

**Micro-habitat Analysis of the Blueside Darter (*Etheostoma jessiae*)**

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## **Abstract**

The Pigeon River lost aquatic biodiversity because of chemical deposition from a paper mill. Most aquatic communities have been restored to the river, but some species have not survived reintroduction. The blueside darter (*Etheostoma jessiae*) has not been re-established to its native range in the Pigeon River. Micro-habitat analyses were conducted where this darter exists in neighboring watersheds. Analysis of habitat is desired to locate acceptable sites on the Pigeon River for reintroductions.

## **Introduction**

The Pigeon River begins in Haywood County, North Carolina, and flows for about 79 miles until it joins the French Broad River (Bartlett 1995). A paper mill established in 1908 began releasing toxic effluents, which led to the degradation of the Pigeon River and had negative effects on the French Broad River as well. It is believed that these contaminants led to the extirpation of many species of fish and mollusks (Coombs et al. 2004). Champion Paper International, currently called Blue Ridge Paper Products, modernized the plant from 1992 to 1994, and while the changes did not entirely eliminate pollution, they drastically improved water quality (Bartlett 1995).

With the improved water quality, many species have begun returning to the river. Surveys have shown a continual increase in the number of fish species until the late 1990s when no new species were observed; an inventory concluded that several, approximately 20-24, of the smaller non-game species were still absent from their native ranges. Some species of fish have limited ability to travel far distances and thus need assistance. The goal of the Pigeon River Recovery Project is to reintroduce as many of

those missing species as possible into locations where successful re-establishment is promising (Coombs et al. 2006). During recent years, several species of fish have been reintroduced by method of relocation from a nearby watershed, but some fish have not “taken” or rather survived the relocation. One fish that did not re-establish a reproducing population was the blueside darter (*Etheostoma jessiae*).

The blueside darter is a member of the subgenus *Doration*. Originally all darters in this complex were identified as a single name, but notable differences in populations of fish could allow the fish to be differentiated into five different species, the blueside darter being one. Etnier and Starnes (1993) stated: “...no group of darters is taxonomically so poorly understood...” This species of fish is benthic and rests on the bottom of streams. The average size of males is greater than 50 mm; the largest in this study was 71 mm.

It is vital to re-establish the native communities of fish to obtain a balanced ecosystem. Each species is a “specialist” and will occupy a specific niche and consume certain prey species. Although it is difficult to determine a complete description of the entire niche, several niche variables can assist in determining the habitat of a species (Solomon et al. 2005). The variables examined in this paper will include abiotic factors that limit the blueside darter to specific habitats in the stream.

The objectives for this study were to:

- locate blueside darters in streams similar to the Pigeon River,
- designate micro-habitat sites where blueside darters exist for analysis of stream characteristics, and
- assess survival following Visible Implant Elastomer (VIE) tagging procedure.

The knowledge of the possible factors that influence the habitat of the blueside darter will assist in the re-introductions of this species into the Pigeon River and provide information about post-tagging survival.

## **Methods**

### ***Study Site***

The Little River flows out of the Great Smoky Mountains National Park through Blount County, Tennessee; this river was used as study site because it is the known to contain populations of blueside darters and because of its easy accessibility. The Little River is a cold-water stream and contains a variety of habitat types including riffles, runs, and pools. Previous sampling of the river demonstrated that the blueside darter exists in several known locations. Preliminary snorkeling surveys confirmed the location of areas in which blueside darters reside. Two different sections of stream reaches were sampled to produce 12 micro-habitat sites. The sections are located 2.5 miles apart and are separated by a milldam; the dam forms a barrier in which the fish from lower sections cannot exchange genetic stock with fish from the upper section. The first stream reach is located approximately 100 yards above Coulters Bridge along Old Walland Highway near Little River Mile 20.5 (LRM 20.5). The second stream reach is located on U.S. Route 321 near Kingdom Lane at LRM 23.

### ***Field Sampling***

Sampling was partitioned into four different principal steps: 1) locating the fish, 2) capturing the fish, 3) tagging the fish, and 4) collecting stream habitat data. The sampling could be done with three people, but having at least four allowed for more

efficient sampling. Seining was conducted using three people, and having one person dedicated to tagging provided enough manpower to efficiently sample an area.

Locating fish was done via snorkeling. Areas were scanned until blueside darters were sighted. Upon locating a fish, the snorkeler would place a marker where the fish was first observed. The marker was constructed with a cork float attached to about three feet of monofilament line and was weighted by egg-sinkers; one-half ounce of weight was sufficient to secure the marker in moderately swift flows. The snorkeler(s) would use the markers to locate as many blueside darters in that area for collection.

After marking the location of a single fish or group of fish, a 4.3-m (5 mm mesh) seine was set 10-15 feet downstream of the area. A person snorkeling would make sure the net was flush against the substrate, and would place small rocks on the bottom of the net's lead line to help secure it. One to three snorkelers would then proceed to "herd" the fish towards the seine; the person(s) herding would approach the fish from upstream and carefully spook the fish towards the net by extending their arms to create a large surface area. Fish were hesitant to enter the net and would stop within a foot of the seine. Once most of the fish were within a foot of the net, the snorkeler(s) would spook the fish by creating fast movements with their arms near the fish; this caused the fish to move into the net, and the seine would be lifted rapidly. This was done several times until snorkelers could make passes without seeing or capturing fish. In areas with heavy silt and no visibility, seine hauls were conducted by beginning outside the area where the fish were seen, seining through the area and then beaching (bringing the bottom of the seine onto a shoal) the seine. After each seining effort, the fish were placed inside a 5-gallon

bucket with fresh river water, which was partially submerged in the stream to maintain a constant temperature.

If desired, the snorkeler(s) could carry small handheld dip nets to collect fish that would not scare towards the seine; multiple ways were used to capture fish with this method. Two methods seemed to have mediocre success, and included: 1) intimidating the fish to rest next to a rock (this provided one side to be secured from escape), then placing the dip net along one side of the rock and using the other hand to guide the fish into the net and lifting fast, and 2) laying the dip net nearly flat a few inches in front of the fish and then in one swift motion, swoop the net towards the fish and then up, The mode that did not work very well was attempting to place the net directly over the fish and then quickly pushing the net towards the substrate to capture the fish between the net and the floor of the stream.

Tagging was performed after successful capture of blueside darters using Visible Implant Elastomer (VIE). Unmixed VIE is a liquid, but after mixing the two components, and setting for a time, becomes a flexible plastic elastomer; these tags are used to mark fish with transparent or translucent tissues. Fish were tagged according to procedures produced by Northeast Marine Technology, Inc. (2006). This process included anesthetizing the fish in a bucket containing 2 liters of stream water and 200 mg of tricaine methanesulfonate (MS-222) to produce a 100mg/L solution. Fish were left in the anesthetic solution until they did not react to being handled. The fish would then be tagged using a 29-gauge needle. Fish were then placed into a bucket with fresh stream water to flush the anesthetic from their system. Upon tagging all fish from a micro-habitat site, the fish were acclimated in selected areas of the stream and released.

Upon completion of searching for blueside darters, stream habitat data were collected. Water quality parameters were collected using a YSI Model 85 to measure dissolved oxygen (mg/L), conductivity ( $\mu\text{S}$ ), and temperature ( $^{\circ}\text{C}$ ) and a YSI Model 60 to measure pH. Habitat data collected included depth, mean water velocity, and substrate composition. Depth was taken using depth stick. Mean water velocity was measured using a Type AA Current Meter. Substrate composition was measured using a 1 x 1-meter grid with 25 cross-sections, each being 25 centimeters apart in a square grid.

A follow-up visit to the stream was conducted to collect additional measurements and to observe for the retention of tags. The visits were made seven days after the initial visit to the stream reaches. Micro-habitat sites were located and visually snorkeled to look for fish. After locating fish, the area was seined to collect blueside darters to better observe tags.

## **Results**

During the study period, measurements were taken at a total of 12 micro-habitat sites. All parameters were recorded during an initial visit; because of time restraints and equipment malfunctions, only several measurements were recorded during a follow-up visit. Because sites were only sampled where blueside darters were found, we could not show habitat suitability and correlations towards certain variables. However, the micro-habitat sites that were surveyed are illustrated using averages and confidence intervals ( $\alpha=.05$ ) where fish were located.

The membrane on the oxygen probe was ruptured in the YSI Model 85, which caused inaccurate dissolved oxygen readings for the first sample effort at Coulters Bridge. These data were excluded from the analysis. Dissolved oxygen ranged from 7.01

to 8.41 mg/L with the average dissolved oxygen from all sampling being  $7.539 \pm 0.187$  mg/L. Conductivity was taken only for the initial visit and ranged from 75.8 to 99.6  $\mu\text{S}/\text{cm}$  with an average of  $92.994 \pm 6.368$   $\mu\text{S}/\text{cm}$ . Temperatures ranged from 23.7 to 28.3 °C with an average of  $25.338 \pm 0.703$  °C; temperature from the lower stream section were warmer than those of the upper stream reach. The pH ranged from 7.07 to 8.56 with an average of  $8.040 \pm 0.135$ . Depth ranged from 20 to 60 cm with an average of  $34.739 \pm 4.35$  cm. Flow ranged from 0.068 to 1.340 feet per second with an average of  $0.376 \pm 0.184$  fps. The average substrate size was medium gravel containing a midpoint of 12mm. Sand was the most predominant substrate making up 16% of the substrate and cumulative fine substrates making up 44% of the substrate.

A total of 82 blueside darters were tagged during the first trip to all of the micro-habitat sites. During the follow-up visit, a total of 112 darters were captured. There were 27 recaptures which is a recapture rate of 0.329 fish.

Table 1. Substrate composition for all sites

<b>Substrate Analysis</b>			
<b>Substrate (Midpoint)</b>	<b>Type</b>	<b>Overall %</b>	<b>Cumulative %</b>
<b>Silt</b>	<b>Silt</b>	<b>13.0%</b>	<b>13.0%</b>
<b>1</b>	<b>Sand (Fine-Course)</b>	<b>15.7%</b>	<b>28.7%</b>
<b>3</b>	<b>Gravel (Very Fine)</b>	<b>9.7%</b>	<b>38.3%</b>
<b>6</b>	<b>Gravel (Fine)</b>	<b>6.0%</b>	<b>44.3%</b>
<b>12</b>	<b>Gravel (Medium)</b>	<b>8.0%</b>	<b>52.3%</b>
<b>24</b>	<b>Gravel (Course)</b>	<b>5.7%</b>	<b>58.0%</b>
<b>48</b>	<b>Gravel (Very Course)</b>	<b>12.7%</b>	<b>70.7%</b>
<b>91</b>	<b>Cobble (Small)</b>	<b>15.0%</b>	<b>85.7%</b>
<b>182</b>	<b>Cobble (Large)</b>	<b>7.3%</b>	<b>93.0%</b>
<b>363</b>	<b>Boulder (Small)</b>	<b>0.0%</b>	<b>93.0%</b>
<b>724</b>	<b>Boulder (Medium)</b>	<b>0.3%</b>	<b>93.3%</b>
<b>1445</b>	<b>Boulder (Large)</b>	<b>0.0%</b>	<b>93.3%</b>
<b>2884</b>	<b>Boulder (Very Large)</b>	<b>0.0%</b>	<b>93.3%</b>
<b>Bedrock</b>	<b>Bedrock</b>	<b>6.7%</b>	<b>100.0%</b>



Figure 1. Location of the lower stream reach and upper stream reach indicated by orange dots

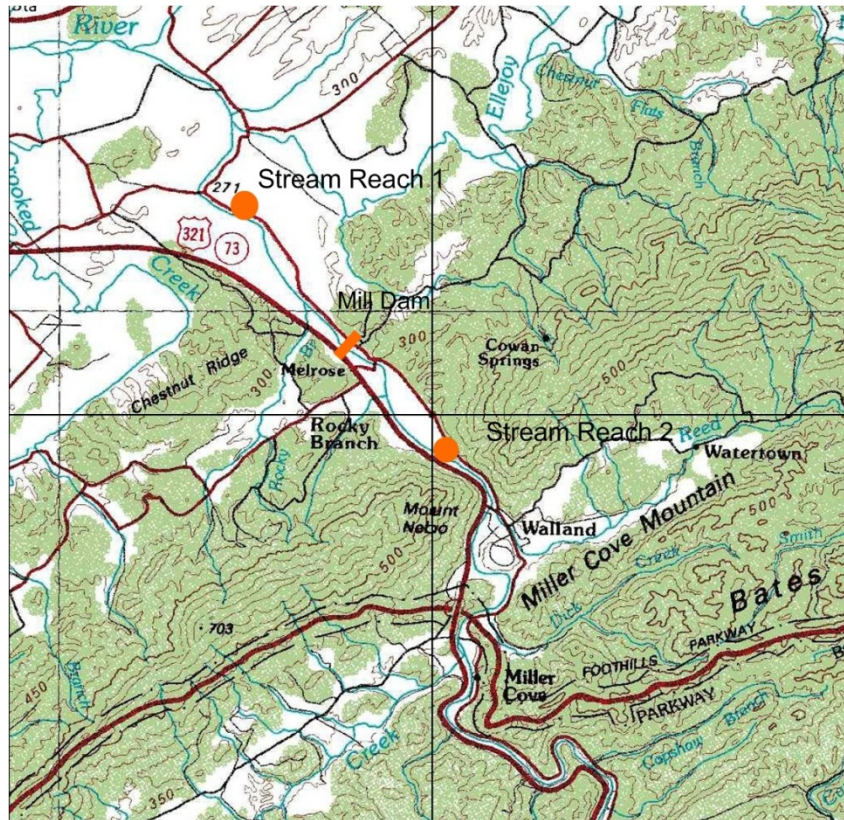


Table 2. Water parameter measurements

Initial Visit to Sites						
	Dissolved Oxygen (mg/L) * <sub>1</sub>	Conductivity (uS)	Temperature ( C )	pH	Depth(cm)	Flow (feet/second)
Average	7.744	92.944	26.058	8.172	32.833	0.441
Std Dev	0.568	9.747	1.861	0.311	12.576	0.424
95% CI	0.498	6.368	1.053	0.176	7.115	0.294
Follow-up Visit to Sites						
	Dissolved Oxygen (mg/L)	Conductivity (uS) * <sub>2</sub>	Temperature ( C )	pH	Depth(cm)	Flow (feet/second)
Average	7.426		24.378	7.864	36.818	0.289
Std Dev	0.331		0.429	0.237	8.134	0.234
95% CI	0.216		0.281	0.155	4.807	0.187
All Data for Sites						
	Dissolved Oxygen (mg/L)	Conductivity (uS)	Temperature ( C )	pH	Depth(cm)	Flow (feet/second)
Average	7.539	92.944	25.338	8.040	34.739	0.376
Std Dev	0.438	9.747	1.645	0.316	10.644	0.352
95% CI	0.187	6.368	0.703	0.135	4.350	0.184

\*1 - DO meter was broken during initial visit to the lower stream reach at Coulter's Bridge.

\*2 - Conductivity was only measured at during the initial visit

## Discussion

The analyses conducted for this project dealt only with locations where blueside darters were found. Because of this, we can only describe the general micro-habitat parameters surrounding the individuals we found, but cannot show preference for habitat. Thus we can only explore what variance occurred among the sites where blueside darters existed.

Blueside darters exist mostly in pools with slow moving water with mostly fine substrate. Some sites did have swifter flows with larger substrates, but were located adjacent to *Justicea* and *Elodea* beds which would slow the movement of water. Because smaller substrates tend to exist in slower water and not in swifter waters, it would be difficult to determine whether substrate or water flow was the defining habitat characteristic for the blueside darter in these locations. Blueside darters also maintained a fairly shallow depth with a maximum of 60 cm. A measurement not taken during the study, but noted in the field, was the relatively short distance to either shore or to an aquatic plant community.

Temperature varied between the two stream reaches by 3-4 °C. Because of seasonal variation, temperature will vary depending on timing of the year and will not limit micro-habitat selection, but needs to remain inside the tolerance levels of the darters. Conductivity differed between the two stream reaches with a lower conductivity in the upper stream reach; this indicated that ions are being added to the stream between the two sites, most likely in the form of runoff, and illustrates that blueside darters have some tolerance to conductivity changes. The average pH for all of the micro-sites was

slightly basic which is indicative of the limestone rock substrate in the area. Both of the stream reaches had comparable DO readings.

Blueside darters retained tags and experienced no short-term mortality due to the tagging. Previous attempts at reintroductions of blueside darters to the Pigeon River have failed and warranted a test trial of tagging to determine if the tagging procedure could have influenced the inability of the darters to re-establish in the river. A recapture rate of 0.329 was not a great success, but not low by any means. A study conducted by Roberts and Kilpatrick (2004) indicated a 0.11 recapture rate after 3.5 months. The lack of more recaptured darters could be attributed to emigration and inadequate sampling.

Further research could be done by examining another watershed in which blueside darters exist. This would allow comparison of water quality parameters. During preliminary snorkel surveys, blueside darters were located in the west prong of Little Pigeon River in Sevierville, TN, just below the intersection of The Great Smoky Mountains Parkway and Chapman Highway. This is the location for genetic stock to reintroduce to the Pigeon River, so it was not desired to conduct the initial portion of this study there in case mortality occurred from tagging. Visual observations did confirm that blueside darters in this drainage also preferred slow moving pools mostly dominated by fine substrate. The darters were also located next to *Justicea* beds at a depth similar to the Little River.

Because data have already been taken for areas where blueside darters exist in the Little River, habitat preference studies could be conducted. Based on a study by Osier and Welsh (2007), one could return to the stream reaches previously sampled, randomly select micro-habitat sites within the stream, and measure water quality and substrate

composition; this would indicate blueside darter preference towards certain variables over others.

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