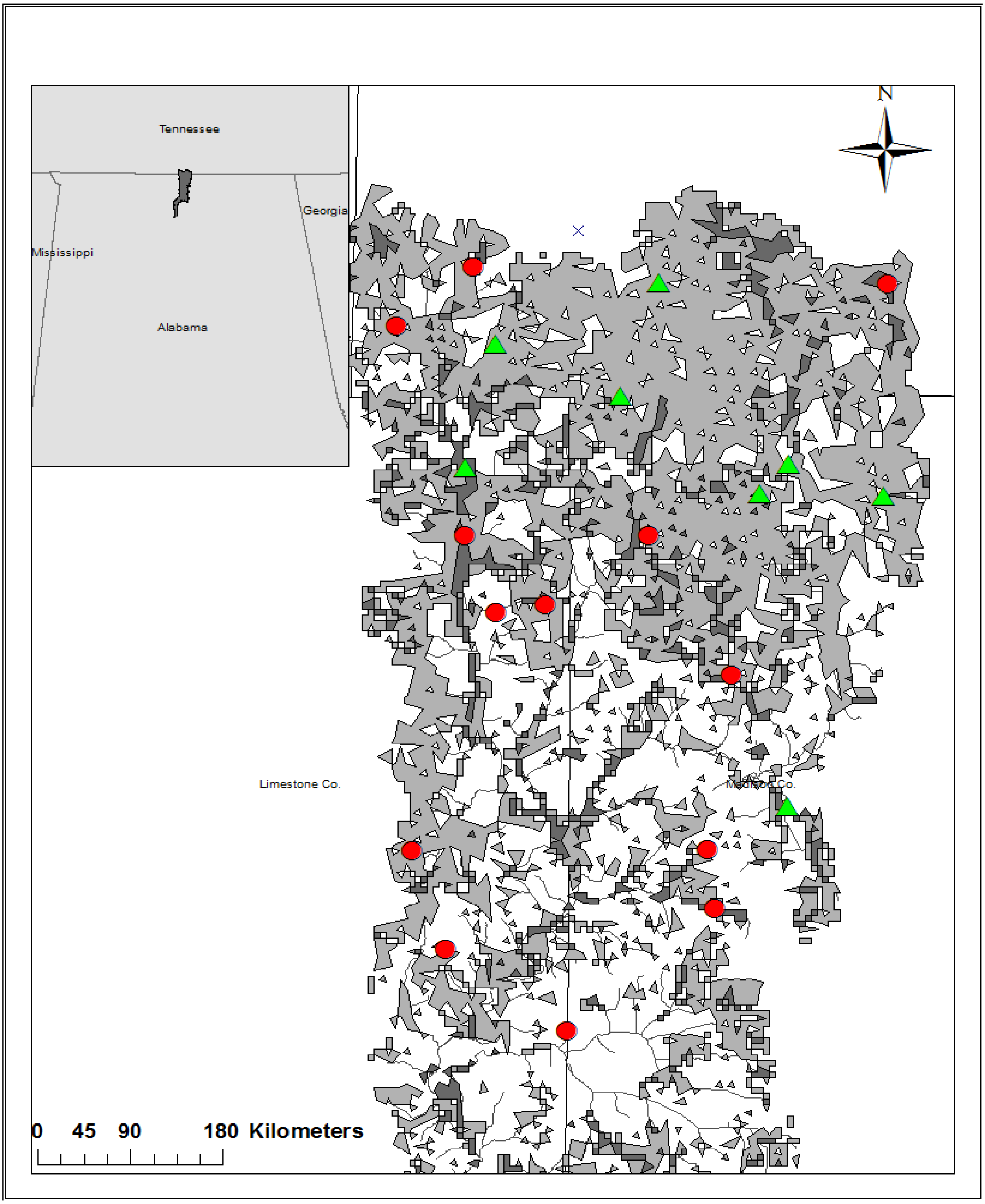

Figure 16. Map of eDNA sample sites in Limestone Creek compared to the viable habitat model. Habitat with the highest suitability rating is indicated by dark grey shading, lighter grey indicates the second-highest habitat suitability rank. Red circles indicate sites where Slackwater Darter was not detected; green triangles indicate positive detections.

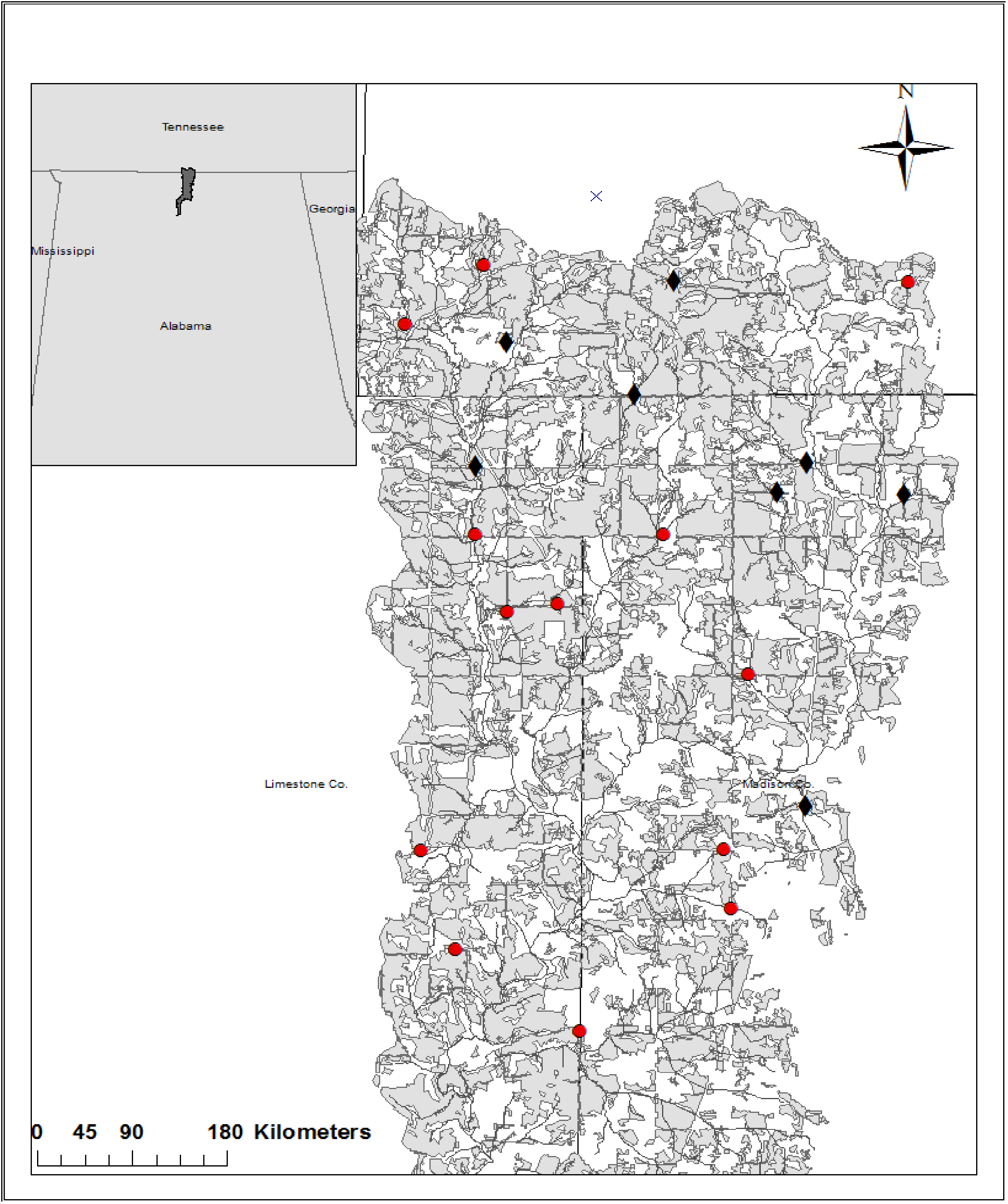

Figure 17. Map of eDNA sample sites in Limestone Creek compared to pasture land within the drainage. Sites with positive detections are indicated by black diamonds; sites with negative detections are indicated by red circles.

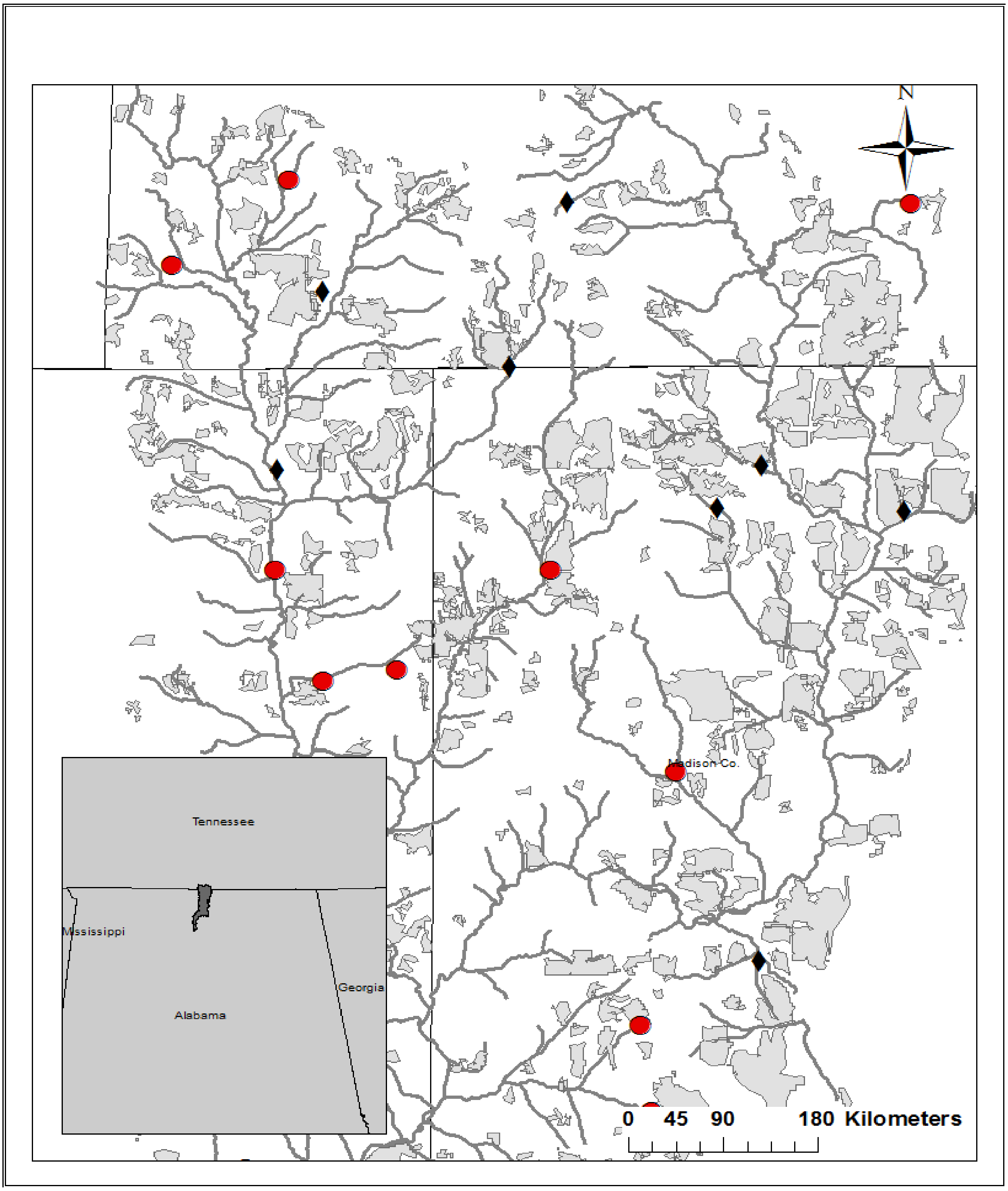

Figure 18. Map of positive eDNA sample sites in Limestone Creek compared to cropland within the drainage. Sites with positive detections are indicated by black diamonds; sites with negative detections are indicated by red circles.

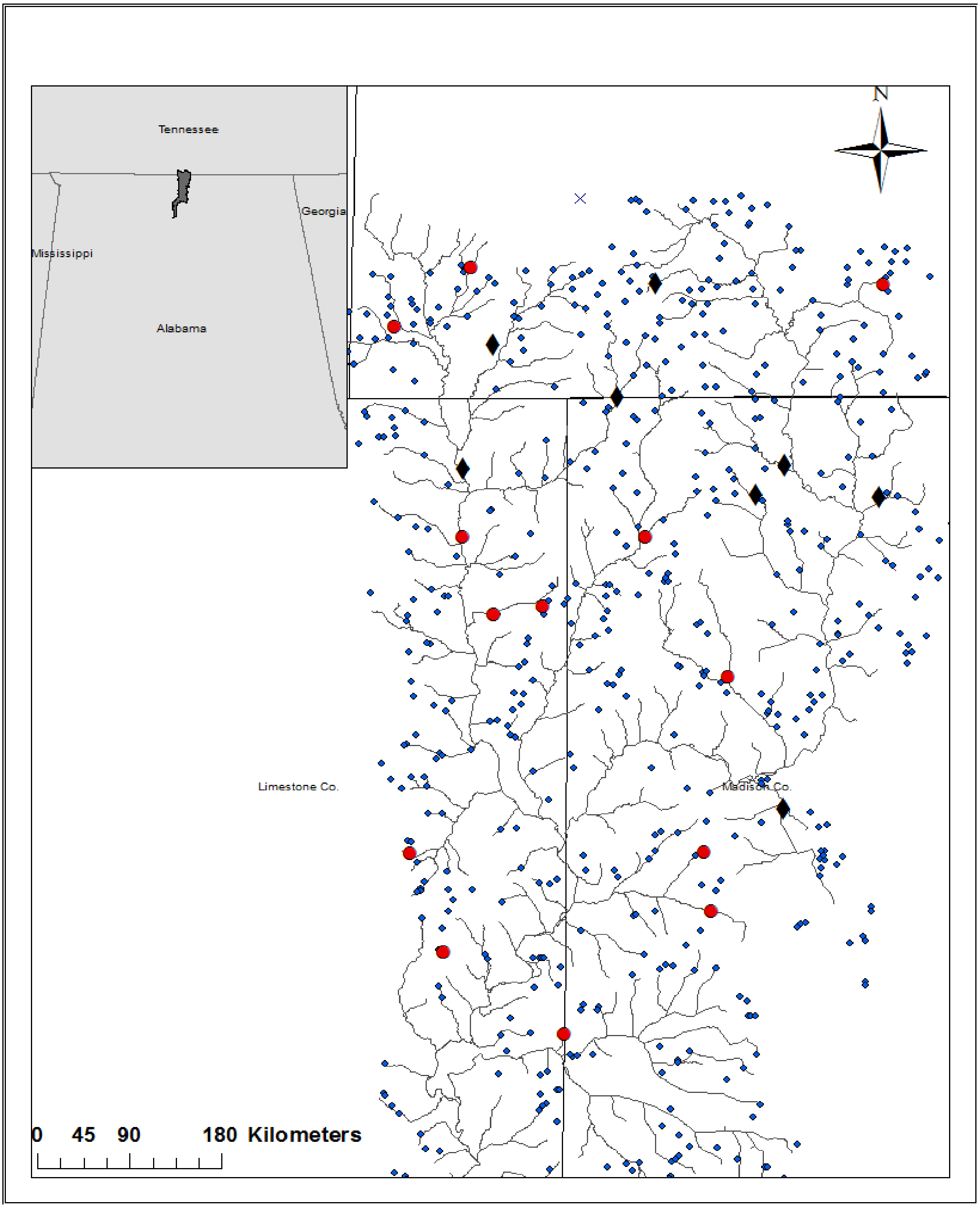

Figure 19. Map of eDNA sample sites in Limestone Creek compared to farm pond location within the drainage. Farm ponds are denoted as small blue circles. Negative Slackwater Darter detections are shown as red circles. Positive Slackwater Darter detections are shown as black diamonds.

Appendix I, Site localities for all previously-collected Slackwater Darter populations considered within the model.

OBJECTID	LAT	LONG	SITE NAME
1	34.91643000	-87.84990000	Burcham Branch, Natchez Trace Pkwy
2	34.95141000	-87.88706000	Burton Branch, Co. Rd. 158
3	34.94245000	-87.82860000	Lindsey Creek, Natchez Trace Parkway
4	34.96104000	-87.88910000	Lindsey Creek, Co. Rd. 60
6	34.92533000	-87.81496000	Lindsey Creek, Co. Rd. 81
7	34.98120000	-87.83470000	Lindsey Creek, Co. Rd. 5
8	34.92650000	-87.81210000	Lindsey Creek, E Natchez Trace Pkwy
9	34.95623300	-87.82156000	Threet Creek at Natchez Trace
10	34.97171000	87.82034000	Cemetery Branch, Natchez Trace Pkwy
11	34.97590000	-87.82275000	North Fork Cypress Creek, Natchez Trace Pkway
12	34.97938000	-87.83064000	Elijah Branch, Co. Rd. 85/5
13	34.98215000	-87.83100000	North Fork Cypress Creek, Co. Rd. 85/5
14	34.99599000	-87.82067000	Trib, Cypress Creek, Natchez Trace Pkwy
15	35.00563000	-87.87130000	Cypress Creek, 0.5 mi SW Cypress Inn
16	35.00527000	-87.81361100	Dulin Branch at Hwy 157
17	34.99924000	-87.76890000	Lathum Branch
18	35.01444400	-87.81555000	Trib, Dulin Branch, N Hwy 227
19	35.00652000	-87.81245000	Cypress Creek, Natchez Trace Pkwy
20	35.01600000	-87.82314000	Trib, Cypress Creek, Natchez Trace Pkwy
21	35.04084000	-87.75489000	Trib, Middle Cypress Creek at powe line, Pigg Rd.
22	35.04931000	-87.76449000	Trib, Middle Cypress Creek, E Gilchrest Rd. and Pigg Rd.
23	35.05290000	-87.86627000	Trib, Middle Cypress Creek, Dodd Rd. and Gilchrest Rd.
24	35.05550000	-87.77062000	Trib, Middle Cypress Creek, Dodd Rd
25	35.06171000	-87.77153000	Trib, Middle Cypress Creek
26	34.86030000	-87.73547000	Cypress Creek, Co. Rd. 16
27	34.99067600	-87.81465200	Cypress Creek, Co. Rd. 10
28	34.94247000	-87.75691000	Middle Cypress Creek, Co. Rd. 8
29	34.94253000	-87.76386000	Greenbrier Branch, Co. Rd. 8
31	35.01580000	-87.82070000	Trib, Cypress Creek, Natchez Trace Pkwy
32	35.05920000	-87.77200000	Trib, Middle Cypress Creek, Dodd Rd. and Gilchrest Rd.
33	35.06041000	-87.74900000	Spain Branch, Gilchrest Rd.
34	35.32787000	-87.28507000	Little Shoal Creek, Dooley Rd.
35	35.28657000	-87.32202000	Little Shoal Creek, Beasley Rd
36	35.32032700	-87.29602100	Little Shoal Creek at Hwy 43
37	35.37278300	-87.42540000	Chief Creek at Hwy 240
38	34.81326000	-87.00705000	Round island Creek, 2.0 mi N Athens
39	34.84381000	-86.93085000	Collier Branch, Bean Rd. just E I65
40	34.84842000	-86.96057000	Swan Creek, Piney Chapel Rd.
41	34.86986000	-86.96970000	Swan Creek, Huber Rd.

42	34.87860000	-86.97186000	Roadside ditch, Swan Creek drainage, Co. Rd. 80
43	34.88084000	-86.92570000	Roadside seep (Swan Creek drainage), Co. Rd. 80
44	34.81300000	-86.92570000	Trib, Swan Creek at Linton drive
45	34.83174000	-86.95181000	Swan Creek, Elkton Rd.
46	34.84840000	-86.96970000	Roadside Seep (Swan Creek drainage), Co. Rd. 55
48	34.93390000	-86.71910000	Limestone Creek at Ready Section Rd
49	34.96230000	-86.66580000	Brier Fork, Bobo Section Rd
50	34.96230000	-86.67070000	Trib, Brier Fork, Elkwood Section rd.
51	34.99170000	-86.67800000	Trib, Brier Fork, Scott Rd. State line Rd.
52	34.99190000	-86.67790000	Brier Fork, Scott orchard
53	34.98110000	-86.67700000	Trib, Brier Fork, Scott Rd.
54	34.99170000	-86.67670000	Trib, Brier Fork, Scott Orchard Rd.
55	35.01540000	-86.65530000	Brier Fork, Fowler Rd.
56	34.99167000	-86.59776000	Copeland Creek, Charity Lane
57	34.90550000	-87.78980000	Lindsey Creek, Co. Rd. 15
58	34.99270000	-87.83540000	North Fork, cypress Creek, Co. R. 10
59	34.95540000	-87.86000000	Lindsey Creek
60	34.96755000	-87.87805000	Lindsey Creek, Co. Rd. 8
61	34.96750000	-87.89666000	Trib, Lindsey Creek, Co. Rd. 8
62	34.94245000	-87.82860000	Lindsey Creek, Natchez Trace Parkway
63	34.92470000	-87.81420000	Lindsey Creek, Co Rd. 154
64	34.96220000	-87.88420000	Burcham Creek, Co Rd. 100
65	34.99001900	-87.79375000	Greenbrier Branch, Co Rd. 10
66	34.96160000	-87.78280000	Greenbrier Branch, Co. Rd. 85
67	34.99924000	-87.76890000	Lathum Branch
68	35.00434000	-87.81348000	Trib, Dulin Branch, Hwy 227
69	35.03039200	-87.82128700	Cypress Creek, Natchez Trace Parkway
70	34.99067600	-87.82465200	Cypress Creek, Co. Rd. 10
71	35.00681000	-87.80080000	Cypress Creek at Holly, Natchez Trace
72	35.02890000	-87.73680000	Middle Cypress Creek at Hwy 227
73	35.05800000	-87.82335000	Trib, Cypress Creek, Natchez Trace Pkwy
74	35.00434000	-87.81348000	Seep, Cypress Creek, State Line Rd
75	35.03266700	-87.79711000	Dulin Branch, Gilchrist Rd.
76	35.06161000	-87.77153000	Trib, Middle Cypress Creek
77	35.32833000	-87.28144000	Trib, Little Shoal Creek at horse farm
78	35.32032700	-87.29602100	Little Shoal Creek, Ethridge Park
79	35.35988000	-87.41940000	Chief Creek, Barnett Rd.
80	34.94751000	-86.65610000	Brier Fork at Charity Lane
81	34.97705000	-86.67060200	Brier Fork, Co. Rd. 118
82	34.98196100	-86.60114400	Fowler Creek, Edgar Gooch Road
83	34.97640000	-86.64130000	Copeland creek, Butter and Egg Rd.
84	34.99123000	-86.63011000	Copeland Creek, State Line Road
85	34.98801000	-86.65186000	Huckleberry Branch, State Line Road
86	34.99195000	-86.67798000	Trib, Brier Fork, State Line Road
87	34.99894000	-86.67660000	Trib, Brier Fork, Scott Road
88	34.97752100	-86.71379000	Limestone Creek, Elkwood Section Road

89	34.97752300	-86.72630000	Limestone Creek, W Elkwood, Elkwood Section Road
90	35.02798000	-86.75727000	Mud Springs Branch, Hwy 110
91	35.02798000	-86.75727000	Mud Springs Branch, Power Station Road
92	35.01537000	-86.76054000	Taft Branch, Jones Road
93	34.86981000	-86.95419000	Trib, Swan Creek, Huber Road
94	34.88067000	-86.95259000	Trib, Swan Creek, Co. R. 80
95	34.88063000	-86.95039000	Trib, Swan Creek, Co. Rd. 80
96	34.73090000	-86.94350000	Swan Creek, E Tanner Crossroads just before I65
97	34.81655000	-87.03615000	Round Island Creek at Glaze Road
98	34.86946000	-86.93729000	Mud Creek
99	34.81300000	-86.92570000	Trib, Swan Creek, Linton Drive
100	34.84009000	-86.92100000	Piney Creek, Johnson Road
101	34.93284000	-86.93295000	Collier Branch
102	34.87870000	-86.97550000	Trib, Elk River, Co. Rd. 55

Appendix 2, External ArcGIS data sources
*All downloaded datasets were transformed and projected into WGS 84/UTM Zone 16N
Roads data

- Tennessee: https://catalog.data.gov/dataset/tiger-line-shapefile-2013-state-tennessee-primary-and-secondary-roads-state-based-shapefile
- Alabama: http://www.alabamaview.org/GISTigerfiles.php

Streams data

- Tennessee: http://www.tngis.org/water.htm
- Alabama: http://www.alabamaview.org/GISTigerfiles.php

County outlines:

- Tennessee: https://www.arcgis.com/home/item.html?id=47aa2778faa74389aa06c34bc767f41c
- Alabama: https://catalog.data.gov/dataset/tiger-line-shapefile-2013-state-alabama-current-county-subdivision-state-based

State outlines were downloaded from the USA State Boundaries Layer Package provided by esri on arggis.com
https://www.arcgis.com/home/item.html?id=540003aa59b047d7a1f465f7b1df1950

Soils data were downloaded from ArcGIS.com as a data download package derived from the 2014 NRCS SSURGO data:
https://www.arcgis.com/home/item.html?id=a23eb436f6ec4ad6982000dbaddea5ea

Land cover data was obtained from the 2011 National Land Cover Dataset:
https://www.mrlc.gov/nlcd2011.php

Watershed boundaries were clipped from the National Hydrography Dataset:
https://nhd.usgs.gov/wbd.html