

MICROHABITAT USE BY BLACKBANDED (*PERCINA NIGROFASCIATA*), TURQUOISE
(*ETHEOSTOMA INSCRIPTUM*), AND TESSELLATED (*ETHEOSTOMA OLMSTEDI*)

DARTERS IN A PIEDMONT STREAM

by

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Under the Direction of Gary D. Grossman

ABSTRACT

We quantified factors affecting microhabitat use for three darter species in a 116m reach of a Piedmont stream, via underwater observation and macroinvertebrate benthos samples during 2001-2002. The 12 month period of sample collections, and the previous 12 months were the first and second driest years on record, respectively. PCA indicated that in Spring 2002, a season with higher amounts of habitat availability, there existed fewer significant differences in darter microhabitat use, whereas in Autumn 2001 and Summer 2002 all darters used significantly different microhabitats. In general, turquoise darters used more scour areas, tessellated darters more depositional, and blackbanded darters utilized intermediate habitats. During Autumn 2001, large blackbanded darters ($\geq 55\text{mm}$) occurred in faster microhabitats with more erosional substrata than smaller specimens, and used a greater variety of depths. In Autumn 2001, large turquoise darters ($\geq 40\text{mm}$) used deeper microhabitats with higher velocities and more erosional substrata than smaller individuals, whereas the opposite was true for Autumn 2002. Blackbanded darters used microhabitats with significantly less macroinvertebrate biomass and abundance in Autumn 2001, but with higher percentages of Dipterans. In Spring 2002, all three

darters occurred in microhabitats with significantly lower macroinvertebrate biomass and numerical abundance (blackbanded and tessellated only) than found at random. We observed no interspecific differences in biomass, numbers, or percent Dipteran composition, nor any size related differences in macroinvertebrate abundance in microhabitats occupied by darters.

INDEX WORDS: Microhabitat Use, *Percina nigrofasciata*, *Etheostoma inscriptum*, *Etheostoma olmstedi*, Macroinvertebrate, Diptera, Length-related Microhabitat Use, Principle Component Analysis,

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CHAPTER 1

INTRODUCTION

Fish are the most abundant and perhaps least well understood class of vertebrates on earth (Maitland 1995). Approximately, 20% of all freshwater fishes are threatened with extinction, which increases at an estimated 4% per decade in North America (Ormerod 2003). Only 2% of US rivers are of high enough quality to be worth federal protection, due in part to land alteration and water development projects which affect approximately 85% of species in the threatened/endangered categories (Tockner and Stanford 2002).

The southeastern (SE) United States supports the highest diversity of freshwater fishes in North America. Of approximately 800 freshwater fish species in the United States, over 60% are found in the Southeast (Burkhead et al. 1997, Warren et al. 1997). Furthermore, over 200 of the named and unnamed species and subspecies in this region are lotic darters (Percidae: Etheostomatinae, Warren et al. 2000), many of which have restricted distributions (Folkerts, 1997, Warren et al. 2000). Despite their high diversity, approximately 34% of the Etheostominae are classified as threatened, endangered, or vulnerable species (Warren et al. 2000). We quantified factors affecting microhabitat use for three darter species because darter habitat and diet requirements are not fully understood, many darters are imperiled, and because recent literature suggest prey abundance may be a better predictor of microhabitat use than physical variables.

CHAPTER 2

LITERATURE REVIEW

Stream Habitat Degradation and Its Effects on Fish

Streams provide a variety of habitat needs for fishes including foraging habitat, reproductive habitat, and shelter from predators. Like other aquatic fauna, fish assemblages are affected by habitat degradation (Matthews 1998). Stream fishes are especially sensitive to habitat degradation, because freshwater is limited, hydrologic inputs are easily disrupted, and landscape alterations strongly influences water quality in streams. Human activity often is responsible for stream degradation. I will discuss a variety of processes that contribute to stream degradation below.

Nutrient enrichment in soils and water bodies often results in eutrophication which may negatively influence fish assemblages (Wade et al. 2002). Eutrophication is a major problem affecting freshwater ecosystems in Europe (Droic et al. 2001; Wade et al. 2002), Asia, (Rose et al. 2004; Dudgeon 1992; Dudgeon 2005), Africa (Koning and Roos 1999), Australia (Sonneman et al. 2001), North America (Maitland 1995; Ormerod 2003) and South America, (O'Ferrell et al. 2002; Agostinho et al. 2005; Olguin et al. 2004; Salibian 2005; Mugetti et al. 2004; Silvano and Begossi 1998). Nutrient enrichment results from two primary processes, domestic/industrial wastewater and agriculture. Domestic and industrial waste water deliver high amounts of Nitrogen and Phosphorous compounds to surface waters (Khan and Ansari 2005; Koning and Roos 1999; Sonneman et al. 2001; Ormerod 2003). Intensive agriculture practices that utilize fertilizers also contribute to nutrient enrichment via over-application or loss due to overland or

subsurface flow (Jha et al. 2005; Smith et al. 2005). In addition, if nutrients are sorbed to sediment particles, they can be transported to streams via sediment erosion (Smith et al. 2005).

Anthropogenic inputs of heavy metals also may negatively affect fish assemblages. The dominant sources of heavy metal inputs in rivers are domestic and industrial effluents, and mining activities. Resulting heavy metal contamination is widespread in Europe (De Vivo et al. 2001; Swennen et al. 1998; Fialkowski et al. 2003), Asia (Rose et al. 2004; Sekhar et al. 2005; Singh et al. 2005; Kannan et al. 2005), Africa (Nriagu 1992; Coetzee et al. 2002; Bell et al. 2002), Australia (Churchill et al. 2004; Sloane and Norris 2003), North America (Maitland 1995; Ormerod 2003), and South America (O’Ferrell et al. 2002; Agostinho et al. 2005; Olguin et al. 2004; Salibian 2005; Mugetti et al. 2004; Silvano and Begossi 1998; Gray et al. 2002; Mol et al. 2001; Smolders et al. 2003). Many heavy metals persist in the environment for decades (Coetzee et al. 2002), as indicated by the prevalence of increased amounts trace metals in surface waters (Singh et al. 2005, Kannan et al. 2005; Nriagu 1992; Mugetti et al. 2004, Churchill et al. 2004), soils (De Vivo et al. 2001), and sediments (De Vivo et al. 2001; Swennen et al. 1998) after inputs have ceased. Churchill et al. (2004) document an Australian catchment with no mining activity since the 1850’s, where one stream continues to exhibit seasonal elevations in mercury concentrations that surpass standards set by the Australian and New Zealand Environment and Conservation Council for safe water use and healthy aquatic assemblages. In addition, metals concentrate at multiple trophic levels (Sekhar et al. 2005), commonly detected in lichens and trees (De Vivo et al. 2001), invertebrates, (De Vivo et al. 2001; Fialkowski et al. 2003), and fish (Mol et al. 2001; Malm 1998). Suspended solids serve as an important vehicle for transport of metals such as mercury (Gray et al. 2002; Mol et al. 2001). Rates of metal contamination

increase with increased catchment urbanization and industrialization (Rose et al. 2004; Nriagu 1992; Agostinho et al. 2005; Silvano and Begossi 1998).

Heavy metal and pesticide contamination negatively influence fish and prey assemblages in streams. Environmental exposure to pesticides is widespread, and pesticide toxicity to aquatic fauna is reflected in decreased animal abundance, increased animal mortality, a shift in assemblage structure to toxicant-tolerant species; the presence of muscle cholinesterase which causes uncontrolled muscular contractions, and fish die-offs. The deleterious effects of heavy metal contamination can be produced by acute events, for example, a single rain event produced pollutant levels in a South African river that were toxic to invertebrates and fishes (Shultz 2004). Increased pesticide concentrations and turbidity associated with agriculture were shown to reduce the number of taxa (decrease species richness) in a benthic invertebrate assemblage, resulting in an assemblage lacking sensitive species (Thiere and Schultz 2004). Benthic invertebrate studies in a North African stream revealed depauperate invertebrate assemblages with regard to species diversity and abundance (Arab et al. 2004). Changing levels of pollution were strongly related to varying invertebrate abundance along their longitudinal distribution (Arab et al. 2004). Invertebrate assemblages in polluted streams in Australia (Sloane and Norris 2003) ranged from severely impaired to not significantly different from reference sites. Seventy percent of the variation between observed versus expected (O/E) taxa ratios was explained by sediment and water metal concentrations, and pH, indicating these factors negatively effect aquatic invertebrate assemblages (Sloane and Norris 2003).

In streams influenced by acid mine drainage, investigators often observe aquatic assemblages which have been deleteriously impacted by decreases in pH and increases in trace metal concentrations in surface waters and stream sediments. Application of a sediment

transplant experiment revealed that toxic sediments had little effect on aquatic invertebrates, whereas an invertebrate assemblage remained depauperate when non-toxic sediments were implanted in a stream with high metal concentrations (Battaglia et al. 2005). These results suggest that transport of pollutants by water or atmospheric deposition are the causal agents behind the low diversity in the affected systems. In the Pilcomayo River in South America, which is highly polluted with heavy metals from mining activities, most aquatic invertebrates were extirpated with Chironomids representing 97% of the benthic macroinvertebrate abundance (Smolders et al. 2003). Guyonnet et al. (2003) showed that size of the pollution tolerant fish *Ethmalosa fimbriata* was negatively correlated with increasing levels of pollution in three lagoons along Africa's Ivory Coast. In addition, fish diversity and assemblage structure decreased with increasing pollution (Guyonnet et al. 2003). Vertebral grow rate analyses showed that populations of the catfish *Clarias gariepinus* suffered decreased fitness after reaching maturity, caused by insufficient water flow, over-harvesting, and pollution (Yalcin et al. 2002). A survey of Suriname rivers indicated that where mining-related increased concentrations of mercury were present, samples of fish flesh, especially piscivorous species, contained higher total mercury concentrations than samples taken from unpolluted waters (Mol et al. 2001). Amounts of mercury in fish specimens clearly reflect levels of surface water contamination throughout the Brazilian Amazon basin (Malm 1998). Coetzee et al (2002) found that levels of metals in fishes respond quickly (within a few months) to changing levels in the environment, and that fishes absorb metals through gills and skin, and also from eating contaminated prey.

Increased sediment erosion and its detrimental effects on aquatic habitats is a main threat to streams and fish assemblages throughout the world. The negative effects of sedimentation

from anthropogenic sources have been observed in Europe (Smith et al. 2005; Haigh et al. 2004; Remondo et al. 2005), Asia (Dudgeon 1992, 2005), South America (Agostinho et al. 2005; Mugetti et al. 2004), and North America (Maitland 1995; Waters 1995). Poor agricultural management, destruction of riparian zones, and road and building construction lead to excess loss of sediment via erosion (Waters 1995). These effects increase stream turbidity and alter substratum composition, which negatively affect fish and macroinvertebrate assemblages (Culp et al. 1986; Sutherland et al. 2002). Sedimentation may be the most important factor affecting threatened/endangered fishes in the SE United States (Walsh et al. 1995; Sutherland et al. 2002).

Hydrological alteration of lotic systems via impoundment constitutes a major problem facing riverine ecosystems and their fish assemblages world wide (Ormerod 2003; Tockner and Stanford 2002; Petts 1990). Impoundments change streams from lotic to semi-lentic or lentic systems. Riparian areas and adjacent wetlands are lost along with their filtering capacity for sediments and pollutants flowing overland (Mugetti et al. 2004, Ormerod 2003; Maitland 1995). The local heterogeneity in terms of current velocity, depth, and substrata composition exhibited by most streams is homogenized (Dudgeon 1992, 2005; Tockner and Stanford 2002). The volume and timing of natural flooding and biogeochemical cycles are disrupted (Dudgeon 1992, 2005; Petts 1990), as are sediment transport patterns (Ormerod 2003).

Hydrological regulation also negatively influences invertebrate assemblages. Dams change river hydrology, channel morphology, and water quality. Dams also block fish passage, reduce connectivity to floodplain habitats, and alter aquatic plants and invertebrate assemblages, all of which impact fish assemblages (Petts 1990 Dudgeon 1992, 2005; Petts 1990; Ormerod 2003; Maitland 1995). Within the La Plata River basin, significant decreases in fish biodiversity and fish kills have resulted from various types of pollution, reservoir operation, and over-

exploitation. Many examples exist of altered fish and invertebrate assemblage structure after river impoundment, almost always accompanied by significant loss of native taxa (Mugetti et al. 2004). Silvano and Begossi (1998) observed decreases in migratory catfish catches and deleterious changes in fish assemblage structure related to impoundments, pollution, and degradation related to deforestation and catchment urbanization. Aarts et al. (2004) cite the loss of river channel and floodplain habitat, hydrologic regulation, and decreased floodplain-river connectivity as factors preventing the restoration of European large river fisheries, despite marked decreases in water pollution. Alternatively, the Pilcomayo River, Bolivia, remains in its natural state, which supports stable migratory fish populations (sabalo, measured in fish catch) despite metal pollution in suspended sediments. This pollution is naturally mitigated by dilution with clean eroded particle inputs, characteristic of a river with naturally high sediment loads (Smolders et al. 2002).

Habitat Use

Stream habitats are patchy mosaics with varying physical and chemical characteristics (Robinson 1986, Smale and Rabeni 1995, Petty and Grossman 1996; Poff et al. 1997, Thompson et al. 2001, Wehrly et al. 2003). A variety of factors affect the use of microhabitat (characteristics at the position of a fish) by stream fishes including: physiological constraints, intra- and inter-specific interactions, predation, and food availability.

Physico-chemical characteristics such as water temperature, dissolved oxygen levels (DO), current velocity and discharge, and substrata composition all may influence habitat use by fishes in streams (Matthews 1998). Changes in thermal and dissolved oxygen regimes may restrict fish species from otherwise suitable areas (Baltz et al. 1982, Feminella and Matthews 1984, Wehrly et al. 1984). In addition, changing dissolved oxygen levels influence seasonal and

diel fish habitat use (Suthers and Gee 1986, Kramer 1987). Alternatively, small warm thermal refugia are critical habitat components for some stream fishes during harsh winter conditions (Peterson and Rabeni 1996, Raibley et al. 1997), although acute increases in water temperature from sources such as power plant effluent may be detrimental to stream fishes (Matthews 1998). Both models and empirical studies indicate that stream discharge also may affect habitat use and survivorship of stream fishes (Horwitz 1978, Grossman et al 1998; Peterson and Kwak 1999).

Substratum composition greatly influences stream fishes, especially benthic foragers and spawners (Page 1983, Quinn et al. 1992, Warren and Burr 1994, Burkhead et al. 1997). Reduced substratum heterogeneity is strongly associated with decreased species richness and abundance within stream fish assemblages (Berkman and Rabeni 1987; Waters 1995), especially in the southeastern U.S. (Folkerts 1997; Warren et al. 2000)..

Current velocity influences habitat use in many lotic fishes, especially water-column species, in that they must balance the energetic cost of holding position in a current against the benefits of drifting food which typically increases with increasing velocity (Merritt and Cummins, 1984; Grossman et al. 2002). The relationship between prey capture success and current velocities may be the greatest factor influencing habitat selection in water column fishes, and species from several families select microhabitats (i.e., focal point velocities) on the basis of maximizing net energy gain (Hill and Grossman, 1993; Grossman et al. 2002).. In contrast, many benthic stream fishes possess behavioral and morphological adaptations that enable them to occupy high velocity microhabitats with little apparent cost (Facey and Grossman 1992; Matthews 1998).

Resource limitation and intraspecific competition also may influence habitat use among stream fishes. As the density of conspecifics increases, organisms often position themselves

within habitats so that the profit for each individual across habitats is equal. This phenomenon, the ideal free distribution (Fretwell and Lucas 1970, Fretwell 1972), has been documented in stream fishes, such as catfish (Power 1984). Intra-specific competition also influences habitat use via size-related interactions. For example, juvenile black surfperch (*Embiotoca jacksoni*) forage for invertebrates in foliose algae, whereas adults feed in algal turf covering rocks (Holbrook and Schmitt 1984, Wootton 1998). In addition, adult mottled sculpin (*Cottus bairdi*) exclude juveniles from high quality patches in a southern Appalachian stream which plays a strong role in density-dependent regulation in this species (Petty 1998; Grossman et al 2006).

Resource limitation also may result in interspecific competition, denoted by a decrease in fitness due to the presence of competitors (Greenberg 1988, Wootton 1998). Evidence for competition is often expressed via a niche shift, after removal of a dominant competitor (Wootton 1998). In Michigan streams with stable flows, resting positions are a limiting resource for salmonids. Fausch and White (1981) defined resting positions in terms of velocity and use of shade. Introduced brown trout (*Salmo trutta*) were competitively dominant over native brook trout (*Salvelinus fontinalis*) but when the former was removed the latter species shifted into the resting positions previously occupied by brown trout. Inter-specific competition may be the reason that brook trout often display decreased abundance, growth, and lifespan in the presence of brown trout (Fausch and White 1981). A similar relationship was identified between brook trout and introduced coho salmon (*Oncorhynchus kisutch*) when salmon were removed (Fausch and White 1981). In addition, riffle sculpin (*Cottus gulosus*) and speckled dace (*Rhinichthys osculus*) exhibited segregation in riffles along stream sections in Deer Creek, California (Baltz et al. 1982). Quantification of niche parameters revealed high overlap with regard to depth, current velocity, and substrata preference, as well as moderate dietary overlap. Laboratory

experimentation demonstrated that riffle sculpin were intolerant of higher temperatures found in the lower reaches of Deer Creek, whereas they competitively excluded speckled dace from cooler riffles (Baltz et al. 1982). Finally, redbase shiners (*Richardsonius balteatus*) were introduced into British Columbia lakes where they competed with rainbow trout (*Oncorhynchus mykiss*) for prey resources. Redside shiners foraged closer to areas of high prey density, foraged among rather than over vegetation, and fed on smaller prey items which potentially reduced the number of prey growing large enough for trout utilization. Dietary shifts to alternate prey types occurred in both species, which probably decreased inter-specific competition between them (Johannes and Larkin 1961).

Darters and Habitat Selection

Darters are benthic insectivores commonly found throughout streams of the southeast and eastern seaboard (Page 1983, Folkerts et al. 1997, Warren et al. 1997, 2000). Darters exhibit high species diversity, including 26 new species described since 1990, with at least 43 extant undescribed species (Burkhead and Jelks, 2000). Many darters display specialized food and habitat requirements that are often linked to the substratum of their native streams (Page 1983). Gause's Principle states that two species with the same niche (identical requirements) cannot coexist indefinitely (Ricklefs 1990), Yet, darters regularly coexist with many closely related species (Page 1983; Warren et al. 2000). In many cases differences in resource use are apparent, however the general relationship between these differences in resource use and coexistence is unknown for most coexisting darters (food use - Smart and Gee, 1979; Greenberg 1991; van Snik Gray et al., 1997; substratum use - Greenberg 1991; Kessler and Thorp, 1993; Chipps et al. 1994; Kessler et al., 1995; Stauffer et al., 1996; Welsh and Perry, 1998; van Snik Gray and Stauffer, 2001; microhabitat depth - Greenberg 1991; Kessler and Thorp, 1993; Chipps

et al. 1994; Kessler et al., 1995; Stauffer et al., 1996; Welsh and Perry, 1998; van Snik Gray and Stauffer, 1999; activity time - Greenberg 1991; current velocity - Chipps et al. 1994; Kessler et al., 1995; Stauffer et al., 1996; Welsh and Perry, 1998; and distance from the substratum - Smart and Gee, 1979; Greenberg 1991; Welsh and Perry, 1998).

Unfortunately, 24% of known darters, and an estimated 35% of undescribed species are imperiled (Burkhead and Jelks, 2000; Warren et al., 2000). Darters suffer higher rates of imperilment because of their increased susceptibility to habitat degradation; primarily substrata homogenization via sedimentation and stream impoundment (Burkhead and Jelks, 2000). This sensitivity stems from the general small size and low fecundity of darters as well as their insectivorous trophic habits (Berkman and Rabeni 1987; Angermeier 1995).

The threatened or endangered status of many darter species substantiates the need for conservation of these fishes and their habitats. At present, however, our knowledge of habitat requirements for many darter species is incomplete. Past darter microhabitat studies have focused on habitat use in terms of physical variables. Nonetheless, in recent years, investigators have shown that the patchy distribution of prey in streams also strongly affects microhabitat selection by benthic fishes (Petty and Grossman 1996; Petty 1998; Thompson et al. 2001) In fact, these studies showed that the prey abundance of a patch was a better predictor of its microhabitat quality than its physical characteristics. Previous studies of darter resource use have not ascertained if darters make similar distinctions. Consequently, I examined microhabitat use of three species of darters in a Piedmont stream in Georgia in relation to both physical characteristics and prey abundance.

Study Species

The blackbanded darter (*Percina nigrofasciata*) is found throughout Gulf and Atlantic coastal drainages from Lake Pontchartrain to the Edisto River, South Carolina (Lee et al. 1980). Adults range from 39-80mm SL, and live 3-4 years. *Percina nigrofasciata* are benthic insectivores, consuming mainly Dipterans, Ephemeropterans, and Trichopterans (Mather 1973), while juveniles feed on crustaceans (Page 1983). *Percina nigrofasciata* is common over gravel, sand (Lee et al. 1980), rock, rubble, mud, and silt (Crawford 1956), but preferred medium gravel in lab studies (Mathur 1973). Suttkus and Ramsey (1967) suggest *P. nigrofasciata* may use still water along stream edges as over wintering habitat. This species does not appear to be active at night, and leaves stream reaches when water temperatures exceed 25 C (Suttkus and Ramsey 1967). Blackbanded darters typically spawn over sand or loose gravel, burying and abandoning the eggs (Suttkus and Ramsey 1967), from May to June (Lee et al. 1980; Rohde et al. 1994), but also as early as February (Mettee et al. 1996; Suttkus and Ramsey 1967).

The tessellated darter (*Etheostoma olmstedi*) is found in Atlantic coastal drainages from Quebec City, Canada, to Oklawaha River, Florida. Members of this species range in size from 44 to 62 mm SL (adults) and live 3-4 years (Lee et al. 1980). *Etheostoma olmstedi* feed primarily on Dipteran, Ephemeropteran, Trichopteran insect larvae (Raney and Lachner 1939). Tessellated darters typically utilize pools of small streams, shallow waters over mud, sand, and rubble, whereas they avoid strong riffles (Lee et al. 1980; Page 1983). *Etheostoma olmstedi* males generally excavate a nest under a stone and guard the eggs. Spawning typically occurs from April or May until June (Lee et al. 1980; Rohde et al. 1994; Raney and Lachner 1939; Atz 1940; Dewoody et al. 2000; Constantz 1985; Schultz 1999), but has been observed in March in the Savanna River (Dewoody et al. 2000).

The distribution of the turquoise darter (*Etheostoma inscriptum*) is limited to the Savannah, Altamaha, and Oconnee river drainages (Lee et al. 1980). No data has been published regarding the lifespan of *E. inscriptum*, but closely related species often live 3-4 years. Adult *E. inscriptum* range from 55-65 mm SL (Lee et al. 1980). This species is insectivorous, and Baker (2002) observed electivity for Chironomids. Turquoise darters are restricted to shallow gravel, rubble, and bedrock riffles with moderate to strong currents (Lee et al. 1980; Baker 2002; Richards 1966; Page 1983). *Etheostoma inscriptum* spawn from late March to early June (Richards 1966).

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CHAPTER 3

MICROHABITAT USE BY BLACKBANDED (*PERCINA NIGROFASCIATA*), TURQUOISE
(*ETHEOSTOMA INSCRIPTUM*), AND TESSELATED (*ETHEOSTOMA OLMSTEDI*) DARTERS
IN A PIEDMONT STREAM¹

¹ Henry, B. E., and G. D. Grossman. To be submitted to *Environmental Biology of Fishes*.

ABSTRACT

We quantified factors affecting microhabitat use for three darter species in a 116m reach of a Piedmont stream via underwater observation and macroinvertebrate benthos samples during 2001-2002. The 12 month period of sample collections, and the previous 12 months were the first and second driest years on record, respectively. PCA indicated that in Spring 2002, a season with higher amounts of habitat availability, there existed fewer significant differences in darter microhabitat use, whereas in Autumn 2001 and Summer 2002 all darters used significantly different microhabitats. In general, turquoise darters used more scour areas, tessellated darters more depositional, and blackbanded darters utilized intermediate habitats. During Autumn 2001, large blackbanded darters ($\geq 55\text{mm}$) occurred in faster microhabitats with more erosional substrata than smaller specimens, and used a greater variety of depths. In Autumn 2001, large turquoise darters ($\geq 40\text{mm}$) used deeper microhabitats with higher velocities and more erosional substrata than smaller individuals, whereas the opposite was true for Autumn 2002. Blackbanded darters used microhabitats with significantly less macroinvertebrate biomass and abundance in Autumn 2001, but with higher percentages of Dipterans. In Spring 2002, all three darters occurred in microhabitats with significantly lower macroinvertebrate biomass and numerical abundance (blackbanded and tessellated only) than found at random. We observed no interspecific differences in biomass, numbers, or percent Dipteran composition, nor any size related differences in macroinvertebrate abundance in microhabitats occupied by darters.

INTRODUCTION

Streams are common aquatic systems, that display high levels of patchiness in their physical and biotic characteristics (Grossman et al 1998). Stream fishes reach their highest diversity in North America in the southeastern United States (Matthews 1998). This diversity is manifested mostly by non-game fishes such as minnows (Cyprinidae) and darters (Percidae). Although, the center of diversity for these families is in the southeastern United States, this region also has a high rate of imperilment for members of these families (Warren et al 2000). The plight of these fishes is compounded by the fact that we know little about their habitat and dietary requirements beyond general surveys such as Etnier and Starnes (1994), Jenkins and Burkhead (1994), or Page (1983).

Extant information indicates that most darters are benthic insectivores that typically inhabit lotic systems (Page 1983, Folkerts et al. 1997, Warren et al. 1997, 2000). Our knowledge of the taxonomy of this family is incomplete and at least 26 new species have been described since 1990, with at least 43 species/subspecies remaining undescribed (Burkhead and Jelks, 2000). In the eastern United States, darters commonly occur sympatrically and in some cases differences in resource use are apparent (diet - Greenberg 1991; van Snik Gray et al. 1997; substratum use - Kessler and Thorp 1993; Chipps et al. 1994; Welsh and Perry 1998; van Snik Gray and Stauffer 2001; microhabitat depth - Kessler et al. 1995; Stauffer et al., 1996; Welsh and Perry 1998; activity time - Greenberg 1991; current velocity - Chipps et al. 1994; Kessler et al., 1995; Stauffer et al. 1996; Welsh and Perry 1998; and distance from the substratum - Greenberg 1991; Welsh and Perry 1998). However the general relationship between differences in resource use and coexistence is unknown for most sympatric darters (but see Greenberg 1988 and Van Snik Grey and Stauffer 2001).

Most studies of stream fish habitat use focus on the physical environment (Grossman et al. 2002). Recently, however, several studies have shown that the distribution of prey may be a better predictor of microhabitat (i.e. patch) use than physical habitat characteristics such as depth, and substratum composition (Petty and Grossman 1996, 2004; Thompson et al. 2001). Temperate streams are known for their heterogeneity in depth, velocity and substratum composition (Grossman et al. 1995, 2006) and this heterogeneity can affect small stream-dwelling fishes such as darters (Greenberg 1991). Given the general paucity of knowledge regarding both microhabitat use in darters and the lack of studies linking prey patchiness to microhabitat use in stream fishes, we examined both of these factors in three darter species (blackbanded darter, *Percina nigrofasciata*, tessellated darter, *Etheostoma olmsteadi* and turquoise darter *E. inscriptum*) found in a Piedmont river in Georgia. Specifically, we asked: 1) Does physical habitat availability and invertebrate abundance vary seasonally? 2) Do darters occupy microhabitats with physical characteristics that differ from those available at random? and 3) Do darters occupy microhabitats with greater invertebrate abundance than those available at random?

METHODS

The Study Site

Our study site was a 116 m section of Cloud's Creek, a third order tributary (Strahler 1964) of the South Fork Broad River (SFBR), Madison and Oglethorpe Counties, Georgia, U.S.A. (830717, 340206) (Fig. 1). The site was located in Watson Mill Bridge State Park, approximately 244 m upstream of Cloud's Creek's confluence with the SFBR, at an elevation of 146 m above sea level (NGVD 1929). The average bankfull width was 19.4 m. Riparian bank vegetation included species typical of Piedmont mixed forests and understory, including loblolly

pine (*Pinus taeda*), water oak (*Quercus nigra*), winged elm (*Ulmus alata*), red maple (*Acer rubrum*), hophornbeam (*Ostrya virginiana*), southern red oak (*Quercus falcate*), river cane (*Arundinaria gigantean*), river birch (*Betula nigra*), *Muscodine spp*, and poison ivy (*Toxicodendron radicans*).

A bedrock outcrop, cobbles and boulders interspersed with sand dominated the upstream half of the site, whereas sand, silt, and occasional cobbles dominated the lower half of the site. Exposed bedrock platforms and boulders delimited natural subsections throughout the site, and we sampled one subsection (unit) at a time. Subsections ranged from 4 to 18 m in length, and 6 to 14 m in width. Sampling at the study site occurred during drought periods. In Georgia, August 2001 to July 2002 was the driest 12 month period on record since 1895. The preceding year, September 2000 to August 2001 ranked second in dryness (National Climate Data Center, 15 August 2002). Low flow conditions during the study are reflected in annual SFBR total precipitation (Fig. 2), whereas seasonal differences in the number of wetted sampling points and fish observed (Fig. 3) illustrate seasonal variation within the site. I sampled only 28 of 116 meters in Summer 2002 (July) due to extremely low flows. Conversely, autumn storms began after I gathered fish use data, preventing collection of habitat availability data. Hence, Autumn 2002 was excluded from microhabitat use analyses.

The SFBR lies in a 245 square mile watershed in the Savannah River Basin (10,577 square miles), with 337 stream miles. The SFBR watershed land cover is deciduous, coniferous, and mixed forests interspersed with pasture, hay, and row crops. Local soils are mainly loamy surface layers over red clay subsoil, characteristic of well-drained, upland soils. Madison County ranks fifth in the state for both poultry and cattle production. Other land uses include

forest production and row crops. The SFBR experienced severe erosion, with an estimated 65% of total topsoil lost from 1900 to 1960 due to conventional tillage practices.

Currently, water impairment results from high rates of runoff delivery of sediment, nutrients, pesticides, and pathogens. Pollutant runoff stems from widespread application of chicken manure to sloped pastureland, septic system effluent, landfill leachate, litter, riverbank erosion, riparian buffer destruction, lack of tributary protection, non-point source agricultural runoff, and livestock. These levels of degradation in the SFBR watershed are reflected in the ecological health of its streams. Assessments of fish and invertebrate assemblages, habitat quality, and algal production indicated that 63% to 95% of wadable streams in this watershed were in fair to poor condition. Using fish assemblages as an index, an estimated 52% of stream miles involved in the study were in poor condition. In addition, SFBR watershed had highest fecal coliform counts in the Savannah River Basin. This watershed is considered to be a sediment-impacted system, in poor ecological condition.

Microhabitat Availability

We quantified the availability of physical microhabitat within the site in each of four seasons: Autumn 2001 (13 – 15 November) , Spring (8 – 9 May), Summer (31 July) , and Autumn 2002. Heavy rains prevented collection of Autumn 2002 habitat availability data. We made microhabitat measurements via commonly used methods (Gray and Stauffer 1999). In brief, we determined uniform sampling locations by establishing transects perpendicular to flow, spaced along the stream banks at 4m intervals, which stretched perpendicular to stream flow, and recorded measurements every 2m for the length of the transect. At each point we measured depth (nearest ± 1 cm, meter stick), average and bottom current velocities (± 0.1 cm/s, Marsh-McBirney Model 201 electronic velocity meter, nearest 1 cm^{-1}), and visually estimated the

percent composition of eight substratum classes (visual estimate of bedrock, boulder, cobble, gravel, sand, silt, debris, and vegetation) in a 20 x 20cm² quadrat (Grossman and Freeman 1987). The number of microhabitat availability quadrats ranged from 199 to 227, for Autumn 2001 and spring 2002, respectively. We classified substratum particles on the basis of their maximum linear dimension (bedrock [embedded to the surface] and boulders [unembedded particles] >30 cm, large cobble <30 and >15 cm, small cobble <15 and >2.5 cm, gravel <2.5 and >.02 cm, sand <.2 cm, and silt – material capable of being suspended in the water column, Grossman and Freeman 1987). Debris and vegetation (*Podostemum spp.*) were not categorized by size class (Grossman and Freeman 1987).

We used the methods of Grossman and de Sostoa (1994a, b) to quantify non-random microhabitat use by fishes during our study. We entered the microhabitat availability data for each season into a principle component analysis using the correlation matrix. We then multiplied the microhabitat use data for each species by the scoring coefficient matrix of the availability data principle component analysis. This gave all species a score for each component. We computed means and 95% confidence intervals for habitat availability, species, and size class on each significant component (Grossman and de Sosta 1994a, b). We plotted the means and confidence intervals using the first principle component as the x-axis, and the second component as the y-axis. Statistical significance among centroids was determined by a lack of overlap of 95% confidence intervals on the means (Grossman and deSostoa 1994). With this methodology we represented fish microhabitat use on a multidimensional habitat gradient scaled by microhabitat availability.

We also used principle component analysis to elucidate length-related differences using the same methods for multidimensional differences, whereas when differences existed on a

single component we used Kruskal-Wallis tests and Tukey-Kramer a posteriori tests (Grossman & Freeman (1987) . Our length classes generally represented year classes as identified by length-frequency histograms (unpublished data) (Fig. X).

Fish Microhabitat Use

We quantified fish microhabitat use by measuring a suite of microhabitat characteristics at the positions of undisturbed fish, which we defined as specimens remaining motionless after first detection. We collected fish data from 24 October – 8 November, 2001 (Autumn 2001), 18 April – 9 May, 2002 (spring 2002), 24 July – 29 July, 2002 (summer 2002), and 3 – 10 October, 2002 (Autumn 2002). To minimize disturbance, we entered the stream below the downstream border of the site's downstream boundary and then began making fish measurements after we entered the site. Upon sighting an undisturbed specimen, we marked its exact position using a painted weight marker and recorded the following measurements: time, species, standard length (± 1 cm, meter stick straightedge, nearest cm), depth (± 1 cm meter stick, nearest cm), distance to substratum (± 1 cm, meter stick), distance to shelter (± 1 cm, meter stick), type of shelter, distance to nearest neighbor (± 1 cm, meter stick), and species of nearest neighbor (fish within 20 cm). We also measured average, focal, and bottom current velocities (electronic velocity meter ± 0.1 cm/s, Bovee and Milhous 1978), and visually estimated percent substratum composition as described in Microhabitat Availability. Focal-point velocity was measured at the nose of the fish. We defined shelter as any object able to conceal at least 50% of the fish's body. All fish observations occurred between 11:00 and 17:00 hours.

Macroinvertebrate Abundance

We quantified macroinvertebrate abundance in the site to determine the relationship between prey abundance at the fish's position and fish microhabitat use. Benthic samples were

collected at both the position of the fish and at random locations in the site. We generated coordinates for random samples via a random number table, and stratified the site into five equal sections to ensure even coverage (i.e., equal number of random samples from each section). We collected benthic macroinvertebrate samples after the fish data from 20 November – 6 December, 2001 (Autumn 2001). For Spring and Autumn 2002 we collected fish microhabitat use and macroinvertebrate samples concurrently, although Spring random benthic samples were collected on 29 May, 2002. Due to drought, benthic samples were not collected in summer 2002.

To examine the relationship between prey abundance and fish microhabitat selection, we compared macroinvertebrate abundance from samples taken directly underneath the fish's position to random samples. Once we completed fish microhabitat measurements, a 20 x 20 cm area directly below the individual was agitated to a depth of 10 cm, rocks and debris scraped, and the loose material captured with a 27cm² square frame net (250 μ m), positioned downstream. Netted materials were transferred into a plastic jar and preserved with a 10% formalin solution. In the laboratory we manually separated macroinvertebrates from inorganic particles and detritus using a dissecting scope, and identified macroinvertebrates to family. To estimate biomass we measured head capsule width (HCW) (measured across the eyes, or largest anterior margin for those without eyes, Petty and Grossman 1996) to the nearest 0.1 mm using an ocular micrometer within a dissecting microscope.

We used power equations to model macroinvertebrate length-biomass relationships, utilizing family-specific constants and insect head capsule widths (Benke et al 1999; Gibson and Grossman 2004). Parameters for equations relating HCW to biomass (Benke et al. 1999) were species- and genus-specific. Therefore, we estimated family-specific parameters by averaging

values for all species (listed in appendix 3, Benke et al. 1999) within families present at the study site. Often, a single value was given for an entire genus. In this case, we discarded all other single-species values given for that genus.

We compared mean total macroinvertebrate numbers and biomass between darter and random samples for each season using Student's t-tests on log-transformed data. Interspecific and size-related differences were examined using ANOVA and Tukey-Kramer *a posteriori* tests (Zar 1997). Because the literature suggests that Dipterans dominate the diet of darters (Page 1993; Kuehne 1993), we also examined the relative contribution of Dipteran and EPT (Ephemeroptera, Plecoptera, and Trichoptera) by calculating the percent of the macroinvertebrate biomass comprised of Dipterans (Dipteran biomass divided by total biomass). We examined gape sizes in museum specimens that covered the size ranges of specimens observed in the field (*P. nigrofasciata*, 41-100mm SL; *E. inscriptum* 49-73mm SL; and *E. olmstedii* 28-56mm SL). We used these data to set a size limit for prey items included in analysis. Gape sizes for all species were sufficiently large that all prey observed except for Odonata, were consumable by darters. Hence Odonata were excluded from analyses.

RESULTS

Site Characteristics

Mean wetted widths in the site ranged from 15.2 to 19.4m between Autumn 2001 and Autumn 2002, whereas water temperature varied from 10 to 23 C. In addition to blackbanded, turquoise, and tessellated darters we also observed yellowfin shiner (*Notropis lutipinnis*), creek chub (*Semotilus atromaculatus*), rosyface chub (*Hybopsis rubifrons*), bluehead chub (*Nocomis leptcephalus*), and northern hog sucker (*Hypentilium nigricans*) in the site. Several other vertebrate taxa also were observed in the site including kingfishers (*Ceryle alcyon*), a northern

water snake (*Nerodia sipedon*), and tracks from raccoons (*Procyon lotor*) and herons (*Ardea spp.*).

Microhabitat Analyses

Seasonal variation in microhabitat availability

Microhabitat availability differed significantly among seasons (Fig. 4). The PCA extracted five significant components that explained 72.3% of the variance in the data set. Only components one (28% explained variance) and two (14% explained variance) generated ecologically interpretable results. In Spring 2002, the site was deepest with the highest average and bottom current velocities with cobble, bedrock and vegetation substratum and little silt and debris (Table 1, Fig. 4). Summer 2002 represented the start of the drought and displayed the opposite suite of habitat characteristics (e.g. shallowest, lowest velocities, etc.), whereas Autumn 2001 possessed intermediate habitat values (Table 1, Fig. 4).

Fish Microhabitat Use

Autumn 2001

The PCA extracted five components that accounted for 68% percent of the variance in the microhabitat availability data. Components one and two (26% and 13% variance explained, respectively) represented velocity-depth-substratum gradients (Fig. 5). Microhabitat availability data showed that the site was dominated by low velocity microhabitats with depositional substrata and bedrock (Fig. 5). Microhabitat use for all darter species differed significantly from each other as well as from availability data. Both blackbanded and turquoise darters occupied microhabitats with higher velocities, greater amounts of cobble, gravel and sand (blackbanded only) and lower amounts of silt and debris, than were randomly available (Fig. 5, Table 1). This trend was less pronounced for tessellated darters, which occupied microhabitats with lower

velocities than randomly available and also were found over more sand and debris and less cobble and bedrock than the other species (Fig. 5, Table 1). Turquoise darters also were found over less sand and more bedrock than either blackbanded or tessellated darters.

Spring 2002

Five components were obtained from the PCA, explaining 66% of the variance in the data, although only two were ecologically interpretable. Component one (20% variance explained) also depicted a velocity-depth gradient with silt and vegetation substrata (Table 1). Component two (15% variance explained) depicted a depth-substratum gradient. Microhabitat use by all species differed significantly from what was randomly available, and tessellated and turquoise darters occupied significantly different microhabitats. Microhabitat use by blackbanded darters did not differ significantly from the other two species. All darters used deeper and sandier microhabitats than were available by chance (Fig. 6). In addition, both blackbanded and tessellated darters used areas with lower average velocities, less vegetation, and more silt than available randomly (Fig. 6, Table 1). By contrast, turquoise darters occupied high velocity microhabitats with greater amounts of vegetation and less silt (Fig. 6, Table 1).

Summer 2002

Microhabitat availability during this season was similar to Autumn 2001 with components one and two (29% and 16% variance explained) elucidating velocity-depth-substratum gradients and being the only interpretable components. Summer 2002 was the beginning of a drought and the site had decreased approximately 45.9% in area (Fig. 3). Fish densities had decreased and we did not observe sufficient turquoise darters for statistical analysis ($N < 5$). Habitat use for both blackbanded and tessellated darters differed significantly from each other and from habitat availability (Fig. 7, Table 1). Both darter species occupied deeper

microhabitats with high velocities, more silt and less sand than were randomly available.

Blackbanded darters also occupied microhabitats with more vegetation and less bedrock than expected by chance (Fig. 7).

Seasonal variation in microhabitat use

All species displayed significant seasonal variation in microhabitat use. These shifts, however, always reflected seasonal shifts in microhabitat availability rather than true seasonal shifts in microhabitat use (Compare Fig. 4 to Fig. 5-7).

Size-Related Variation in Microhabitat Use

Sample sizes were sufficient to quantify size-related variation in microhabitat use only for blackbanded and turquoise darters. During Autumn 2001, large blackbanded darters ($\geq 55\text{mm}$) occurred in faster microhabitats with more erosional substrata than smaller specimens (40-55mm) (Fig. 8). Large blackbanded darters also used microhabitats that were both shallower and deeper than smaller individuals (Figure 8). There were no significant size-related differences for blackbanded darters in other seasons. During Autumn 2001, large turquoise darters ($\geq 40\text{mm}$) used deeper microhabitats with higher velocities and more erosional substrata than smaller individuals (Fig. 9). During Autumn 2002, large turquoise darters used microhabitats with lower velocities and greater amounts of cobble and depositional substrata than smaller specimens (Fig. 10).

Macroinvertebrate Analyses

Gape size

Gape size differed between darter species (Fig. 11). Blackbanded darters had significantly larger gapes than tessellated darters, with turquoise darters intermediate and not

significantly different from the other species. All gapes were sufficiently large enough to consume the largest non-Odonate prey item sampled.

Macroinvertebrate Analyses

Invertebrate biomass and percent Dipteran composition were significantly higher in Autumn 2001 and Spring 2002, whereas invertebrate numbers did not (Fig. 12). Although blackbanded darters used microhabitats with significantly lower macroinvertebrate biomass and numbers than randomly available in Autumn 2001, (Fig. 13), they did occupy microhabitats with significantly higher percentages of Diptera than those in random samples (Fig. 13).

Spring 2002

In Spring 2002, all three darters occurred in microhabitats with significantly lower macroinvertebrate biomass and numerical abundance (blackbanded and tessellated only) than found in random samples (Fig. 14). Nonetheless, tessellated darters occupied microhabitats with significantly higher amounts of Diptera, whereas the same was true for blackbanded darters and Dipteran biomass (Fig. 14). There were no interspecific differences in biomass, numbers, or percent Dipteran composition during this season.

Size-related variation in macroinvertebrate abundance in microhabitats

There were no significant size-related differences in macroinvertebrate abundance in microhabitats occupied by any size classes of any darter species.

DISCUSSION

Our results demonstrated that blackbanded, tessellated and turquoise darters in Cloud Creek use microhabitat in a non-random manner, generally choosing areas with faster current velocities and more complex substrata. The species also occupied statistically distinct microhabitats from one another in every season. Finally, we also observed size-related

differences in microhabitat use for blackbanded and turquoise darters. With the exception of turquoise darters in Autumn 2002, smaller darters tended to use slower microhabitats with greater quantities of depositional substrata. Finally, both blackbanded and tessellated darters displayed a tendency to occupy microhabitats in which the macroinvertebrate assemblage was dominated by Diptera.

Temperate stream fish assemblages can often be divided into separate benthic and water column microhabitat guilds (Grossman and Freeman 1987; Grossman et al. 1998; Grossman and Ratajczak 1998). A variety of factors such as species-specific evolutionary constraints, behaviour and morphology, determine guild membership. Most darters are benthic guild members and multiple species commonly occur in sympatry (Greenberg 1991). Page (1983) posited that substratum type was the most important variable influencing darter habitat use, corroborated by Greenberg (1991) who found higher overlap on food resources than habitat. A variety of habitat use differences have been observed in darters. *Percina* in the Elk River, West Virginia, used riffle and pool transition habitats, in contrast to riffle or runs occupied by Etheostomids (Welsh and Perry 1998). In Cloud's Creek, blackbanded darters exhibited greater variation in microhabitat use than tessellated or turquoise darters. Several other investigators have noted flexibility in habitat use by this species (Crawford 1956; Suttkus and Ramsey 1967; and Mathur 1973).

Etheostomids also commonly display non-random habitat use with respect to depth, velocity and substratum. Grey and Stauffer (1999) found tessellated darters in deeper habitats than other darters, and patterns of depth selection varied among sites. In our study, tessellated darters used different velocities from either blackbanded or turquoise darters during at least one season. Interspecific competition also may influence habitat use for tessellated darters, because

Grey and Stauffer (2001) found that this species shifted from larger to smaller substrata when banded darters were present in aquarium studies. Their field studies also suggest tessellated darters are aggressively displaced from preferred microhabitats to slower, shallower habitats by banded darters (Grey and Stauffer 2001). Despite many hours of observation we did not observe a single act of interspecific aggression among blackbanded, tessellated or turquoise darters in Cloud's Creek. Orangefin and spotted darters displayed similar habitat use patterns to darters in Cloud's Creek with non-random use occurring along a substratum size and depth (Kessler and Thorp 1993) gradient.

The relationship between changes in habitat availability and darter habitat use is unclear. Stauffer et al. (1996) observed increased habitat specialization in the month with the greatest habitat availability, however, we observed the opposite trend among darters in Cloud's Creek (greatest overlap in month with greatest availability). Spawning behaviour and differential timing of peak discharge may be responsible for the disparities. Kessler et al. (1995) asserted that decreased spatial breadth and decreased resource overlap in darters occurred with decreased discharge (i.e. reduced habitat availability), implying that biotic interactions influenced habitat use among darters. We observed little evidence of interspecific interactions among darters in Cloud's Creek.

The presence or absence of a swim bladder may affect habitat use by darters. In general, *Percina* species have swim bladders and are less benthic than members of the genus *Etheostoma* (Page 1983; Greenberg 1991; Grey and Stauffer 2001; Smart and Gee 1979; Chipps et al. 1994). Interestingly, Greenberg (1991) observed that *Percina* spent more time in the water column and less time under cover than Etheostomids, yet the two genera displayed no differences in the number of bites taken off the bottom, pitch and yaw of feeding bite, or type of feeding surface.

Nonetheless, morphological differences, including an air bladder probably affect the more generalized habitat use patterns and broad distribution of blackbanded darters.

We tested the hypothesis that darters would occupy microhabitats with higher abundances of prey, but the only relationships observed involved positive selection for patches with a higher percentage of Dipteran prey in some seasons. Darters in other habitats exhibit generalized feeding habitats with dipterans generally dominating the diet (Matthews et al. 1982; Hlohowskyj and White 1983; McGehee 1989; Gray et al. 1997). Several other benthic stream fishes display significant relationships between prey abundance on both a microhabitat and channel unit scale (Petty and Grossman 1996, 2004; Thompson et al. 2001). Our prey abundance-fish microhabitat use results were weaker than those of the aforementioned authors but macroinvertebrate availability in Cloud's Creek appeared to be much lower than that of Coweeta Creek North Carolina (site of the aforementioned studies). For example, mean head capsule width for Cloud's Creek macroinvertebrates was less than .5mm in contrast to the many larger prey in Coweeta Creek (Hill and Grossman 1993, Petty and Grossman 1996).

Page (1983) hypothesized that differences in darter diets are related to habitat use. Greenberg (1988) found that species which used more fine particle sized habitats fed more on Dipterans, and species using more coarse substrata ate more Ephemeroptera. He postulated that changes in temperature and discharge, which affect the amount of available habitat, could influence prey available for darters. We observed little overlap in microhabitat use by darters, however this segregation occurred within channel units rather than among channel units in the site (pers. Obs.). Although some researchers have found that riffles have higher macroinvertebrate densities than runs and pools (Schlosser 1987, Vogt and Coon 1990), we found prey distributed throughout the study site.

Similar to other investigators, we observed length-related differences in microhabitat use for species in our site. Freeman and Stouder (1989) suggested that length-related depth preferences of *Cottus bairdi* were influenced by intraspecific competition; a view supported by the field removal experiment of Grossman et al. (2006). We cannot speculate on the causal mechanisms producing length-related shifts in microhabitat use of blackbanded and turquoise darters and they may be related to ontogeny or intraspecific competition.

Management implications

Low stream discharge may affect darter populations differentially, and knowledge of darter microhabitat use requirements may assist in determining minimum flow levels for dam operation in temperate streams. Our data indicate that all three darter species used microhabitats which were statistically different from those randomly available, and also from each other, most commonly choosing areas with higher current velocities and more complex substrata. Turquoise darters, which naturally exhibit a narrow geographical distribution, also used more specialized scour microhabitats than blackbanded or tessellated darters. Low discharge more acutely affects the availability of riffles and riffle-like stream habitats for fish use, in contrast to deeper habitats (e.g., pools). Therefore, darters that require scour microhabitats may be more prone to the effects of drought. In accordance with this hypothesis, we found only one turquoise darter within the site during Summer 2002. Provided fish microhabitat use information, natural resource managers may more effectively determine minimum flow levels for dam operations, ensuring important stream habitat will remain submerged and available for fish use. In addition, changes in land management practices that decrease stream sedimentation may help prevent homogenization of complex substrata required by many darter species.

Conclusion

In conclusion, we studied microhabitat use by three sympatric darter species during a drought. Habitat availability differed seasonally, and this was reflected in darter microhabitat use. PCA indicated that in Spring 2002, a season with higher amounts of habitat availability, there existed fewer significant differences in darter microhabitat use, whereas in Autumn 2001 and Summer 2002 all darters used significantly different microhabitats. In general, turquoise darters used more erosional areas, tessellated darters more depositional, and blackbanded darters utilized intermediate habitats. We observed size related differences in microhabitat for blackbanded darters in Autumn 2001, and for turquoise darters in Autumn 2001 and 2002. Blackbanded darters used microhabitats with significantly less macroinvertebrate biomass and abundance in Autumn 2001, but with higher percentages of Dipterans. In Spring 2002, all three darters occurred in microhabitats with significantly lower macroinvertebrate biomass and numerical abundance (blackbanded and tessellated only) than found at random.

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CHAPTER 4

GENERAL CONCLUSIONS

We quantified factors affecting microhabitat use for three darter species because darter habitat and diet requirements are not fully understood, many darters are imperiled, and recent literature suggests prey abundance may be a better predictor of microhabitat use than physical variables. The southeastern United States supports the highest diversity of freshwater fishes in North America (Burkhead et al. 1997, Warren et al. 2000) and more than 200 of these species are darters (Warren et al. 2000). For many darters, little or no information has been published regarding life history, habitat, diet, and/or reproduction requirements. Approximately 34% of Etheostominae are classified as imperiled (Warren et al. 2000). In addition, most researchers consider only physical variables when investigating fish microhabitat use. In other species, recent literature suggests that prey abundance is a better predictor of habitat use in some seasons (e.g., for *Cottus bairdi*, Petty and Grossman 1996; *Rhynchithyes cataractae*, Thompson et al. 2001). We attempted to evaluate the generality of this model, extending the idea to Etheostominae in the Piedmont region.

We examined microhabitat use with regard to physical habitat characteristics and prey abundance for three darter species via underwater observation and macroinvertebrate benthos samples during 2001-2002. All sampling occurred under drought conditions. Principle Component Analysis indicated that each species used statistically different microhabitats than found at random, and that statistically significant interspecific differences existed for each season except Summer 2002. In general, turquoise darters used more scour areas, tessellated

darters used more depositional habitats, and blackbanded darters utilized intermediate habitats. When we observed size-related differences in microhabitat use, smaller individuals typically used slower areas with more depositional substrata. Blackbanded darters used microhabitats with significantly less macroinvertebrate biomass and abundance in Autumn 2001, but with higher percentages of Dipterans. In Spring 2002, all three darters occurred in microhabitats with significantly lower macroinvertebrate biomass than random samples and blackbanded and tessellated darters utilized areas significantly lower in macroinvertebrate numerical abundance than found at random. We observed no interspecific differences in biomass, numbers, or percent Dipteran composition, nor any size related differences in macroinvertebrate abundance in microhabitats occupied by darters.

Our results corroborate those found in the literature. Darters primarily are insectivorous members of benthic microhabitat guilds. Page (1983) postulated that substratum type was the most important factor influencing darter habitat use, but darters also segregate along other resource gradients (e.g., diet, depth, distance from substratum). We most commonly found differential microhabitat use with regard to current velocity and substrata complexity. Seasonal differences in fish microhabitat use appeared to mirror seasonal variation in habitat availability. Darters occupied areas with significantly lower prey abundance than randomly available, and that were dominated by Dipterans (for blackbanded and tessellated darters only). The size-related differences in microhabitat use we observed followed ontogenetic differences documented for other stream fishes.

Freshwater stream fishes face multiple threats. Successful management applications should address nutrient enrichment and eutrophication, and the improved treatment of domestic and industrial wastewater. Eliminating wastewater effluents will also decrease heavy metal

inputs into streams. Improving agricultural chemical application processes will decrease nutrient and toxin runoff into local streams. Advancing agricultural and urbanization land management practices to minimize soil losses is crucial to sustaining the health of local fish assemblages. In addition, river regulation poses perhaps the largest threat to stream fish assemblages, as every dam project drastically changes habitat upstream and down. In order to mitigate damage done by hydrologic regulation, management actions should include watershed planning to minimize the total number of streams that are regulated. Also, dam operation should be controlled to provide inundation periods that approximate natural cycles. Restoration of hydrologic integrity is especially important in restoration of streams in urban watersheds (Roy et al. 2003; Booth 2005;). Furthermore, each of the above phenomena concurrently impacts benthic macroinvertebrate communities, decreasing prey resources in addition to the direct negative consequences to the fishes themselves.

Table 1. Means of microhabitat use data for PCA categories in which species were significantly over-or under-represented. We only present data for variables with component loadings > [0.40].

Fall 2001	<i>n</i>	PC	Score category range	Significant categories	No. exhibiting non-random use	Over (R)- or under (U)- represented	Average velocity (cm/s)	Bottom velocity (cm/s)	Depth (cm)	>300mm % bedrock	>300mm % boulder	150-300mm % large cobble	25-150mm % small cobble	2-25mm % gravel	<2mm % sand
availability data	198						18.64 (.89)	8.86 (.79)	15.17	17	2	2	10 (.42)	4	34
		1	-2.25 to 1.75												
		2	-2.25 to 1.75							(-.57)					(.86)
		3	-2.25 to 2.75							(-.58)			(.70)		
		4	-7.25 to 1.75						(.57)		(-.84)				
		5	-1.75 to 6.75									(.85)			
<i>P. nigrofasciata</i>	78	1	-0.50 to 2.00	-2.00 to -.50	0	U	18.64	8.86					10		
		2	-2.00 to 1.50	-1.50 to -1.00	3	U				17					34
		3	-1.50 to 3.00	-0.50 to 0.50	43	R				12			3		
<i>E. inscriptum</i>	53	1	-0.50 to 2.00	0.50 to 1.50	38	R	34.58	9.5					33		
		3	-1.00 to 2.50	1.50 to 2.50	19	R				0			66		
		5	-2.00 to 5.00	0.00 to 0.50	4	U						0			
<i>E. olmstedii</i>	33	3	-1.0 to 2.00	0.00 to 0.50	12	R				4			3		

Table 1.
(cont)

Season	n	PC	Score category range	Significant categories	No. exhibiting non-random use	Over (R)- or under (U)- represented	Average velocity (cm/s)	Bottom velocity (cm/s)	Depth (cm)	>300mm % bedrock	>300mm % boulder	150- 300mm % large cobble	25- 150mm % small cobble	2- 25mm % gravel	<2mm % sand
Spring 2002							27.58	11.35	20.38	24	2	3	13	6	30
availability data	227														
		1	-4.50 to 2.00				(0.85)	(0.58)							
		2	-2.50 to 2.00					(0.51)	(-0.82)						(0.72)
		3	-2.50 to 3.00				(0.65)							(0.63)	
		4	-3.00 to 4.50							(0.43)	(0.67)	(-0.64)			
		5	-4.00 to 4.00												
<i>E. inscriptum</i>	20	2	-0.50 to 1.50	0.50 to 1.00	8	R			21.25	0					33
		4	-2.50 to 3.50	0.00 to 0.50	0	U					2	3.66	13		
		5	-3.50 to 2.00	-3.50 to -0.50	5	R									
<i>E. olmstedii</i>	14	2	-1.00 to 2.00	0.50 to 1.00	7	R			22.57	1					42
<i>P. nigrofasciata</i>	54	1	-1.50 to 1.50	0.00 to 0.50	25	R	21.84	12.76							
		2	-1.50 to 2.00	-2.50 to -1.00	0	U			20.38	24					30
		4	-1.50 to 4.00	0.50 to 1.00	30	R					1	0	3		

Table 1.
(cont)

Season	n	PC	Score category range	Significant categories	No. exhibiting non-random use	Over (R)- or under (U)- represented	Average velocity (cm/s)	Bottom velocity (cm/s)	Depth (cm)	>300mm bedrock	>300mm boulder	150-300mm % cobble	25-150mm % gravel	2-25mm sand	<2mm silt	% del
Summer 2002							4	2.91	10.71	11	0	5	7	44	28	
availability data	93	1	-2.00 to 3.00				(0.76)	(0.82)	(-0.49)			(0.73)		(-0.94)	(-0.56)	
		2	-2.50 to 2.50							(0.70)						
		3**	-2.50 to 4.00						(0.47)				(0.64)			
		4	-2.00 to 3.50							(-0.41)					(0.45)	(0.00)
<i>E. olmstedii</i>	5	2**	0.50 to 1.50	1.00 to 1.50	3	R				0				0		
		3	0.00 to 2.00	1.00 to 2.00	4	R			23.75				4			
		4	-1.00 to 3.00	2.00 to 3.00	4	R				0					89	1
<i>P. nigrofasciata</i>	46	1	-1.50 to 3.00	-0.50 to 0.00	0	U	4	2.91	10.71			5			28	
		1	-1.50 to 3.00	1.00 to 1.50	8	R	12.75	7.63	17.88			9			16	
		2	-2.50 to 2.00	1.00 to 1.50	18	R				7				1		
		3	-2.00 to 3.50	0.50 to 1.50	26	R			19.77				5			
		4	-2.00 to 3.50	2.00 to 3.00	18	R				0					84	9

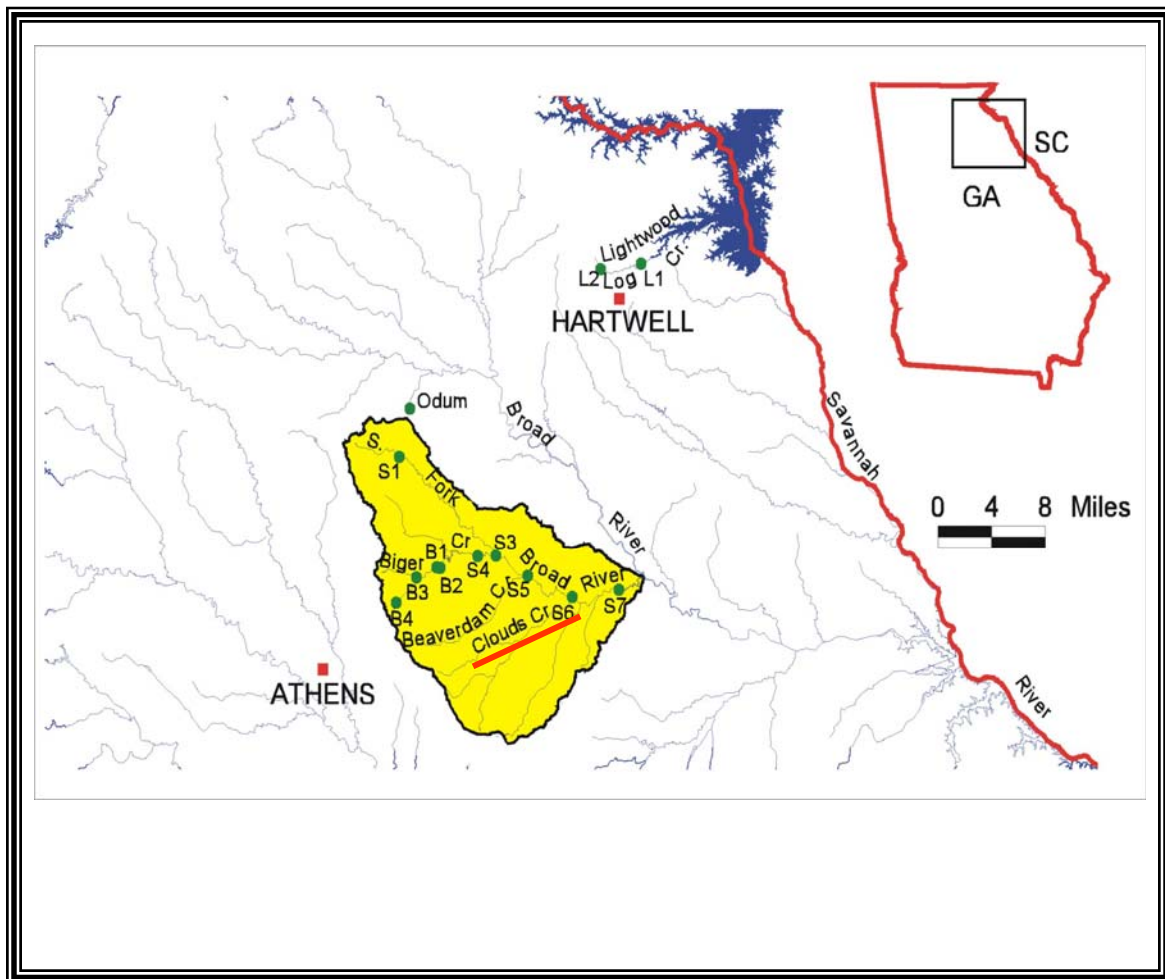


Figure 1. Map of study site. Location of the South Fork Broad River watershed in northeast Georgia. The bold line within the watershed represents Cloud's Creek.

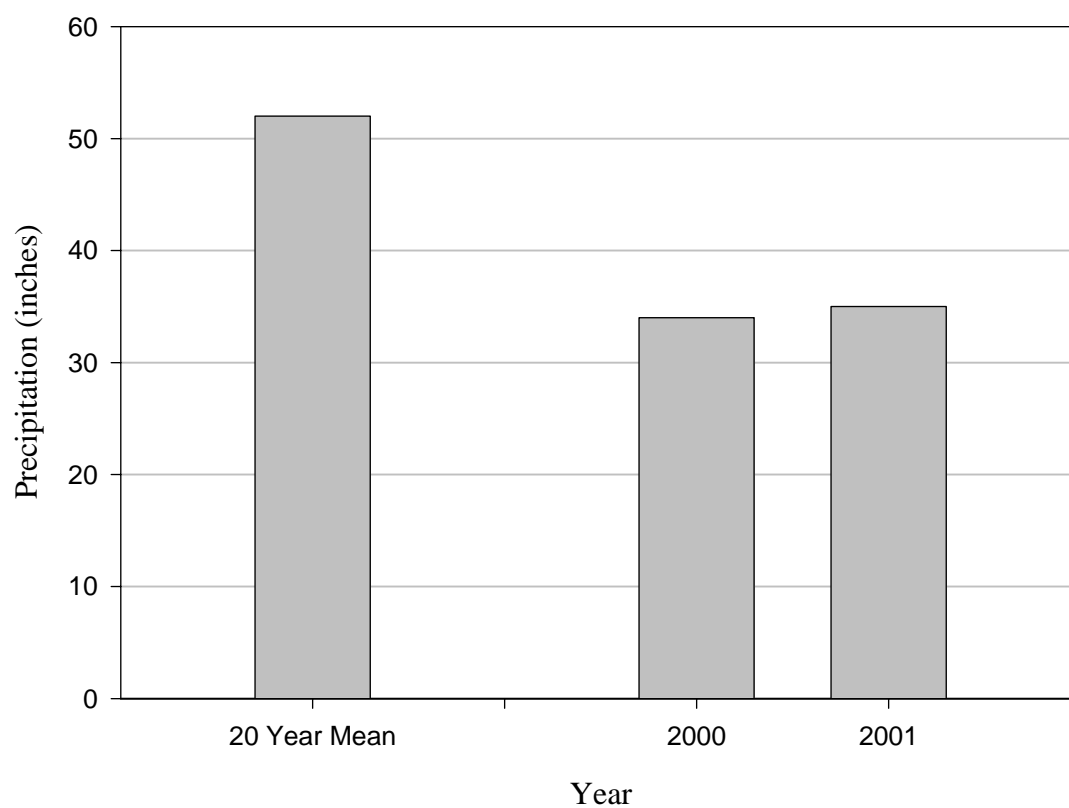


Figure 2. Total annual precipitation in SFBR 2000 and 2001, with the 20-year average included for reference. Measurements recorded near Danielsville, GA. Courtesy of Mark A. Jenkins, National Weather Service Cooperative Observer, and the U.S. EPA National Exposure Research Laboratory.

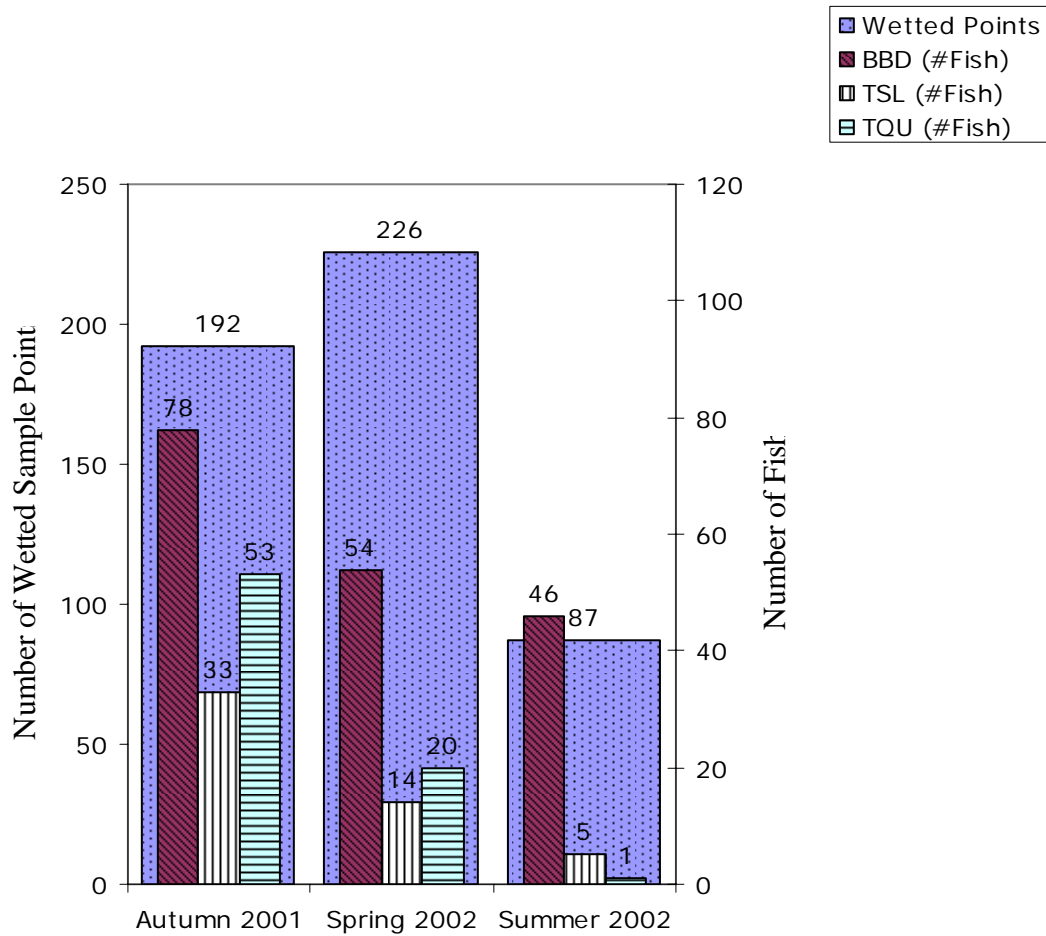


Figure 3. Seasonal variation of wetted points / fish by species. The histogram represents a seasonal comparison between the number of wetted sampling points in the site, and the number of specimens found for each fish species per season. The total number of sampling points was 303 for all seasons.

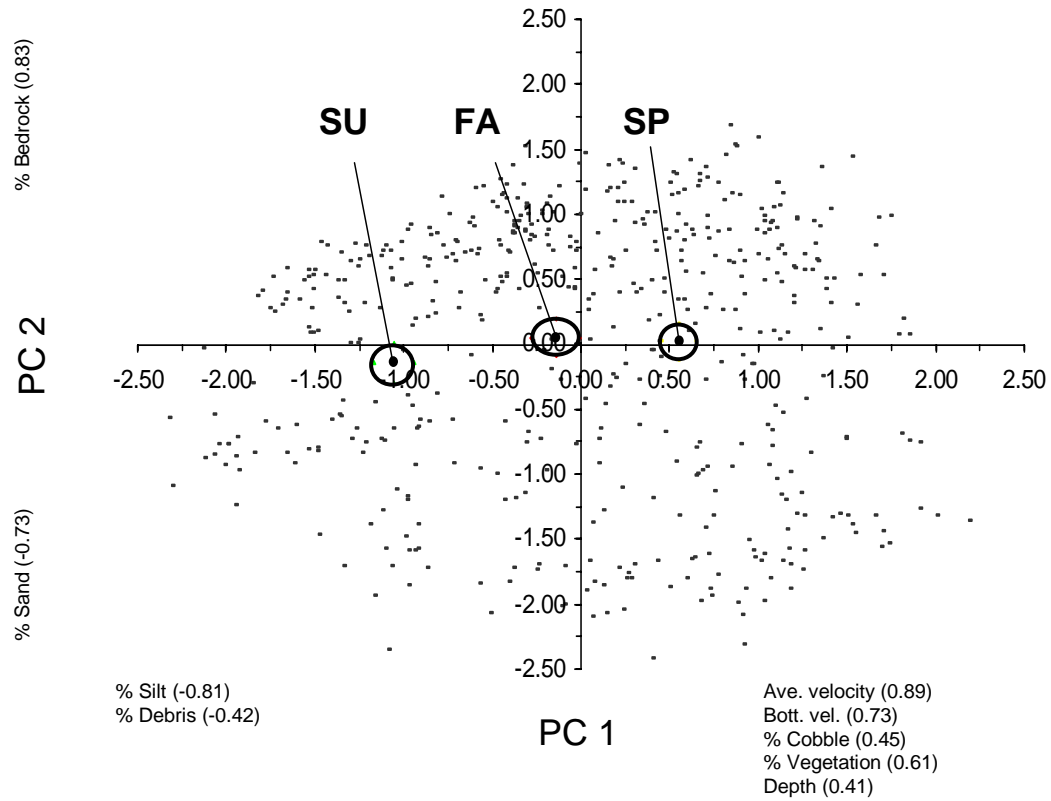


Figure 4. Seasonal differences in microhabitat availability. Mean component scores are presented for each season, as well as a 95% confidence centroid. Axis loadings > 0.40 are listed. Seasonal abbreviations are as follows: FA = Autumn 2001 (n=198); SP = Spring 2002 (n=227); and SU = Summer 2002 (n=93).

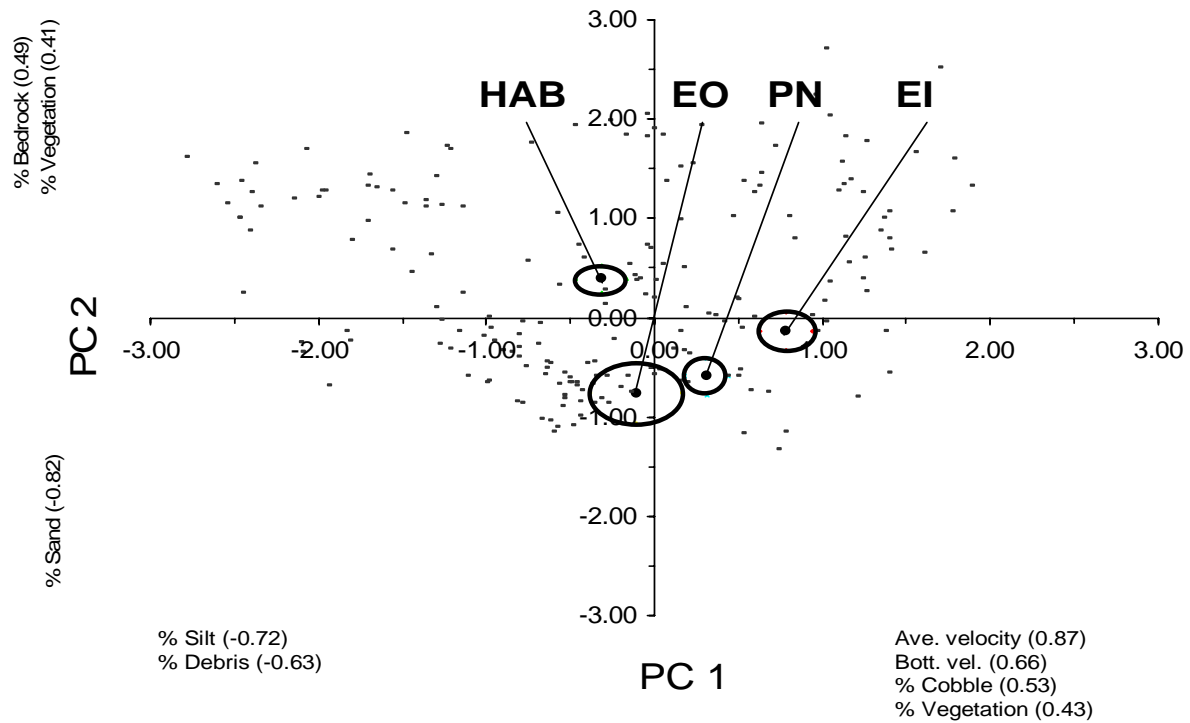


Figure 5. Interspecific differences in microhabitat use, Autumn 2001. Mean component scores are presented for each species, as well as 95% confidence centroids. Axis loadings > 0.40 are listed. Species abbreviations are as follows: PN = *P. nigrofasciata* (n=78), EI = *E. inscriptum* (n=53), EO = *E. olmstedii* (n=33), HAB = random (n=198).

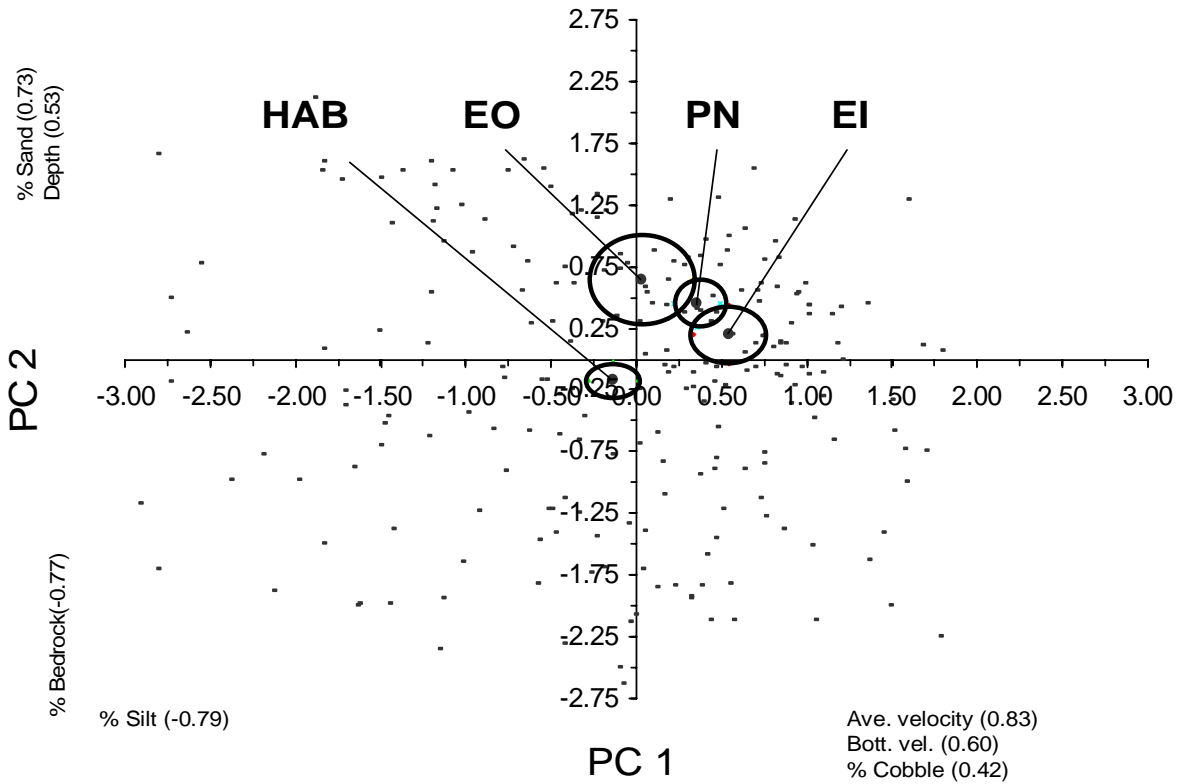


Figure 6. Interspecific differences in microhabitat use, Spring 2002. See Fig. 5 for species abbreviations and further explanation of the figure.

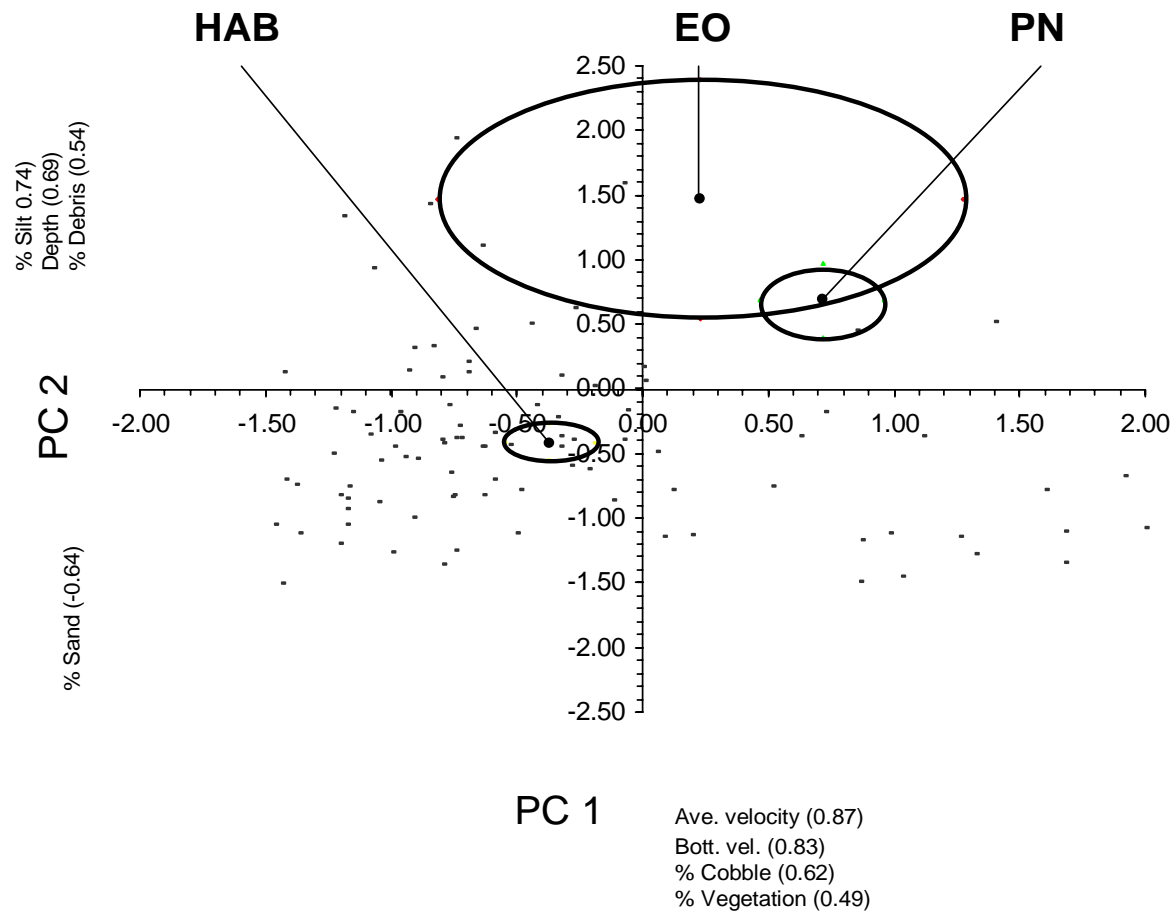


Figure 7. Interspecific differences in microhabitat use, Summer 2002. See Fig. 5 for species abbreviations and further explanation of the figure.

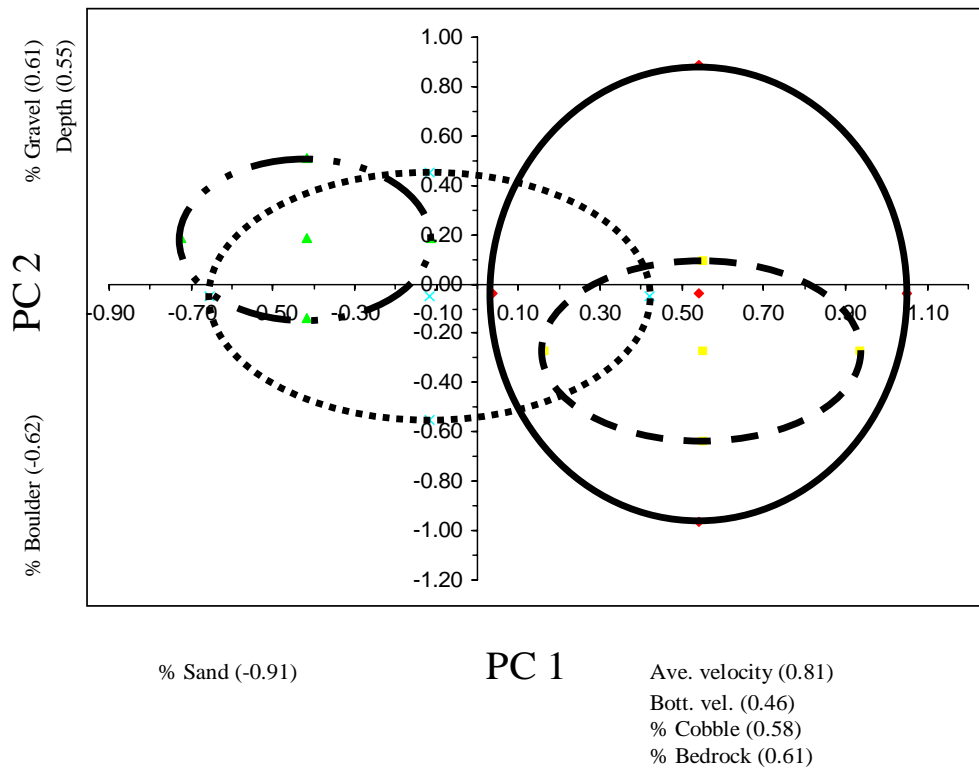


Figure 8. Size-related differences in microhabitat use for *P. nigrofasciata*, fall 2001. Mean component scores are presented for each species, as well as a 95% confidence centroid. Axis loadings > 0.40 are listed. Size abbreviations are as follows:
 = < 40mm (n=12), - . - . - . = 40-55mm (n=36),
 - - - - - = 56-65mm (n=21), ————— = > 65mm (n=9).

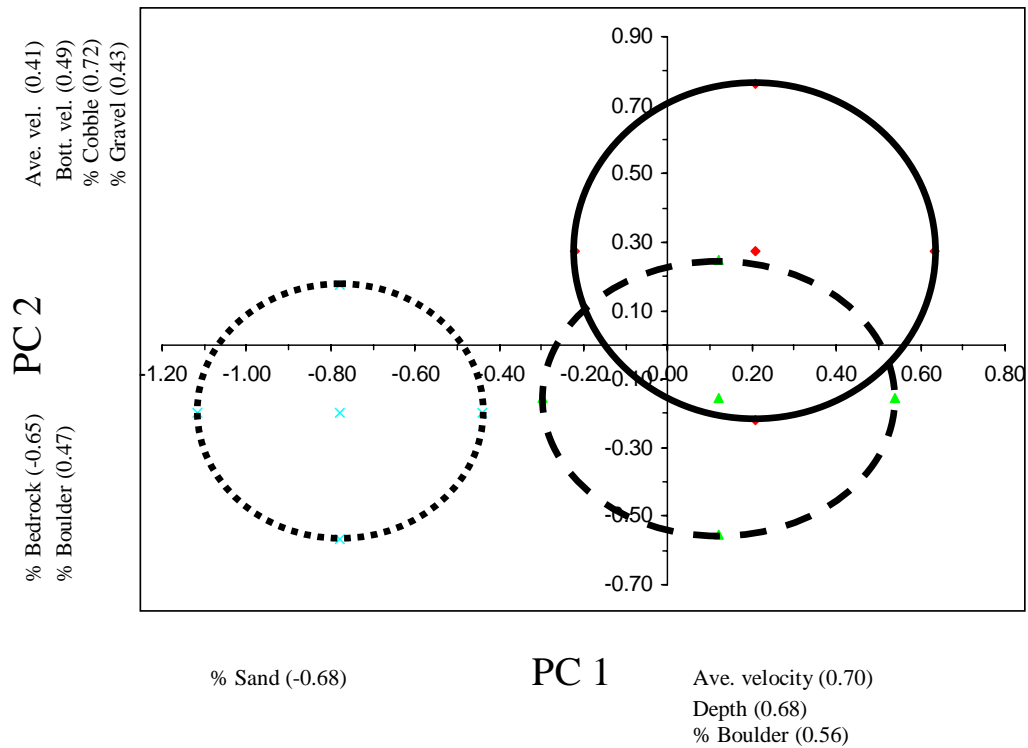


Figure 9. Size-related differences in microhabitat use for *E. inscriptum*, fall 2001. Mean component scores are presented for each species, as well as a 95% confidence centroid. Axis loadings > 0.40 are listed. Size abbreviations are as follows:
 = < 40mm (n=9), - - - - - = 40-55mm (n=24),
 ————— = > 55mm (n=20).

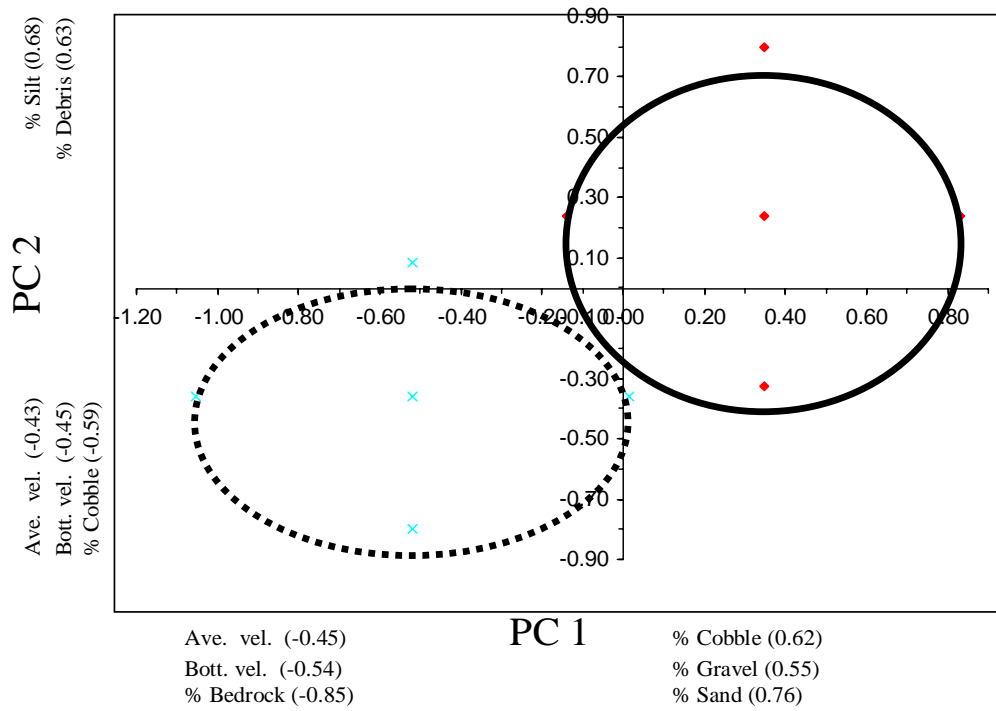


Figure 10. Size-related differences in microhabitat use for *E. inscriptum*, fall 2002. Mean component scores are presented for each species, as well as a 95% confidence centroid. Axis loadings > 0.40 are listed. Size abbreviations are as follows:
 = ≤ 50mm (n=10), ————— = > 50mm (n=15).

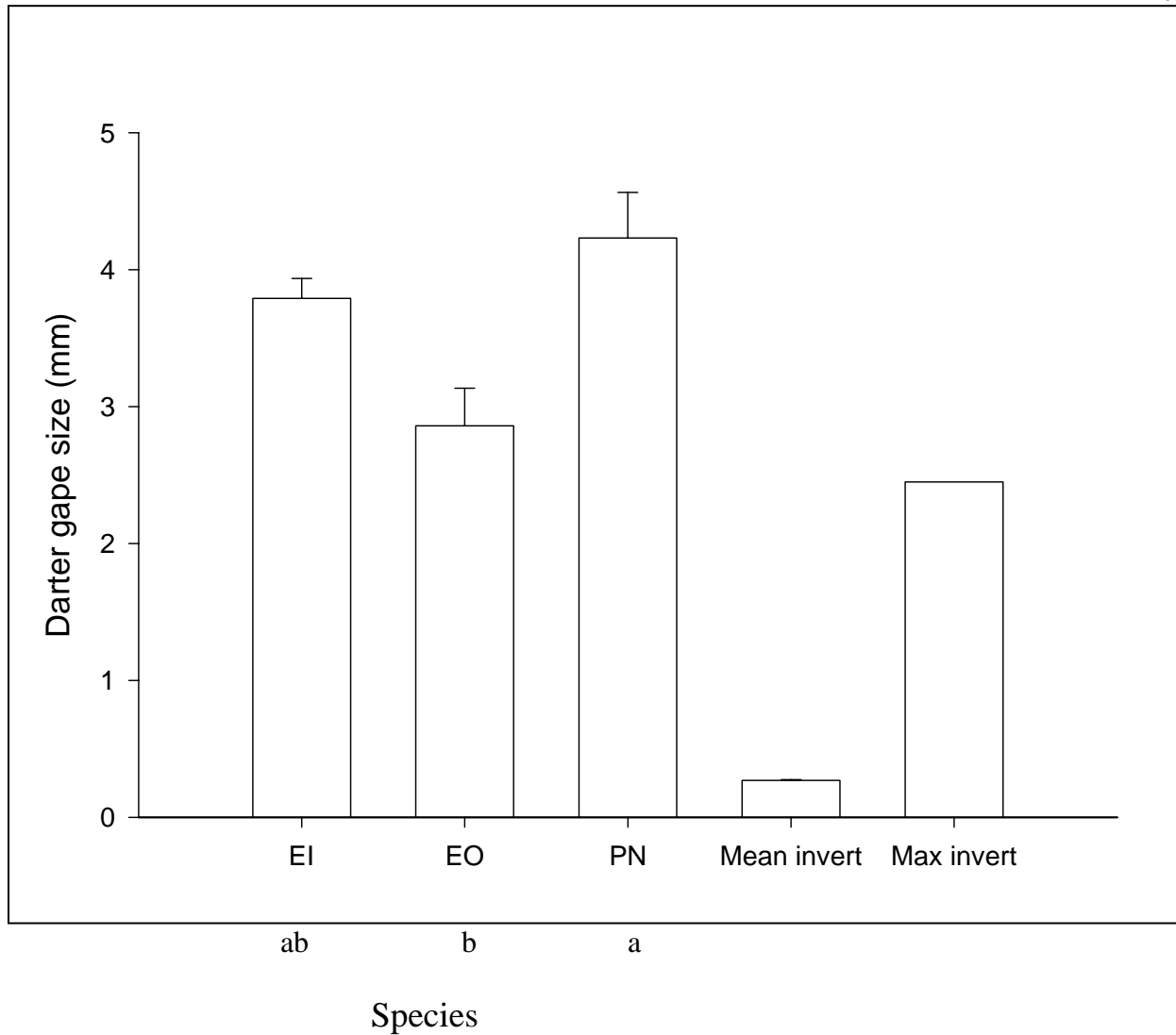


Figure 11. Test of darter gape size. Histograms represent mean darter gape size of museum specimens. Vertical lines represent ± 1 standard error. Maximum and mean invertebrate head capsule widths are included for reference. Species abbreviations are as follows: EI = *Etheostoma inscriptum* (n=16), EO = *E. olmstedii* (n=9), PN = *Percina nigrofasciata* (n=16). (Tukey-Kramer, F-value=5.62, p=0.0073). “Max invert” denotes head capsule width of the largest non-Odonate invertebrate sampled. Differing letters indicate significant differences.

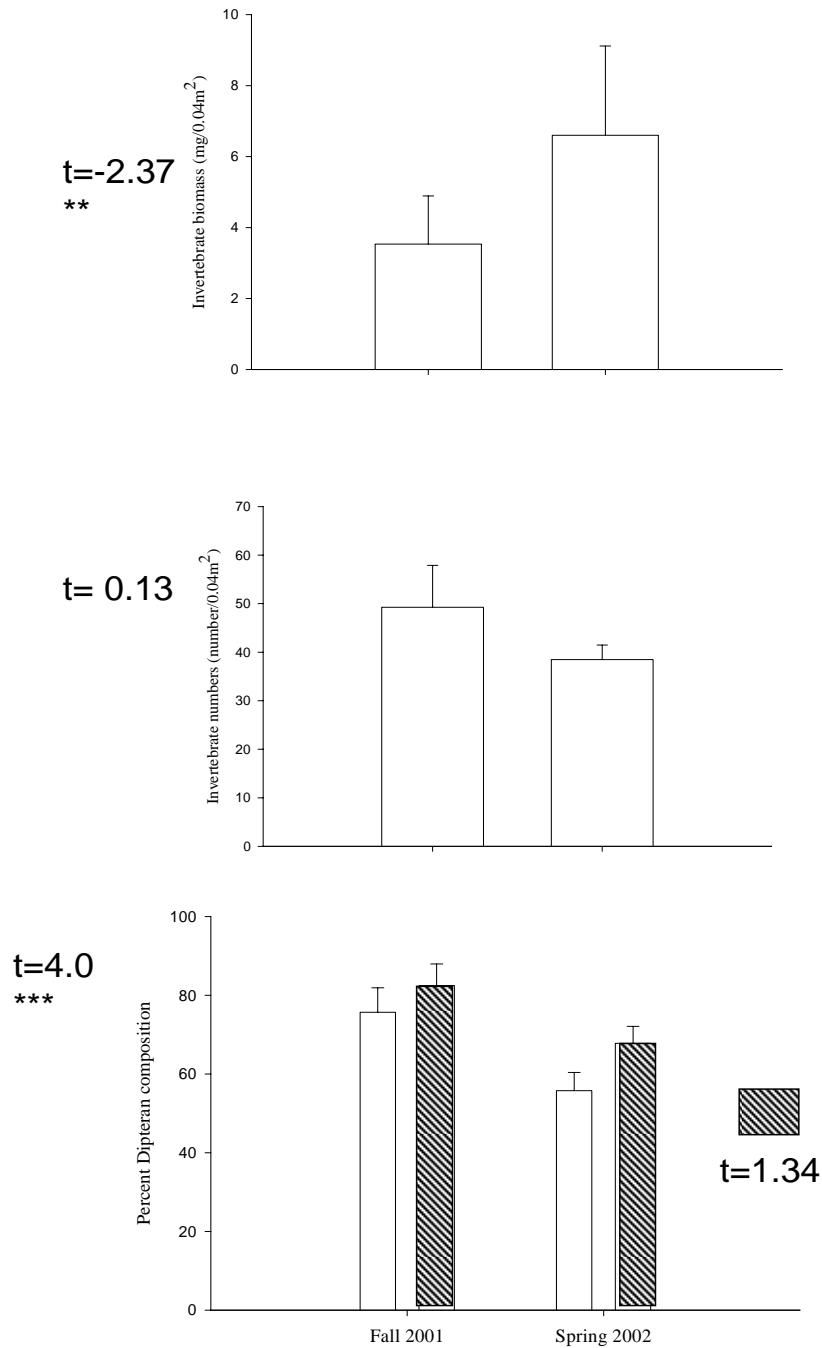


Figure 12. Test of invertebrate abundance for fall 2001 (n=48) and spring 2002 (n=116). Histograms represent mean invertebrate biomass (top), numbers (middle), and mean ratio of Dipteran/total biomass () and number (//) (bottom). Vertical lines represent ± 1 standard error. * = $p \leq 0.10$, ** = $p < 0.05$, *** = $p < 0.01$.

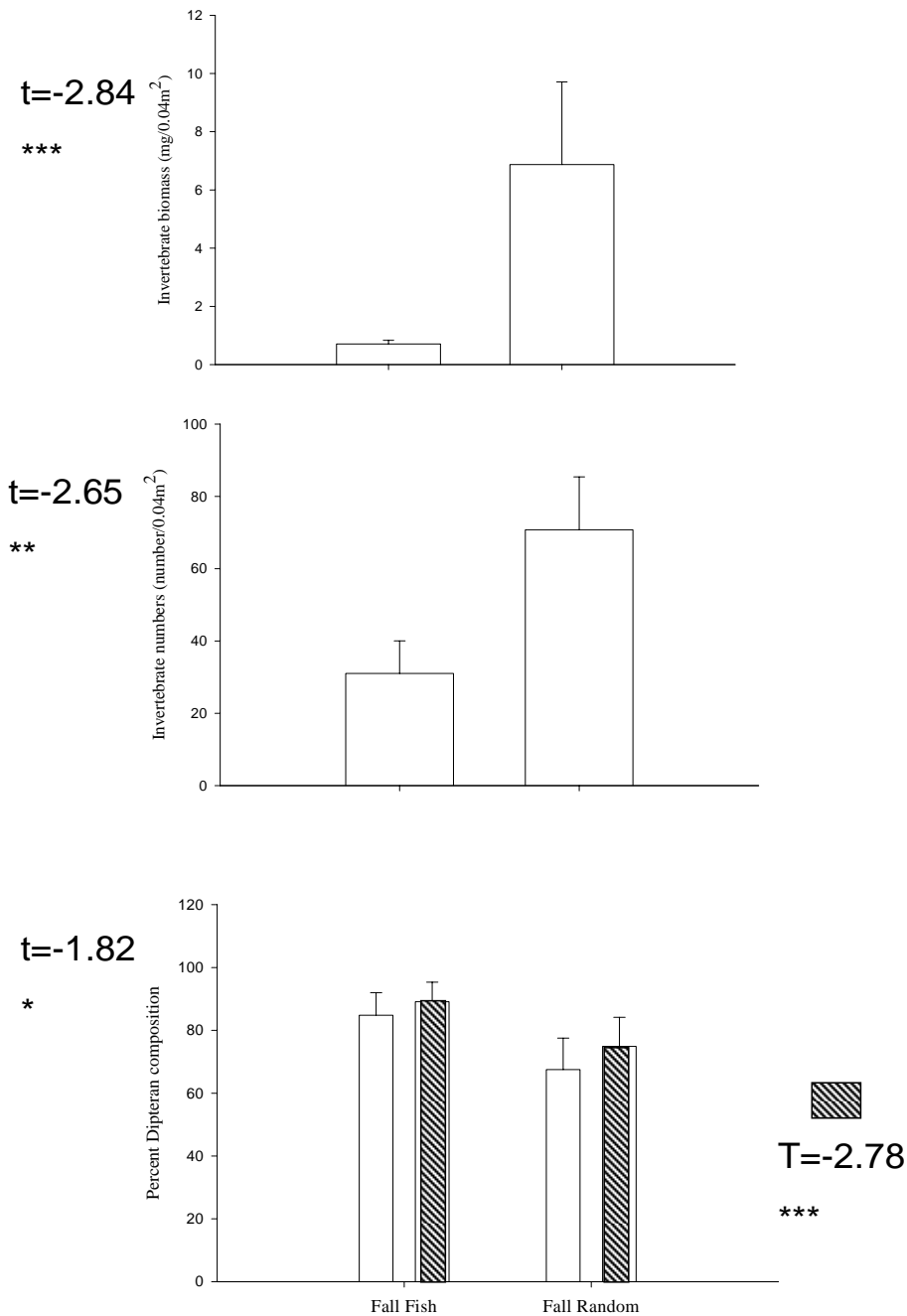


Figure 13. Test of *Percina nigrofasciata* (n=25) and random (n=22) invertebrate abundance for fall 2001. Histograms represent mean invertebrate biomass (top), numbers (middle), and mean ratio of Dipteran/total biomass () and number (//) (bottom). Vertical lines represent ± 1 standard error. * = $p \leq 0.10$, ** = $p < 0.05$, *** = $p < 0.01$.

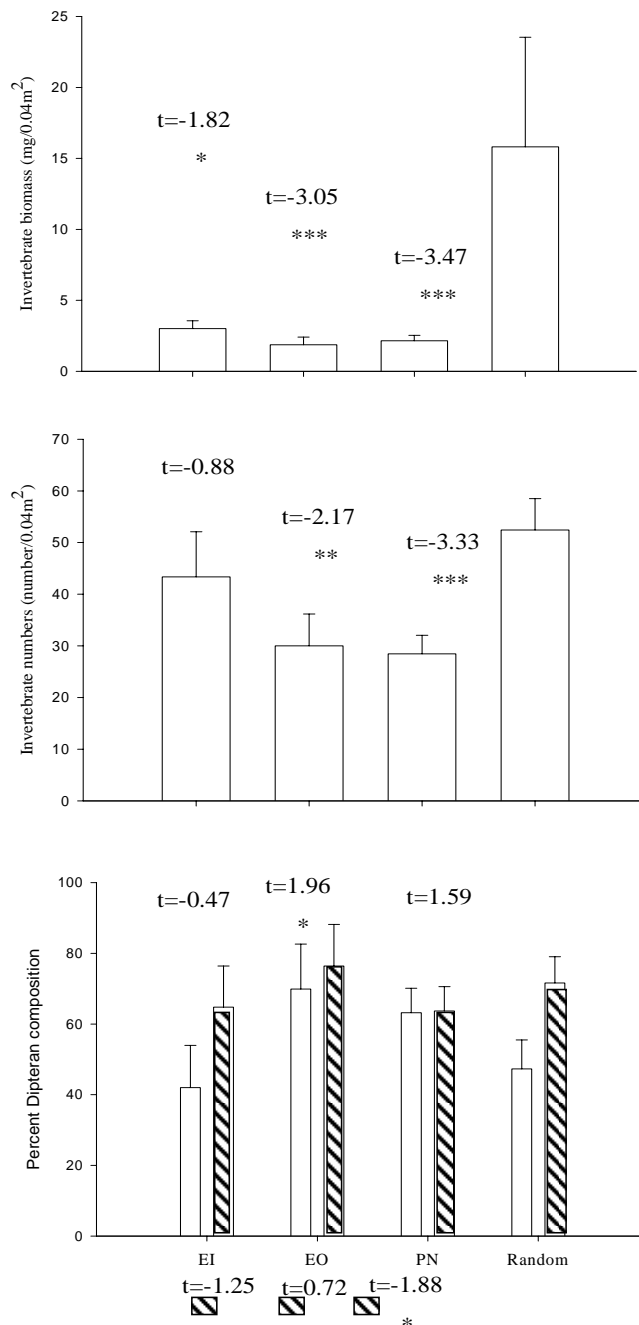


Figure 14. Test of darter and random invertebrate abundance for spring 2002. Histograms represent mean invertebrate biomass (top), numbers (middle), and mean ratio of Dipteran/total biomass () and numbers (//////) (bottom). Vertical lines represent ± 1 standard error. Student's t-tests were used to compare each species to random. Species abbreviations are as follows: EI = *Etheostoma inscriptum* (n=17), EO = *E. olmstedii* (n=13), PN = *Percina nigrofasciata* (n=49), and R = random (n=37). * = $p \leq 0.10$, ** = $p < 0.05$, *** = $p < 0.01$.