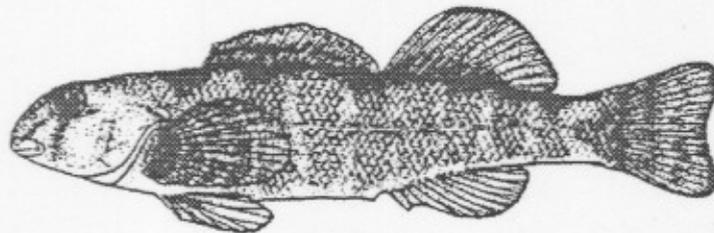


**The Candy Darter (*Etheostoma osburni*) in Stony Creek ,  
George Washington - Jefferson National Forest, Virginia**

**- Trout Predation, Distribution, and Habitat Associations -**



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**- Trout Predation, Distribution, and Habitat Associations -**

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## Introduction

Candy darters *Etheostoma osburni* are rare benthic riffle fish endemic to the New River drainage of Virginia and West Virginia. Candy darters are a species of special concern in Virginia and West Virginia (Williams et al. 1989) and are Category 2 on the Federal Endangered Species List (USF&WS 1989). Because of their rarity, Burkhead and Jenkins (1991) suggested that candy darters be considered for additional protection in Virginia.

Candy darters historically occurred in nine streams in Virginia (Burkhead and Jenkins 1991; Jenkins and Burkhead 1994). Current records, however, suggest that the species is restricted to Stony and Laurel Creeks in southwest Virginia (Burkhead and Jenkins 1991; Jenkins and Burkhead 1994). More recent surveys indicated that the species was well distributed in Stony Creek but was highly localized in Laurel Creek (near its confluence with Wolf Creek; Robert E. Jenkins, personal communication).

Stony Creek, located in Giles County, Virginia, is a fourth order tributary of the New River characterized by relatively cold water flowing over coarse substrate. Stony Creek probably contains the largest and most stable population of candy darters in Virginia (Bruenderman and Lookabaugh, unpublished report), in part because most of its watershed is undeveloped and lies within the George Washington-Jefferson National Forest (GWJNF). Potential threats to candy darters in Stony Creek include turbidity, siltation, and predation by stocked trout (Burkhead and Jenkins 1991).

Our study objectives were to 1) determine the extent of stocked trout predation on candy darters, 2) determine the distribution and relative abundance of candy darters in Stony Creek on the GWJNF, and 3) investigate relationships between candy darter abundance and stream habitat.

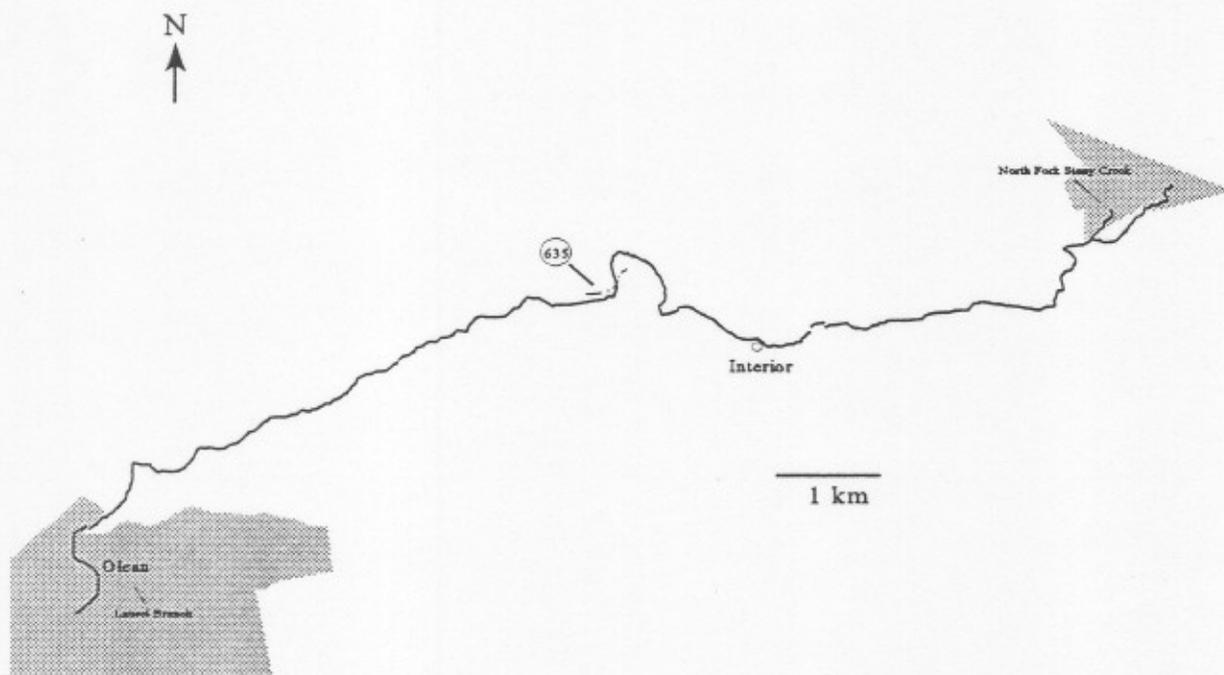
## Methods

*Candy Darter Predation* - On the opening day of trout season, 18 March 1995, personnel from the U.S. Forest Service (USFS), Virginia Department of Game and Inland Fisheries (VDGIF), and Virginia Polytechnic Institute and State University (VPI) collected stomachs from 421 recently stocked rainbow trout (*Onchorhynchus mykiss*) caught by anglers in Stony Creek. The study section began at the downstream USFS boundary and ended 9.7 river kilometers (rkm) upstream near Interior, VA (Figure 1). Stomachs were removed from fish and individually packaged with an identification label, placed on ice, and transported to VPI and stored in a deep-freezer. Stomachs were later examined by laboratory technicians and the contents were identified and preserved in a 70% ethanol solution. In addition, the stomachs of all trout captured during our 1995 Stony Creek electrofishing surveys were evacuated by displacement with filtered stream water delivered by a self-filling syringe (Meehan and Miller 1978). The stomach contents were preserved in a 70% ethanol solution and later examined in the laboratory.

*Habitat Survey* - Visual estimation techniques were used to estimate total surface area of selected habitat types and distribution of candy darters in Stony Creek between 15 August and 15 September 1995. Stony Creek was surveyed from the downstream boundary of the Jefferson National Forest, upstream about 13.8 rkm to the Glen Alton property line (Figure 1). Sampling strata were based on naturally occurring habitat units such as pools, glides, riffles, and runs:

- Pool - an area in the stream with low water velocity, streambed gradient near zero, and a smooth water surface.
- Glide - morphologically similar to pools but with swift flow through most of the unit.

- Riffle - an area in the stream with relatively steep gradient, shallow water, relatively high velocity, and turbulent surface .
- Run - morphologically similar to riffles but with rapid, non-turbulent flow.



**Figure 1.** Stony Creek on the George Washington-Jefferson National Forest, Virginia. Shaded areas indicate private land.

All habitat in the study section was classified and inventoried by a two-person crew. On the first pass through the study section one crew member identified each habitat unit by type, visually classified the dominant and subdominant substratum (using a modified Wentworth scale ranging from organic material to bedrock), and estimated surface area.

Another crew member estimated the average and maximum depth of each habitat unit. Average depth of each habitat unit was estimated by taking depth measurements at various places across the channel profile with a graduated staff marked in 0.05-m increments. The length (0.1

m) of each habitat unit was measured with a hip chain.

The first unit of each habitat type selected for intensive sampling (e.g. accurate measurement of surface area, diver estimation of fish species and distribution) was determined randomly; additional units were selected systematically. Selected habitat units were measured and marked with an identification flag at the upper and lower boundaries. The width of these systematically selected habitat units was measured with a 15-m measuring tape at intervals ranging from about 3 m to 5 m. Interval size was determined by the length and the morphology of the unit (i.e. intervals of measured widths increased with increasing unit length and complexity).

The relationship between the estimated surface area and the measured surface area of each habitat type in the study section was first determined by simple linear regression because the visual estimation techniques developed by Hankin and Reeves (1988) for estimating total habitat area are based on the assumption that visual estimates and measurements are strongly and positively correlated. If these assumptions are met, then visual estimates can be corrected by calculating a calibration ratio. The calibration ratio ( $\hat{Q}$ ), the estimated true total area ( $\hat{M}$ ), and the variance of the area estimator  $\hat{V}(\hat{M})$  were calculated separately for each habitat type.

*Underwater Survey* - Underwater observations were made in systematically selected habitat units (underwater observations were in 39 riffles, 39 runs, 35 glides, and 32 pools). When a sample unit was encountered, two observers, using face masks and snorkels, started at the downstream end and proceeded slowly upstream to the head of the unit while searching for candy darters. Every rock large enough to provide cover was flipped or jarred (when possible) in an attempt to locate all fish.

*Electrofishing Survey* - Candy darter densities (number per 100 m<sup>2</sup>) were determined in systematically selected snorkeled habitat units (11 riffles, 12 runs, 11 glides, and 11 pools) by three-pass removal using two AC backpack electrofishing units (Zippin 1958). The upper and lower boundary of each of the selected habitat units were blocked with two 1 X 5 meter, 6 mm mesh nets to prevent immigration and emigration of fish during the three-pass removals. All fish captured during the three-pass depletions were identified and candy darters and salmonids were weighed (g) and measured (mm).

*Statistical Analyses* - We used a Spearman Rank Order Correlation to examine associations between candy darter densities and individual ecological attributes. Variables used in the analyses were habitat type, substrata (dominant and subdominant), longitudinal position, relative abundance of sympatric species, and maximum depth. Average and maximum depth were highly correlated ( $p < 0.001$ ,  $r = 0.93$ ); thus, only maximum depth was used in the analyses. Significant correlations between candy darter densities and ecological attributes were further investigated using either Kruskal-Wallis One Way ANOVA on Ranks or Chi-Square Goodness-of-Fit tests.

## **Results and Discussion**

*Candy Darter Predation* - No darter remains were observed in any of the salmonids captured during either the angler or electrofishing survey. Of the 421 rainbow trout stomachs taken from Stony Creek anglers less than five percent contained fish remains none of which were from percids. The stomach contents of eight brown trout (*Salmo trutta*), four rainbow trout, and two brook trout (*Salvelinus fontinalis*) captured during electrofishing surveys were also examined. Only one rainbow trout contained fish (cyprinid) remains.

Our results show no direct effect on the Stony Creek candy darter population from predation by newly stocked rainbow trout. These results, however, do not suggest that predation by salmonids does not occur in Stony Creek. The data collected from Stony Creek represents one point in time; two weeks after the trout were stocked. Hatchery trout are accustomed to an abundant food source that can be obtained with minimum effort and therefore are probably less successful foragers than wild trout (Klak 1940). Further, LaRoche (1979) observed limited feeding activity in rainbow trout stocked in two central Virginia streams.

Second, darters have been observed to overwinter under the cover of stones and woody debris in deep water habitats; thus, candy darters may not have been available between the stocking date and opening day of trout season. This postulation is supported by an underwater reconnaissance in Stony Creek conducted about two weeks before opening day in which no candy darters were observed (Michael Bye, Graduate Research Assistant, Per. Comm.).

The effect of hatchery trout predation on candy darters in Stony Creek is still unresolved. Our limited data provides little insight into predation threats posed by acclimated trout. Further, we believe that, given the opportunity, trout will prey on this and similar species. For example, one of three brown trout collected during a preliminary survey in Stony Creek contained candy darter remains (Leftwich et al. 1994). Likewise, Jenkins and Burkhead (1994) reported that the closely related Roanoke darter (*Percina roanoka*) are eaten by stocked rainbow trout. A more comprehensive study is needed to determine the threat posed by trout to the candy darter.

Nevertheless, two weeks before opening day of trout angling season in 1995, 23,200 rainbow trout were stocked in Stony Creek. We estimate the surface area of the stocked section to be about 84,000- m<sup>2</sup>. Thus, the stocking density for Stony Creek, 1995, would be about 28 rainbow trout per 100- m<sup>2</sup>. However, trout densities averaged less than one per 100 m<sup>2</sup> in our

electrofishing sites. Rainbow trout densities ranged 0 - 3 per 100 m<sup>2</sup>, brown trout 0 - 5 per 100 m<sup>2</sup>, and brook trout 0 - 2 per 100 m<sup>2</sup>. This suggests that survival of hatchery rainbow trout in Stony Creek is relatively low. Our data indicates only about a 4% over-summer survival for stocked rainbow trout in Stony Creek in 1995. Stony Creek has a long history of trout stocking with no apparent effects on the candy darter population; although prestocking data is limited. This may be partially due to a poor survival rate of hatchery fish in Stony Creek.

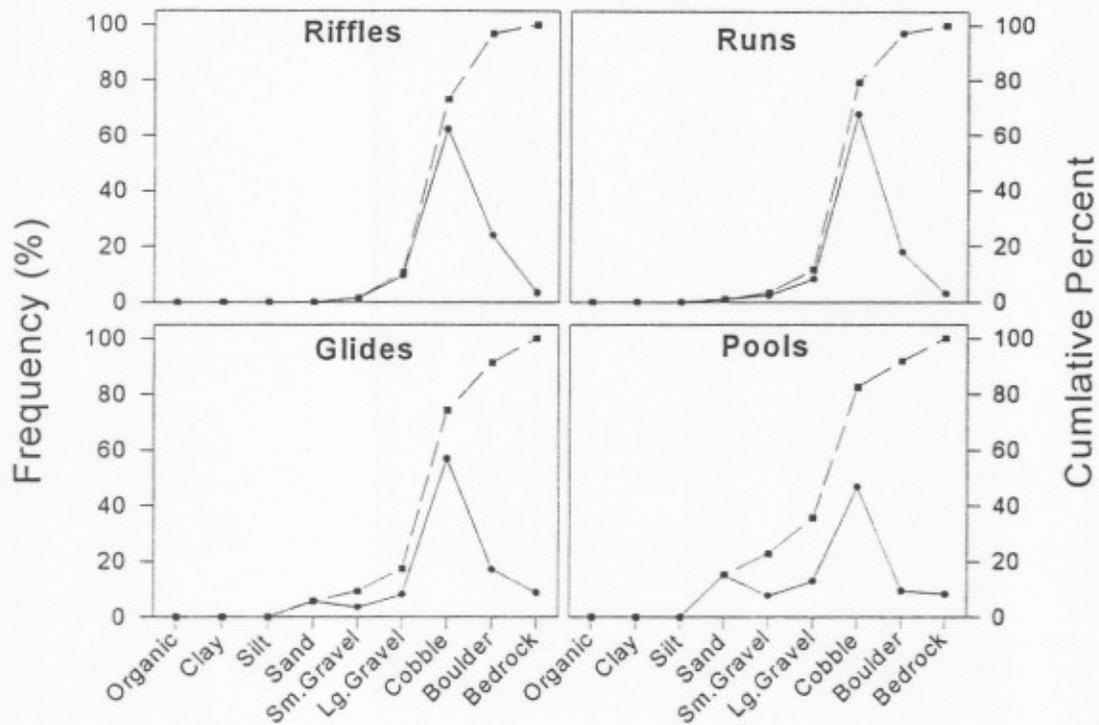
*Habitat Survey* - We identified 172 pools, 346 glides, 208 runs, and 216 riffles in the 13.8 km study section. Visual estimates of habitat area were paired with measured habitat area for 34 (20%) pools, 62 (18%) glides, 38 (18%) runs, and 38 (18%) riffles. Paired observations for all habitat types were highly correlated ( $r > 0.97$ ,  $p < 0.0001$  in all cases). Total area was estimated for each habitat unit using correction factors ( $Q$ ) that ranged from 0.97 to 1.03 (Table 1).

**Table 1. - Total number of habitat units (N), number of units measured (n), degrees of freedom (Df), estimated total habitat area ( $\bar{M}$ ), estimated variance of the estimated total habitat area ( $\hat{V}(\bar{M})$ ), and 95% confidence intervals (C.I.) for the estimated habitat area for the Stony Creek study area.**

Habitat type	N	n	df	$\bar{M}$ (m <sup>2</sup> )	$\hat{V}(\bar{M})$	95% C.I.
Pools	172	34	39	27564.2	173165.7	± 1403.6
Glides	346	62	34	28140.0	49800.3	± 833.8
Runs	216	38	30	32019.7	69081.3	± 1997.4
Riffles	208	38	16	21757.8	103858.4	± 1373.9

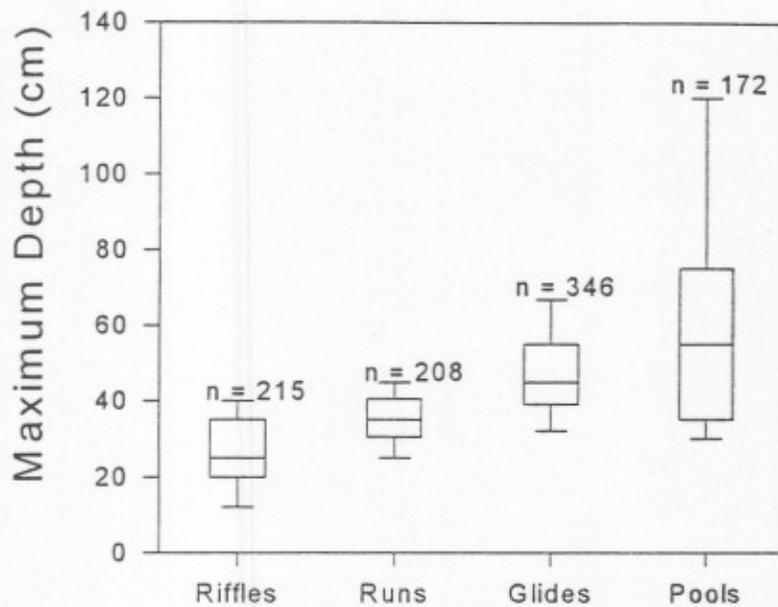
Stony Creek substrata is primarily cobble which accounted for a minimum of 48% of the observations in pools to a maximum of 69% of the observations in runs (Figure 2). Maximum

depths ranged from 8-cm in riffles to 200-cm in pools (Figure 3).

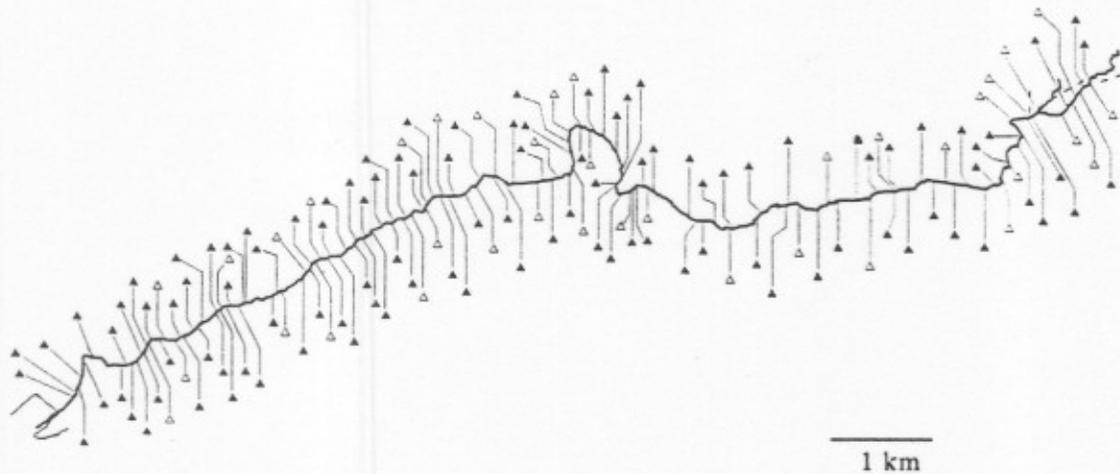


**Figure 2.** Dominant substrate composition by habitat type in Stony Creek. Dots represent frequency and squares represent cumulative percent.

*Species Distribution* - Candy darters are widely distributed throughout the Stony Creek study area (Figure 4). Although we observed candy darters in 74% of the habitat units we sampled, the percentage of candy darters observed varied by habitat type. We observed at least one candy darter in 90% of the runs, 82% of the riffles, 79% of the glides, and 41% of the pools that we sampled. Although we sampled a greater number of sites, the overall distribution of candy darters in Stony Creek did not differ from that reported by Jenkins and Burkhead (1994).



**Figure 3.** Box plots for habitat unit depth in Stony Creek. The box encloses the middle 50% of the observations, the capped lines below and above the box represents the 10% and 90% quantiles, respectively, and the solid line in the box represents the median. Sample sizes are denoted by n.

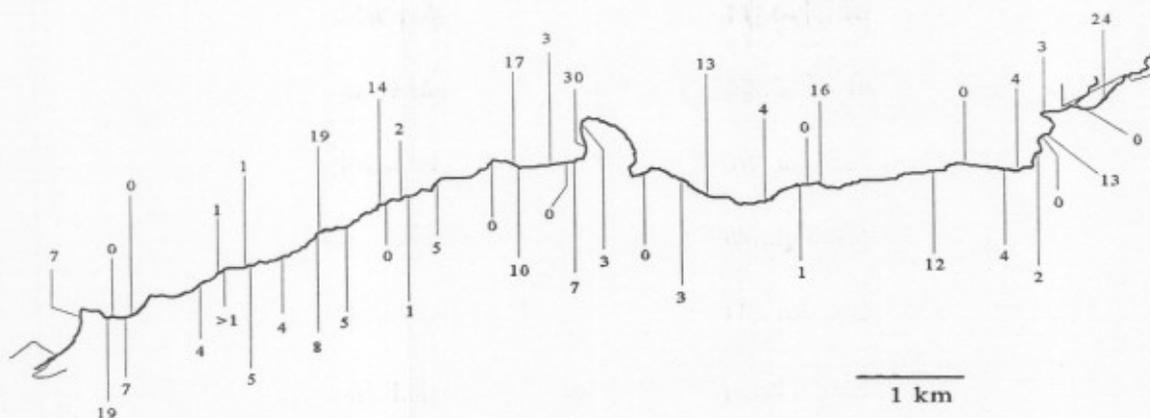


**Figure 4.** Location of underwater observations sites. Solid triangles represent sites where candy darters were present and open triangles represent sites where the species was not observed.

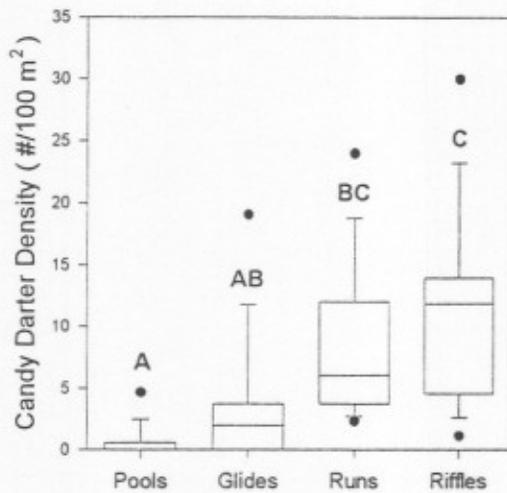
*Species Abundance and Habitat Associations* - Candy darter densities ranged from 0 - 30/100m<sup>2</sup> in the electrofishing sites (Figure 5). There is significant evidence that candy darter density differs

among habitat types (ANOVA on Ranks,  $df = 3$ ,  $p < 0.0001$ ). The mean density of candy darters observed was highest in riffles ( $\bar{x} = 10/100m^2$ ) and significantly different (Dunn's Test,  $p < 0.05$ ) from pools ( $\bar{x} = 2/100m^2$ ) and glides ( $\bar{x} = 4/100m^2$ ; Figure 6). Our habitat classification is ordinal in nature ranging from slow moving deep water habitat (pools) to fast moving shallow water habitat (riffles). If we assume that habitat type is a surrogate for flow and depth, then it appears that candy darters are positively associated with shallow habitats with high velocities.

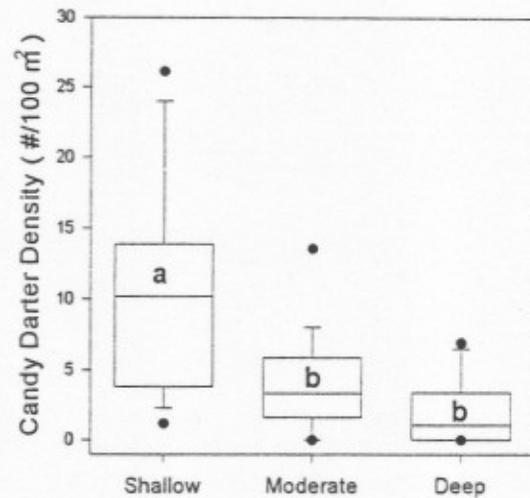
As predicted, we observed statistical differences between candy darter densities according to habitat unit depth (ANOVA on Ranks,  $df = 2$ ,  $p = 0.0002$ ). The mean density of candy darters were highest in shallow ( $\leq 22$  cm) habitats ( $\bar{x} = 10.9/100m^2$ ) and significantly different (Dunn's Test,  $p < 0.05$ ) from densities in moderate (23 - 42 cm,  $\bar{x} = 4.2/100m^2$ ) and deep ( $> 30$  cm) habitats ( $\bar{x} = 2.0/100m^2$ ; Figure 7).



**Figure 5.** Locations of electrofishing sites in Stony Creek. Numbers represents candy darter densities (number per 100 m²).



**Figure 6.** Box plots for candy darter densities by habitat type in Stony Creek. The box encloses the middle 50% of the observations, the capped lines below and above the box represents the 10% and 90% quantiles, respectively, the dot below and above the vertical bar represents the 5% and 95% quantiles, and the solid line in the box represents the median. Box plots with the same letters are not significantly different (ANOVA,  $p < 0.05$ ).



**Figure 7.** Box plots for candy darter densities by habitat depth in Stony Creek. The box encloses the middle 50% of the observations, the capped lines below and above the box represents the 10% and 90% quantiles, respectively, the dot below and above the vertical bar represents the 5% and 95% quantiles, and the solid line in the box represents the median. Box plots with the same letters are not significantly different (ANOVA,  $p < 0.05$ ).

Candy darter density and dominant substratum were positively correlated ( $r = 0.90$   $p < 0.01$ ).

But because Stony Creek substrata is primarily coarse, the dominant substrata in our systematically selected sample sites were primarily coarse. To test statistical relationships between substrata and candy darter densities without violating test assumptions, we divided the substrata categories into two groups: boulder and nonboulder. Chi-square test showed that densities were higher than expected in riffles that were predominantly boulder ( $df = 1$ ,  $p < 0.05$ ). The relationship between candy darter densities and substrata in all other habitat types was not significant ( $p > 0.05$ ).

The observed relationships between candy darter densities and habitat variables are based on the overall characteristics of individual habitat units. At this level of resolution (mesohabitat) we

observed positive relationships between density and swift flows, relatively shallow depths, and coarse substrata. Chipps et al. (1994) observed similar relationships for candy darters in West Virginia streams where they measured habitat variables at a finer resolution (microhabitat) than in this study. The similar findings of these two studies, conducted at different scales, suggest that fast moving, shallow water areas and large stones are important habitat for adult candy darters.

The data collected in our habitat survey show that Stony Creek contains an abundance of suitable habitat for adult candy darters. Because a large portion of Stony Creek lies within the GWJNF, it is also the only stream in Virginia that has both a viable population of candy darters and protection from most agricultural and industrial perturbations.

The strongest correlation between candy darter densities and the densities of any other sympatric species was with fantail darters ( $p < 0.005$ ,  $r = 0.65$ ). In general, candy darter densities were positively associated with riffle species densities and negatively associated with pool species densities; however, the correlations were poor and accounted for less than 22% of the variation in all cases.

Similar associations between candy darters and fantail darters have been observed in West Virginia Streams (Chipps et al. 1994). The significance of this relationship is unknown but suggest that these species prefer similar microhabitats.

### **Conclusions and Recommendations**

The absence of candy darters in the diet of trout collected in this study, the apparent poor survival rate of rainbow trout, and the history of stocking in Stony Creek suggest that a put-and-take fishery and the protection of candy darters in Stony Creek may not be mutually exclusive. Brown trout, however, tend to be more piscivorous than rainbow trout; thus, the VDGIF has

limited the stocking of Stony Creek to rainbow trout in an effort to minimize the threat to candy darters. Due to the popularity of the Stony Creek put-and-take fishery, we recommend that this protocol be followed until 1) a more comprehensive study is conducted to determine the extent and effect of trout predation on candy darters, and 2) candy darter populations can be established in other streams in Virginia within in their historical range.

The results of this study and that of Chipps et al. (1994) suggest that adult candy darters may prefer swift, shallow habitats with large stones; habitats that are common in Stony Creek. Little is known, however, about the habitat requirements of juvenile candy darters or the conditions necessary for successful spawning. We believe these gaps in our knowledge warrant investigation before we can truly understand 1) the reasons this species has declined in Virginia and 2) what efforts are needed to ensure protection of the remaining populations. We also suggest that the potential impact of management activities such as recreation, road improvement, and timber harvest in the Stony Creek watershed be considered before implementation.

Finally, efforts are underway at VPI to propagate candy darters in the laboratory for ultimate translocation to streams within the historic range. We recommend that habitat surveys, similar to those used in this study, be conducted and compared to the Stony Creek data to maximize successful colonization.

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Appendix A. Length and weight of candy darters captured in Stony Creek, Virginia, September 1995.

Sample size = 241.

