HABITAT USE OF LARVAL AND JUVENILE CAPE FEAR SHINERS (*NOTROPIS MEKISTOCHOLAS*)

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HABITAT USE OF LARVAL AND JUVENILE CAPE FEAR SHINERS (*NOTROPIS MEKISTOCHOLAS*)

Andrew Reid Henderson

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HABITAT USE OF LARVAL AND JUVENILE CAPE FEAR SHINERS

(*NOTROPIS MEKISTOCHOLAS*)

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VITA

Andrew Reid Henderson was born in Birmingham, AL, on July 25, 1980, and raised in Springville, Alabama. He attended Auburn University and graduated with a bachelor's degree in Fisheries and Allied Aquacultures in 2004. After graduation, he worked as an intern for the Stream Survey Team of the Georgia Department of Natural Resources. In July 2005 he began graduate school in the Department of Fisheries and Allied Aquacultures.

THESIS ABSTRACT

HABITAT USE OF LARVAL AND JUVENILE CAPE FEAR SHINERS

(NOTROPIS MEKISTOCHOLAS)

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The factors responsible for imperilment of freshwater fishes are diverse, but most can be categorized as some form of habitat loss or alteration. Understanding effects of various forms of habitat alteration on single fish species is difficult, since basic knowledge of habitat requirements is usually lacking for non-game fishes. Information on larval and juvenile habitat use is virtually absent for fishes, particularly those listed as imperiled. Cape Fear shiners are a federally endangered species restricted to just five localities in the Cape Fear River drainage, North Carolina. My objectives were to document habitat use of larval and juvenile Cape Fear shiners (*Notropis mekistocholas*), in both natural and lab settings so that a better understanding of habitat requirements in all life stages can be achieved for conservation purposes. I measured habitat parameters of areas used by Cape Fear shiners in the summers of 2007 and 2008 in the Rocky River, NC. Field data suggest larvae use more shallow depths and have a tendency to use reduced water velocities than adults. Juveniles 15-25 mm TL often school with adults. Experimental tanks were used to separate habitat variables in a lab setting with captive-bred individuals in order to validate field observations. In the mesolarval, metalarval, and multiple juvenile stages, Cape Fear shiners preferentially chose flow and depth microhabitats. In the laboratory larvae showed preferences for moderate current velocities and shallow depths. Juveniles showed preferences for moderate and swift current velocities and deep depths. These findings were consistent with field observations. Data suggests that like adults, Cape Fear shiners are patch restricted in early life stages, and movement and dispersal between patches is limited. Protection of habitat patches is important for conservation of this species.

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INTRODUCTION

Loss of native freshwater fauna is occurring in the southeastern United States and worldwide, especially in areas where exponential increases in human populations have occurred in recent decades (Folkerts, 1997). Conservation of endemic freshwater fishes is a challenge throughout North America, particularly in the southeast where the fish fauna is very diverse (Etnier, 1994; Warren, et al. 2000). Information regarding ecology and early life history of imperiled fishes is essential for their protection. Investigations of habitat use at multiple developmental stages allow scientists and resource managers not only to protect and restore habitat, but also to determine factors affecting species persistence. Decline of effective population sizes and regional extirpation of fishes have been attributed to habitat loss or alteration (Angermeier, 1995; Winston et al., 1991). Geographic restrictions and isolation of freshwater fishes also greatly increases risk of extinction (Burkhead et al., 1997).

Species that exist in small numbers, such as Cape Fear shiners, are especially vulnerable to extinction due to demographic and environmental stochastic events (Burridge and Gold, 2003). The Cape Fear Shiner (*Notropis mekistocholas*) is federally listed as a critically endangered species, due to a very restricted distribution (Fig. 1). The species is known from just five populations in the Cape Fear River drainage, North Carolina, only four of which are currently self-sustaining (Hewitt et al., 2006). These five populations occur in the main stem and tributaries of the Deep, Haw, and Rocky Rivers (Pottern and Huish, 1985, 1986, 1987). The largest and most viable population is located at the Confluence of the Deep and Rocky rivers in Chatham and Lee counties in North Carolina, U.S.

Historically the Cape Fear shiner is thought to have had a very restricted distribution and was not described until recently (Snelson, 1971); it is unique in the genus *Notropis* in that it is a Piedmont endemic. Its long coiled intestine has been thought to be an adaptation to a predominately herbivorous diet, but others have observed opportunistic feeding behavior on both plant and animal matter in the wild and captivity (Mandy Hewitt, John Groves, personal communication). Habitat fragmentation and degradation are proposed to be the greatest threats to the Cape Fear shiner, which has the smallest range of any species of *Notropis*. The building of impoundments in the Cape Fear watershed has fragmented the landscape and isolated populations of the species (Pottern and Huish, 1985). Demographic and genetic consequences of river fragmentation have been reduced effective population sizes for cyprinids and resulted in regional extirpation (Alo and Turner, 2005; Winston et al. 1991). Adult cyprinids that are patchily distributed among favorable habitats have displayed limited short and long-term movements and are generally sedentary (Hill and Grossman, 1987; Johnston, 2000). Additionally, reduced water quality in the region has had deleterious effects, particularly in the Haw River watershed, where habitat is unsuitable and the current status of the Cape Fear shiner is uncertain (Hewitt et al., 2006).

There is little documentation of the Cape Fear shiner life history, and habitat use has been assessed only for adults (Howard, 2003). *Notropis mekistocholas* live about three years in the wild (Snelson, 1971), and reproduce after their first year, spawning over coarse substrata in the spring and summer months when water temperatures are warm (Pottern and Huish, 1985). Early life-history habitat requirements are not known for most freshwater fishes. This is especially true for cyprinids, such as Cape Fear shiners. Members of the genus *Notropis* are known to broadcast their eggs over a preferred substrate, and some species are nest associates (Johnston and Page, 1992). Previous studies with the Cape Fear shiner have focused on adult habitat use, toxicology, and genetic considerations (Howard, 2003; Burridge and Gold, 2003; Saillant et al, 2004).

Within their range, Cape Fear shiners are clumped in distribution in habitat patches characterized by clean flowing water over mixed coarse substrates, and are associated with aquatic vegetation, particularly during the breeding season (Howard, 2003). The most common aquatic vegetation encountered within the Cape Fear shiner range is American water-willow (*Justicia americana*) and to a lesser extent Arrowhead (*Saggitaria spp*.), and Cattail (*Typha spp.*). Riverweed, (*Podestomum ceratophyllum*) is abundant on the riverbeds, covering coarse substrates year-round. Larvae and juveniles may use more shallow areas than adults in slack water or no flow (Pottern and Huish, 1986), but description of larval and juvenile habitat use is lacking for this species. Although habitat is limiting at some historical localities, there are habitat patches that appear suitable for occupation but are not used (Howard, 2003). Such vacant habitat patches may be outside the dispersal range of the species, or may not be used due to a lack of nearby or adjacent habitat appropriate for larvae or juveniles. Fragmentation of habitat by construction of impoundments has isolated populations of Cape Fear shiners (Fig. 1).

Genetic studies to date indicate that while genetic diversity appears relatively high in the populations of Cape Fear shiners at two localities (Confluence of Deep/Rocky Rivers and Upstream Deep River), both populations appear to have undergone significant recent decline in effective size (Saillant et al., 2004). A major concern for small populations of threatened or endangered species like *N. mekistocholas* is the potential for reduced fitness and accumulation and fixation of slightly deleterious recessive mutations, warranting continual monitoring of populations (Frankham, 1995; Burridge and Gold, 2003). Genetics typically affect populations on a longer time scale than ecological and habitat degradation impacts. Population fragmentation from the building of impoundments results in demographic consequences for aquatic organisms, especially cyprinids (Schrank et al., 2001, Winston et al, 1994). There is sufficient gene flow between populations of Cape Fear shiners but declines in effective population sizes (Saillant et al., 2004). There is adequate adult habitat available but not used, and suitable habitat is fragmented by impoundments (Howard, 2003). Habitat requirements for critical early life-history stages are potential limitations to dispersal and colonization of new habitats.

Despite recent investigative research on this species (Howard, 2003; Burridge and Gold, 2003; Saillant et al., 2004), there are still unknown factors determining the success and persistence of the Cape Fear shiner. Habitat required for early life may be crucial to regional persistence of species. The Cape Fear shiner is frequently observed in and near patches of American water-willow (*Justicia americana*), which seems to be important in all components of the life history of the fish, presumably because of the shiner's opportunistic feeding behavior. Adults are observed in velocity breaks, areas of swift water adjacent to areas of slack water. If no suitable habitat exists within the maximum dispersal distance, larval and juvenile dispersers are lost to the population at a constant rate, which decreases first and second year survival (Taylor et al., 2002). If Cape Fear shiners have low dispersal ability and are restricted to five distinct populations, assessment and description of larval and juvenile habitat choice is a link between recent research efforts with this species. These aspects will be important for establishing future management priorities.

Habitat required for early life stages of many species of cyprinids is not known, in particular species that are of conservation concern. The breeding behavior and aspects of life history of closely related wide-ranging sympatric species of the Cape Fear shiner such as the swallowtail shiner (*Notropis procne*) and the sand shiner (*Notropis stramineus*) have received close examination (Raney, 1947; Summerfelt and Minckley 1969). *Notropis mekistocholas*, because of its limited distribution and endangered listing status since 1987, has received relatively little attention. Snelson (1971) considered *N. procne* and *N. stramineus* to be the closest relatives to *N. mekistocholas.* The early development of *N. procne* and *N. stramineus* has been documented from wild-caught individuals, but their habitat associations are limited to general observations (Loos and

Fuiman, 1978; Perry and Menzel, 1978). The range of *Notropis mekistocholas* is much smaller than these species (Hocutt and Wiley, 1986), and specific habitat requirements for early life of *N. procne* and *N. stramineus* have not been investigated.

Habitat preferred in early life has been documented for the Coastal shiner, (*Notropis petersoni*) from the Cape Fear River basin. Comparisons were made regarding life history and ecology of stream tributaries, Cape Fear River, and Waccamaw Lake populations of *N. petersoni* (Davis and Louder, 1971). *N. mekistocholas* is sympatric with *N. petersoni* in a few reaches within the Cape Fear watershed, and both are associated with aquatic vegetation during the breeding season. However, *N. mekistocholas* prefers coarse substrates and swift current velocities (Howard, 2003), while *N. petersoni* is associated with black-water streams in which sand substrates and slow current velocities are common (Davis and Louder, 1971). Also, unlike *N. mekistocholas, N. petersoni* has a wide distribution in many river basins along the Atlantic slope (Hocutt and Wiley, 1986). Howard (2003) investigated adult habitat use and conducted population estimates of *N. mekistocholas* throughout their range in the Deep, Haw, and Rocky Rivers, along with tributary populations in both breeding and non-breeding seasons. Limitations of that study were young of year abundances and habitat use was not included.

Reproductive habitat has not been directly assessed for *N. mekistocholas*. Howard (2003) did not document a spawning event, and made assumptions regarding the duration of breeding and non-breeding seasons for the Cape Fear shiner. Members of the genus *Notropis* typically 'broadcast' eggs and sperm in the water column, off of the substrate in flowing water. Minnows of the genus *Notropis* are fractional spawners, and females spawn more than one clutch of eggs during the reproductive season (Heins and Rabito, 1986). The eggs of many members of this genus are adhesive or semi-adhesive and settle in the substrate or attach to submersed aquatic vegetation. *Notropis mekistocholas* eggs are semi-adhesive, and settle and stick to vegetation or mixed substrates (Pat Rakes and J.R. Shute, unpublished data). Cape Fear shiners have been observed in breeding coloration and seined multiple times in shallow water less than 10 cm deep over American water-willow (*J. americana*). It is likely that this species spawns in and around American water-willow beds and eggs attach to vegetation, although this has not been directly observed. Howard (2003) confirmed adult Cape Fear shiners were closely associated with *J. americana* during the breeding season.

Emergent aquatic vegetation is common on the river margins in the Rocky River. Shallow depths, slow current velocities, and boulder and cobble substrates are common around emergent vegetation often within close proximity to riffle habitat. Gentle current velocities with emergent vegetation are a good source of oxygen, and cover supplied by vegetation no doubt provides refuge from predators for young-of-year cyprinid species (Garner, 1996; Scheidegger and Bain, 1995; Watkins et al., 1997).

Objectives

The objectives of this study with Cape Fear shiners followed the recovery plan for the species developed by Biggins (1998). My objectives were: to investigate larval and juvenile habitat use and habitat choice *in vitro* using captive bred individuals and to validate field observations and measurements of habitat used by larvae and juveniles in the Rocky River, North Carolina. Laboratory experimentation allowed assessment of variables not controlled in nature, and examination of dispersal potential by young of year fishes. Available habitat was measured within habitat patches used by adult Cape Fear shiners in the Rocky River, NC and compared to habitat use in summers of 2007 & 2008 using frequency distributions and non-parametric statistical tests. Habitat variables used by larval, juvenile, and adult Cape Fear shiners for each sampling date were compared in 2007 and 2008 and across years to assess differences in temporal habitat preferences differ among size classes. Conservation efforts that include early life history habitat requirements will provide needed insight into this critically endangered species (Hilton-Taylor 2000).

MATERIALS AND METHODS

Field Study Sites

The extant populations of the Cape Fear shiner are in few tributaries and main-stem reaches of the Deep, Haw, and Rocky Rivers (Fig. 1). The largest and most viable population of Cape Fear Shiners is at the confluence of the Deep and Rocky rivers (Pottern and Huish, 1985), where the river channel is wide with a large shoal. The Cape Fear River is the largest river basin located entirely within the state of North Carolina, with 9,735 km of freshwaters and streams. The Rocky River is located almost completely within Chatham County, North Carolina. The Rocky River is in the Carolina Slate Belt and is approximately 56 kilometers in length. Land use within the Rocky River watershed is primarily agriculture, dairy production, and forest (NCDWQ, 2004). The Rocky River is a large tributary to the Deep River, and these rivers meet to form the Cape Fear River near the Fall Line, bordering Chatham and Lee counties, NC. There is a large non-hydropower impoundment high in the watershed, the Rocky River reservoir, which is the water supply for Siler City, NC. There are also several small, low-head impoundments located throughout the river that were once used as mill dams, which have fragmented Cape Fear shiner populations and contributed to loss of habitat suitable for Cape Fear shiners (Howard, 2003; Pottern and Huish, 1985).

The primary study sites for all habitat observations and available habitat and habitat use measurements are approximately 450 (35.61867 N, -79.15883 W) and 600

(35.61845 N, -79.16124 W) meters upstream of the confluence of the Deep and Rocky Rivers on the Rocky River. These habitat patches are the first with abiotic habitat heterogeneity proceeding upstream from the Confluence of the Deep and Rocky Rivers, and are the first areas supporting populations of Cape Fear shiners. There are coarse substrates breaking the water surface with aquatic vegetation, and variable current velocities in riffle and run habitats. The substrates used by adult Cape Fear shiners in the Rocky River are predominately bedrock with varying amounts of boulders, cobble, and gravel (Howard, 2003). Water flows are moderate to low throughout the summer. American water-willow (*Justicia americana*) as well as other emergent aquatic and terrestrial vegetation is abundant on river margins, particularly in summer months, and beds of *J. americana* are common around boulders and cobble that break the water surface throughout the river channel.

Field Work

Monitoring of Cape Fear shiners was conducted by snorkeling in spring and summer months of the years 2007 and 2008. Snorkeling is a non-intrusive method for observing endangered fishes, and has been used by previous investigators of this species (Howard, 2003), and others (Johnston, 2000). Schools of Cape Fear Shiner larvae were flagged and monitored at the first study site on the Rocky River 550 m upstream from the confluence of the Deep/Rocky Rivers in May and August 2007, and April and July 2008 (35.61867 N, -79.15883 W). This habitat patch is 150 m in length, and approximately 6,300 m^2 in total area. The study site upstream from this location was monitored April -

July 2008 (35.61845 N, -79.16124 W). This habitat patch is 200 m in length, approximately 5,200 m^2 in total area, and is 175 m upstream from the downstream monitoring site. Cape Fear shiners adults are known to occur in these patches (Howard, 2003), and microhabitats within these patches are favorable for young of year fishes. Pool and deep run habitats were searched snorkeling upstream and downstream from these patches to determine if Cape Fear shiners occurred outside of these patches, and no *N. mekistocholas* were observed upstream or downstream. Deep run/pool habitats upstream and downstream of suitable habitat patches were not favorable for Cape Fear shiner occupation (Howard, 2003), and within these habitats large numbers of fishes known to be predators of minnows in larval, juvenile, and adult stages (e.g. *Micropterus salmoides*) were present (Harvey, 1991; Schlosser and Angermeier, 1990).

I used a small ruler when snorkeling to measure lengths of Cape Fear shiners. Within favorable habitat patches, Cape Fear shiners were commonly observed schooling in early life with other minnows, notably the swallowtail shiner (*Notropis procne*), river chub (*Nocomis leptocephalus*), and striped shiner (*Luxilus chrysocephalus*), which are the numerically dominant cyprinids within the study area. The use of underwater visual sampling has been used to assess habitat specificity for aquatic species where traditional methods (seine, electrofishing) are inefficient, the habitat is complex, or the fish species is threatened or imperiled (Freeman and Grossman, 1993; Johnston, 2000). Distance sampling techniques are non-intrusive and provide a way to assess age-0 fish abundance that cannot be obtained using traditional techniques (Pink et al., 2007). Habitat suitability for adult Cape Fear shiners in patches in the Rocky River is high (Howard, 2003). Cape

Fear shiners were observed by snorkeling in an upstream direction to minimize disturbance, and colored weights and/or plastic flagging were used to mark microhabitats occupied by fish. Similarities in microhabitat use of multiple size classes of fishes within and among species, including cyprinids, has indicated recommendations for resource management should be based on microhabitat use data collected in conjunction with availability data (Moyle and Baltz, 1985).

Physical habitat variables along random transects greater than 5 meters apart within habitat patches occupied by adults were measured in May and August 2007, and April and July 2008 on the Rocky River, NC. Available habitat transects included the following abiotic variables: wet river width, current velocity (m/s), water depth (cm), substrate composition, and % cover. Current velocity was measured with a Marsh-McBirney 2000 flow meter. Water depth was measured with a meter stick to the nearest centimeter, and substrate composition and % cover were visually estimated. Substrate was categorized according to a modified Wentworth scale: gravel 1-50 mm, cobble 50-250 mm, boulder 250-2500 mm, bedrock > 2500 mm. Point measurements of these variables were also taken on these dates for larvae, juvenile, and adult *N. mekistocholas*.

Where schools of Cape Fear Shiners were observed, I took point measurements of habitat variables. Three size classes of Cape Fear shiners were monitored: larvae 1-15 mm TL, juvenile 15-35 mm TL, and adult > 35 mm TL. Measurements of habitat use included fish focal water velocity (m\s), substrate characterization based on a modified Wentworth scale (cm), fish focal depth (cm), and percent canopy/vegetative cover. Current velocity was measured with a Marsh-McBirney 2000 flow meter. Fish focal depth and substrate size were measured with a meter stick to the nearest centimeter. Percent cover included terrestrial vegetation canopy cover, aquatic macrophytes, algae, rock overhang, roots, and woody debris. Substrate was categorized according to a modified Wentworth scale listed above. A classification of substrates including bedrock in most combinations was chosen because Cape Fear shiners often were found over a mixed matrix of substrates, and bedrock was included in most combinations because it is the dominant substrate in the Rocky River. Silt and sand are uncommon in the Rocky River (Howard, 2003). Cover was categorized and given a ranking from 0-100%, and corresponding categories 1-10 were used in data analysis. To determine if young of year fishes occupy near shore habitats more frequently than adults, distance to bank was included in point measurements in 2008 for larvae, juvenile, and adult *N. mekistocholas*. To validate species field identification larval and juvenile *N. mekistocholas* were collected from the Rocky River, NC on 28 June 2007. In 2008 larval and juvenile Cape Fear shiners were preserved on the following dates: 2 May, 12 June, and 18 July (USFWS permit # TE163433-0).

Available habitat and use data were analyzed with principal components analysis (PCA) with varimax rotation. PCA was performed for habitat availability and use data compiled and separated by year for a total of four analyses. These analyses illustrated habitat variable loadings. Additionally, frequency histograms were constructed for each habitat variable by date to compare available habitat to habitat used for 3 life-history stages of the Cape Fear shiner: larvae 1-15 mm TL, juvenile 15-35 mm TL, adult > 35 mm TL. Kolmogrov-Smirnov two-sample tests were used to compare depth, flow, and substrate, which were treated as continuous habitat variables, to compare each life-history stage use to available habitat. Chi-square tests were used for cover, which was treated as a categorical habitat variable. Each life history stage, larvae, juvenile, and adult was compared to other size classes for each of the four habitat variables measured using K-S tests to see if habitat use at stages differed for all dates. Fish distance to bank for point measurements was analyzed using K-S tests. All data were analyzed with SPSS (11.0). Alpha for all statistical analysis was 0.05, and Bonferroni correction for multiple comparisons procedures was applied to K-S and chi-square analysis.

Captive Population Transfer

The Cape Fear Shiner recovery plan calls for propagation and maintenance of a captive population to establish a genetic reservoir of the species in case of the occurrence of a catastrophic event in the wild, and for possible re-introduction efforts in the future. This work began at the North Carolina Zoological Park in Asheboro, NC in 1998, under John Groves, using specimens collected from the Deep/Rocky River confluence site. The Zoo provided eggs and recently hatched fry for experimentation on 5 May 2008. Eggs and fry were siphoned from the bottom of 5 tanks of age classes 3-4 years of adult *N. mekistocholas,* and a small aquarium net was used to collect eggs and fry with water from tanks and they were deposited in 10 g plastic storage bags. Bags were filled and placed in a 50 gallon cooler. There were 20-25 eggs per bag. Five bags contained 10-15 fry. These 20 bags were then pumped with oxygen for transport, and bags were sealed with rubber bands. Condition of fish eggs and fry in addition to water temperature were checked periodically during transport.

Upon arrival in Auburn, AL, bags were allowed to acclimate in 10 g aquaria, and then poured into large (8-10 cm) aquarium nets suspended in 10 g tanks. One tank had fungus on eggs within 12 hours after arrival in Auburn so all tanks were immediately treated for fungus with an anti-fungal fish medication. The remaining tanks had 60-80% survival, but 95% mortality occurred in the tank with fungus. Natural lighting was provided from adjacent windows. Water temperature in tanks holding eggs and fry was monitored every 12 hrs. Water quality in holding tanks including ammonia, nitrate, nitrite, and pH was checked 3 times weekly. Fry developed to the mesolarval and metalarval stages, which were tested for habitat preferences in the laboratory in an experimental tank. There were 20 trials each conducted at various times of day for 2 larval size classes, mesolarvae (7-12 mm), metalarvae (14-16 mm). Descriptive terms of intervals of development follow definitions by Loos and Fuiman (1978) and Fuiman et al. (1983).

After complete mortality of all larvae by June 9 (day 35), the Zoo made an additional 125 larval Cape Fear shiners available on 15 June 2008. These fish were hatched between May 1 and 15 2008. Larvae were transported using the same methodology as listed above. Fish were allowed to acclimate, and then placed directly into 10 g aquaria for holding. Cape Fear shiner larvae were fed Hikari freeze-dried daphnia until day 85 then were shifted to Tetramin Flake Fish Food. Also, larvae commonly fed on algae growing on holding tank walls. The exact ages of the larvae were not known, but all were between 35-50 days old upon arrival in Auburn, AL. Water temperature and pH were monitored daily in holding tanks, and ammonia, nitrite, and nitrates were monitored weekly. Larvae developed to early, mid, and late juvenile stages, which were tested for habitat preferences in the laboratory in an experimental tank. There were 20 trials each conducted at various times of day for 3 juvenile size classes: early juvenile (20-24 mm), juvenile (23-32 mm), and late juvenile (31-36 mm). Descriptive terms of intervals of development follow definitions by Loos and Fuiman (1978) and Fuiman et al. (1983).

Larval/Juvenile Habitat Choice Tanks

Cape Fear shiner eggs and fry were obtained from the captive population to conduct a simulated stream tank experiment to facilitate comparisons of habitat choice in early life in the laboratory to that of natural conditions. Larval/juvenile habitat choice was measured in a 20 g aquarium, 0.78 m long, 0.32 m wide, 0.32 m deep, and a large indoor 270 g tank, 1.8 m long, 0.7 m wide, 1 m deep; Aquatic Eco-Systems, Inc. Initially I planned to use only the large tank, but mortality of small larvae upon introduction into the large tank was high in preliminary trials. As a result, the larger tank design was 'scaled-down' to a 20 g aquarium for larval trials at 30 and 56 days development (Fig. 2). Tanks had identical designs in terms of arrangement of habitat variables, with the exception of current velocity. Both tanks contained appropriate habitat as determined from field observations, including monitoring efforts of larvae and juveniles made in the summers of 2007-2008 on the Rocky River. A factorial design with all treatment combinations possible for each trial was used to assess habitat use within both tanks.

Response to two flows, two depths, and two substrates were tested in a 20 g tank for the first two age classes of Cape Fear shiners at 30 days and 56 days development

(Figure 2). Two Second Nature 640 powerhead pumps 0.02 hp, suspended in the water column were used to generate current. Shallow depths were achieved by using clay bricks 19.5 cm long, to elevate the substrates on one side of the tank. Gravel and small cobble substrates were placed in random design determined by coin toss within the small tank. Cape Fear shiner larvae were randomly placed in groups of three in trials lasting 10 minutes each. Fish were randomly chosen from holding tanks. Larval fish were introduced in random locations throughout the tank for each trial. General observations of fish behavior were noted during the acclimation period. After 5 minutes, the pump providing water flow was turned on. Fish habitat selectivity was noted every 30 seconds for 5 minutes for a total of 10 observations per trial. A total of 40 trials were conducted with larval Cape Fear shiners. Larval flow preferences, depth, and substrate choice were observed at the mesolarval and metalarval stages. Specific habitat measurements along with comparisons of habitat use were made within the tank in relation to fish choice. Time in seconds was used as a response variable corresponding to how long fishes occupied a specific set of habitat variables. The effect of size of mesolarvae or metalarvae was also tested as a response variable in a three-way multivariate analysis of variance (MANOVA).

Juvenile habitat preferences were tested within the large tank in response to three flows, 2 depths, and 2 substrates (Figure 3). Cape Fear shiner juveniles were tested at 70, 90, and 125 days development. A 1/3 horsepower low head pump was used to generate water flow on one end of the tank. Water was pumped through 2 PVC tubes 0.6 m long with holes every 2.5 cm for uniform flow the length of the tank. Shallow depths were achieved by using 61 cm long solid cinder blocks stacked 2 deep the full length of the tank. Gravel and mixed small and large cobble substrates were placed in 37.9 L Rubbermaid tubs, and separated in tubs with plastic lawn edging. Tubs were randomly arranged with respect to substrate combinations within flow and depth treatments by coin toss. Groups of three fish were introduced in random locations into the experimental tank for trials lasting 10 minutes each. Juvenile fish were introduced and allowed to acclimate for 5 minutes. General observations of fish behavior were noted during the acclimation period. After 5 minutes, the pump providing water flow was turned on, and fish habitat selectivity was noted every 30 seconds for 5 minutes for a total of 10 observations per trial. A total of 60 trials were conducted with Cape Fear shiner juveniles. Flow preferences, depth, and substrate choice were observed at 3 juvenile developmental stages, 20 trials per size class. Habitat measurements along with comparisons of habitat use were made within the tank in relation to fish choice. I also measured the maximum distance moved, in meters, from initial deposition to where fish settled or the habitat combination where fish spent the most time during trials. Time in seconds and size were treated as response variables. Time corresponded to the amount of time fish spent in a specific suite of habitat variables. A three-way multivariate analysis of variance (MANOVA) was used for analysis. Post-hoc comparisons for early, mid, and late juvenile Cape Fear shiners were conducted using Bonferroni multiple comparisons procedure to identify the size class responsible for the largest amount of variation in results.

The only difference in the experimental design of the small and large tank was the small tank tested response to 2 current velocities (low, medium) and the large tank tested response to 3 current velocities (low, medium, high). All other variables were functionally identical. Mesolarvae and metalarvae trial data were separated from juvenile trials because the larger tank tested preference of three current velocities in contrast to the smaller tank, which only tested preference of two current velocities. Time in seconds and size were treated as response variables in both small and large tank analyses. Time corresponded to the amount of time fish spent in a specific suite of habitat variables. Larval and juvenile tank data were analyzed separately using a general linear model (GLM) multivariate analysis of variance (MANOVA) in SPSS (Ramsey and Schafer, 2002). There were a total of 100 trials conducted at various times of day for 5 size classes: mesolarvae (7-12 mm), metalarvae (14-16 mm), early juvenile (20-24 mm), juvenile (23-32 mm), and late juvenile (31-36 mm). Maximum distance moved, measured in meters from the introduction point to where fish settled in juvenile trials, or spent the majority of time during trials, was tested with K-S tests.

RESULTS

Habitat Use - Field

Results from comparison of available habitat to use indicated Cape Fear shiners preferred depths that were significantly different from available in 2007 but not 2008 (Tables 1-8). Temporal variability in microhabitat selection by size classes of *N. mekistocholas* was apparent in 2007 and 2008. In May 2007, Cape Fear shiner adults preferred depths that differed from available (p = 0.006) (Table 3). In July 2008, juveniles used habitats associated with higher cover than available (p = 0.004) (Table 7).

Frequency histograms illustrating available habitat and use in each life history stage by date reveal differential use of habitat variables by all size classes and shifts in use of these variables over time (Figs. 4-27). Trends from frequency histograms of habitat used by each life history stage indicated juveniles used gravel/bedrock and cobble/bedrock substrates more than larvae or adults (Fig. 16), and larvae used more shallow habitats than juveniles or adults in May 2007 (Fig. 4, Table 1). Also, adults appear to use deeper depths than larvae and juveniles (Fig. 4, Table 1). In August 2007, juveniles again used gravel/bedrock and cobble/bedrock substrates differing from larvae and adults (Fig. 17). Larvae used habitats in August 2007 with cover more than juveniles and adults (Fig. 23, Table 2). Trends from frequency histograms of April 2008 comparisons of habitat used by each life history stage indicated larvae used significantly different depths from juveniles and adults, and juveniles used deeper depths than larvae and more shallow depths than adults (Table 2, Fig. 6). In July 2008, juveniles used habitats with larger amounts of cover that differed from larvae and adults (Table 2, Fig. 25).

Available habitat and use by larval, juvenile, and adult Cape Fear shiners was combined for each habitat variable measured and compared across years for 2007 and 2008 (Table 9). Available depth, current velocities, substrata, and cover were not significantly different ($p > 0.05$) across years. Distance to bank measured for larvae, juvenile, and adult Cape Fear shiners in 2008 was not significantly different ($p > 0.05$). Habitat use for each size class was not significantly different in 2007 (Tables 3-5). Juvenile *N. mekistocholas* used habitats with cover significantly more than adults in July 2008 (Table 8).

Principal components analysis (PCA) of available habitat and use variables in 2007 revealed combination of available and use resulted with component 1 scores had positive loadings for substrate and depth. Component 2 scores had positive loadings for cover and current velocity habitat variables (Table 10, Fig. 30). In 2008, component 1 scores had positive loadings for current velocity and depth, and component 2 had positive loadings for substrate and cover habitat (Table 11, Fig. 31). A second PCA combined all habitat use only variables for larval, juvenile, and adult Cape Fear shiners in 2007 & 2008. In 2007 & 2008 when all life stages were separated by size class, larvae and juveniles were associated with coarse substrates, shallow depths, slow current velocities, and high cover (Figs. 32 and 33).

Habitat Use - Lab

Experiments involving Cape Fear shiner mesolarvae at 30-32 days development (5-12 mm TL) were tested in the 20 g tank on 5 June 2008 (Fig. 2). Metalarvae were tested on 18 June 2008 at 50-54 days development (14-16 mm TL). Results from the small tank trials indicate that at an early age and small sizes, mesolarvae, 7-12 mm TL, and metalarvae, 14-16 mm TL, Cape Fear shiner choice of flow was statistically significant, indicating strong selection of habitat variables ($p = 0.013$) (Table 14). Depth preferences for mesolarvae and metalarvae were also significant ($p = 0.001$) (Table 14). Mesolarvae and metalarvae showed preferences for moderate current velocities 0.05-0.10 m/s (Figure 2). Larvae also preferred shallow depths 10-15 cm. There was also an effect of larvae size on depth preference ($p = 0.04$), indicating shallow depth choice as a function of fish size (Table 14). Specific choice of gravel or cobble substrates was not significant for mesolarvae or metalarvae.

Cape Fear shiner juveniles were tested in the large tank on 11 July (70 days development, 20-24 mm TL) 29 July (90 days development, 23-32 mm TL), and 5 September 2008 (125 days development, 31-36 mm TL). Results from the large tank experiment indicate that Cape Fear shiners in early, middle, and late juvenile stages preferentially chose flow and depth habitats (Table 15). Juveniles showed preferences for moderate and fast current velocities 0.05-0.10, and > 0.13 m/s ($p = 0.015$). Juveniles also chose deeper water column depths 35-55 cm ($p < 0.001$), in contrast to mesolarvae and metalarvae, who chose shallow depths. Specific choice of gravel or cobble substrates was not significant for all stages of juveniles. Specific substrate preference was not significant for mesolarvae, metalarvae, or all three sizes of juveniles in tank trials in the laboratory (Tables 14 and 15). Maximum distance moved from introduction into the juvenile test tank was statistically insignificant for all three juvenile stages. Similar to larvae, solitary juveniles generally moved shorter distances than fish in pairs, who were more likely to exhibit greater movement and explore larger areas of the experimental tank. Results from post-hoc multiple comparisons indicate significance of mid and late juveniles on selection of habitat variables.

DISCUSSION

Young of year Cape Fear shiners monitored at study sites in the Rocky River were found consistently in the same microhabitats, and development through the juvenile stages was observed in the summers of 2007 and 2008. In this study, field habitat use data for 2007 and 2008 years suggests depth and cover habitat variables measured on at least one date accounted for persistence of *N. mekistocholas* in differing life history stages. Results from field and laboratory components of this study suggest that Cape Fear shiners spend their early life in the same mesohabitat patches occupied by adults, but summary statistics, frequency histograms and observations reveal segregation by differing size classes within these patches. Protection of in-stream abiotic physical habitat variables measured is critical for persistence of early life history stages of this species.

The influence of habitat features on the distribution of fishes has been widely studied, and influence of habitat configuration in comparisons of microhabitat assemblages have been documented (Angermeier and Schlosser, 1989). Members of the genus *Notropis* have shown patterns of non-random microhabitat use, including young of year fishes (Grossman and Freeman, 1987; Gorman, 1988). Cape Fear shiner adults are almost exclusively found in riffle and shallow run habitats throughout their range in both spawning and post-spawning seasons, are clumped in distribution, and favor patchy habitats with high heterogeneity (Howard, 2003). Clumping behavior of cyprinids at low population densities in patchy habitats is influenced by shelter characteristics and predation risk (Fraser and Sise, 1980). Many adult cyprinids are sedentary in favorable habitat patches, and distribution can be restricted to these patches (Johnston, 2000).

A drought throughout the southeast in 2007 contributed to low water levels and reduced current velocities, and also a dramatic increase in abundance of blue-green filamentous algae (*Cladophora spp.*) in the Rocky River, which reduced abundances and detectability of Cape Fear shiners in all developmental stages in late summer in the Rocky River. Annual variation in physical habitat variables is common in North American freshwater rivers and streams, and environmental variability has been shown to affect fish assemblage structure (Grossman et al., 1998). However, dissolved oxygen levels were drastically reduced in late summer in the Rocky River in 2007 to 4 ppm, resulting in decreased abundance of Cape Fear shiners in all life stages, in addition to other rheophilic species. Larval and juvenile abundance of minnow species has been shown to be particularly sensitive to flow conditions and habitat requirements (Harvey, 1987; Schlosser, 1985). Low water levels, slow current velocities, and coarse substrates; all characteristics of habitat in the Rocky River in late summer 2007, favor *Cladophora* blooms. These filamentous algae blooms have been associated with increased nutrient levels and community-level habitat shifts (Dodds, 1991).

The overlap of interspecific young-of-year cyprinids in microhabitat use during reduced river discharge has been associated with limitation of access to littoral vegetation (Copp, 1992). Ability of age-0 cyprinids to determine variations in current velocity over small spatial scales for multiple developmental stages is dependent upon discharge

(Freeman et al., 2001; Garner, 1991). Cape Fear shiners in differing size classes did not use substrates significantly different from available or other size classes. Specific substrate preference was not significant for mesolarvae, metalarvae, or all three sizes of juveniles in tank trials in the laboratory. This was consistent with field data. Variability in cobble substrate sizes in the larval and juvenile test tanks could have affected results, but small and large cobble substrates are abundant in the Rocky River, and presence and abundance of young of year Cape Fear shiners over certain substrates may be more of an association with riffle microhabitat than with particular substrata. Shifts to more coarse substrates corresponding with ontogenetic development (Simonovic et al., 1999) in the juvenile and adult stages were not observed for Cape Fear shiners.

Focal depth was significantly different from available for Cape Fear shiner larvae, juveniles, and adults in the Rocky River in 2007, and larval and juvenile size classes chose depths preferentially in lab tank experiments. Cape Fear shiner larvae chose shallow habitats < 5 cm deep in both the mesolarval and metalarval stages in an experimental setting. This was in contrast to the choice of deep habitats > 35 cm by three age classes of juveniles. The use of shallow water habitats by cyprinid larvae in floodplains and regulated and unregulated rivers has been widely documented in the U.S. and Europe (Scheidegger and Bain, 1995; Garner, 1996; Copp, 1997; Turner et al., 1994). Interspecific use of differing vertical positions in the water column by cyprinids has been assessed for guilds of minnows in temperate and tropical climates (Welcomme, 1985). Ozark minnow species showed significant changes in vertical distributions between depth zones and also showed changes in vertical distributions in response to hetersospecifics within each depth zone (Gorman, 1988).

Examination of frequency distributions reveals Cape Fear shiner juveniles had greater focal depth overlap with larvae in early summer, and this shifted to overlap with adults in summer in 2007 and 2008. PCA component plots are consistent with these observations, and juveniles were closer in habitat use to adults than larvae in 2008. Cape Fear shiner adults occupy lower-pelagic habitats than *Notropis procne* or *Luxilus chrysocephalus* (A. Henderson, personal observation). Cape Fear shiner adults occupy a wide range of depths in the Rocky River in spawning and post-spawning seasons (Howard, 2003). In July and August of 2007 & 2008, at total lengths of 15-25 mm, Cape Fear shiner juveniles were observed schooling with adults lower in the water column than larvae. A shift to deeper depths was possibly accompanied by occupation of higher current velocities and cooler water temperatures, although juvenile current velocity use in the Rocky River did not significantly differ from available or larvae and adults in 2007 or 2008.

I took a conservative approach to habitat measurements and only measured habitat variables where Cape Fear shiners were known to occur in the Rocky River. These conservative measures could have affected field results, but it is apparent Cape Fear shiners do not chose to move from favorable habitat patches in any life history stage. Adults did not move out of suitable habitat patches in the Rocky River in 2008 (A. Henderson, unpublished data). Colonization of microhabitat patches within study sites used by young of year Cape Fear shiners in 2007 was apparent in 2008. It is not likely young of year fishes of this species move distances greater than 150 meters unless they are displaced by a high flow event (Harvey, 1987; Scheidegger and Bain, 1995). Maximum distance moved from introduction in the experimental test tank did not significantly differ across juvenile size classes, indicating limited movement and low dispersal potential by juvenile *N. mekistocholas*. Habitat variables were not measured in the same months in both years, and variability in freshwater ecosystems is high. However, contrary to expectations, all habitat variables measured were not significantly different across years.

Juvenile and adult cyprinids are known to exhibit high overlap between age groups in use of riffle habitats (Schlosser, 1987). *Notropis petersoni* exhibited a similar ontogenetic shift in juvenile stages, and schooled with adults after their second month of life in the Cape Fear River at approximately 18 mm TL (Davis and Louder, 1971). Cape Fear shiner juvenile shifts in habitat use are probably associated with changes in ontogeny and development (Werner and Gilliam, 1984), and reveal important intraspecific habitat segregation and possible resource partitioning among size classes. The Eurasian minnow, (*Phoxinus phoxinus*) differed greatly in its microhabitat use among developmental stages in the River Lee, Ireland (Simonovic et al., 1999). In contrast to larvae that favored shallow, near-shore habitats with filamentous algae, juvenile *P. phoxinus* occupied significantly deeper and wider areas without filamentous algae. In addition, adults differed from juveniles, occupying faster flowing areas, further from bank, and over more coarse substrata. These ontogenetic shifts are likely a response to lower predation risk, and greater swimming capability of juvenile and adult minnows

(Schlosser, 1987). The roach (*Rutilus rutilus*), a cyprinid in the River Great Ouse, UK, exhibited a similar shift in water column depth in juvenile stages and also moved to the littoral interface where steep banks occurred (Copp, 1997).

Cover was measured as a habitat variable for field data only, and was used significantly more than available by juveniles in July 2008. The use of vegetative cover as a shelter from predation by young of year cyprinids has been assessed for *R. rutilus* (Copp 1997), and others (Santos et al., 2004). Cape Fear shiner juveniles and adults were often found in close proximity to vegetative cover on river margins during field observations, as opposed to mid-channel habitats. Young-of-year Cape Fear shiners were observed in microhabitats with aquatic vegetation in summer months following the breeding season during this study, but not exclusively. This is consistent with cover use by adult Cape Fear shiners in the Rocky River (Howard 2003). Location of schools of *N. mekistochoas* larvae, juveniles, and adults were often flagged on mid-channel islands or boulder and cobble substrates supporting vegetative cover. Use of aquatic macrophyte beds as a food source is a factor in juvenile fish abundance, and the influence of trophic variables affects spatial distribution of fishes (Grennouillet and Pont, 2001). Trophic level interactions and dynamics influence habitat use and cyprinid abundance (Power et al., 1985).

Cape Fear shiner distance to bank was measured in field data for 2008, and contrary to expectations young of year did not use distances to river margins that were statistically different from adults. Size-structured use of riverbanks by cyprinid larvae is common, as near-shore areas provide nursery habitats important to species survival

(Childs et al., 1998). Diel use of riverbanks by cyprinids has also been investigated, as has larval drift to near-shore areas, both responses to predation risk and light levels (Copp and Jurajda, 1999; Manteifel et al., 1978). Extensive larval drift by Cape Fear shiners was not observed during the summers of 2007 & 2008, as young of year fishes were most often found in microhabitats previously monitored, and habitat outside of these patches was not suitable. Potential to disperse out of suitable habitat patches by young of year *N. mekistocholas* is low, but dispersal within suitable habitat patches is possible.

During field observations for this study, Cape Fear shiners fed opportunistically on both plant and animal matter in riverweed (*Podestomum spp.*) beds in the Rocky River. In addition, *N. mekistocholas* has fed on both plant and animal matter in captivity, and faster growth has been documented for individuals fed a mixture of plant and animal matter (John Groves, unpublished data). Foraging and seasonal differences in behavior have been shown to influence the spatial distribution of cyprinids, in contrast to overall availability of suitable habitat (Freeman and Grossman, 1993). Foraging relationships among co-existing species of *Notropis* have shown that behavioral adaptations to predators partially explain intraspecific spatial separation (Gorman, 1988; Mendelson, 1975).

Investigators have used experimental stream systems in research on fishes for a wide range of applications (Gelwick and Matthews, 1993), and these have potential for advanced knowledge of fish ecology and behavior. With replication and reasonable configurations of habitat variables, these systems allow control of variables not possible under natural conditions. Using experimental streams for research on species that are endangered, threatened, or of conservation concern has the ability to provide insight otherwise unattainable (Knight and Gido, 2005). The use of an experimental stream system was effective at separating important habitat variables for Cape Fear shiners in the laboratory as well as validation of important habitat parameters measured in the field, in particular the importance of variability in water depth.

Some interesting behaviors that were noticed of Cape Fear shiners held in captivity was that of burying in the substrate, and greater distances moved by pairs of Cape Fear shiners in experimental trials in the laboratory. Burying behavior was exhibited by roach *Rutilus rutilus* as a response to predators and varying light levels in laboratory experiments (Manteifel et al. 1978). Cape Fear shiners held in captivity had never been exposed to any natural predators, so this behavior may be innate. This species always buried in substrate immediately preceding death in the laboratory. Young of year cyprinids have used interstitial spaces in the substrate to survive drought events (Tramer, 1977). Distances moved by pairs of *N. mekistocholas* were always greater than that of single individuals during experimental trials, although this was not directly measured. The ability to find one another and explore habitats in pairs could be a possible explanation for clumped Cape Fear shiner distribution in nature.

Conclusions

In summary, Cape Fear shiners in early life stages may use slightly different microhabitats than that of adults, but occupy the same mesohabitat patches. Larval and juvenile *N. mekisocholas* depend on variable river channel depths, the availability of shallow water habitats, and proximity to deeper waters. Reduced current velocities associated with submersed aquatic vegetation although not statistically significant are important microhabitat variables for young of year of this species. Habitats with large amounts of cover were important for juvenile Cape Fear shiners, but this was not tested in the laboratory. Movements into deeper waters are likely associated with increased predation risk, and juveniles may recognize the need for microhabitats with cover. The use of deeper waters by adults of an epi-benthic species such as the Cape Fear shiner was expected. However, frequency histograms reveal habitat shifts by juveniles of this species, and schooling in larval stages with other minnow species in the upper water column.

Availability and spatial arrangement of habitat patches has the potential to strongly influence habitat suitability for stream animals more than a range of microhabitat conditions, and proximity to a specific suite of habitat variables can influence habitat used by organisms (Hoss, 2007). Differential use of habitat variables by multiple age classes over time highlights the importance of heterogeneity among in-stream physical habitat for intraspecific microhabitat preferences (Mallet et al., 2000). Temporal stability of abiotic habitat variables strongly influences juvenile fish abundance (Freeman et al., 2001), and it is unclear if shifts in habitat use in 2007 are a function of reduced current velocity and water levels. Microhabitat use in 2008 is perhaps a better indication of habitat use by young of year Cape Fear shiners. Larvae, juvenile, and adult *N. mekistocholas* occupied habitats that differed from available in 2007 and 2008.

Management of habitats for adult Cape Fear shiners should include aspects of early life history microhabitat use in order to validate species protection. Construction of small dams and impoundments on rivers and streams has resulted in the decline of the Topeka shiner (*Notropis topeka*), which was related to dispersal (Schrank et al., 2001). Anthropogenic impacts have fragmented riverine habitats, reduced water quality, and altered the natural flow regime within the Cape Fear watershed (Howard, 2003; Pottern and Huish, 1985). These effects have prevented dispersal of the Cape Fear shiner, and potentially resulted in recent local extirpation (Saillant et al., 2004). Pairing of laboratory and field studies has great potential for further knowledge of critically endangered species such as the Cape Fear shiner. Larval and juvenile *N. mekistocholas* occupied riffle and run habitats, similar to adults, but their use of these habitats differed both within and across years in the Rocky River and the laboratory. It is apparent that young of year Cape Fear shiners use a smaller set of habitat variables that is similar to adults, and are restricted to suitable mesohabitats within their range. Dispersal potential outside of suitable habitat patches is extremely low. Future conservation efforts focused on the Cape Fear shiner should include aspects of habitat management that account for early life history stages.

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Table 1. Available habitat and use summary statistics (mean, standard deviation) May, August, and combined 2007.

Summary Stats	Larvae		Juvenile		Adult		Av. Habitat	
5/07	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Depth	32.42	14.04	37.01	15.83	61.05	8.167	56.06	24.05
Current Velocity	0.034	0.064	0.069	0.054	0.114	0.050	0.059	0.063
Substrate	7.000	1.333	7.200	1.033	7.300	1.160	6.820	1.804
Cover	3.500	2.121	3.400	3.471	2.100	1.524	3.700	3.671

Summary Stats	Larvae		Juvenile		Adult		Av. Habitat	
8/07	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Depth	42.84	16.26	23.15	8.077	52.03	7.136	32.45	23.76
Current Velocity	0.020	0.021	0.087	0.059	0.085	0.050	0.039	0.055
Substrate	5.930	1.163	6.670	0.900	6.870	1.302	6.260	1.103
Cover	2.730	0.704	2.070	2.404	2.000	1.134	2.620	2.423

Summary Stats	Larvae		Juvenile		Adult		Av. Habitat	
2007	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Depth	38.67	15.98	28.69	13.42	38.31	20.54	44.26	26.58
Current Velocity	0.026	0.049	0.080	0.057	0.097	0.051	0.049	0.060
Substrate	6.360	1.319	6.880	1.013	7.040	1.241	6.540	1.514
Cover	3.040	1.457	2.600	2.887	2.040	1.274	3.160	3.142

Table 2. Available habitat and use summary statistics (mean, standard deviation) April, July, and combined 2008. Distance to bank (D statistic and P-values) measured for larval, juvenile and adult Cape Fear shiner point measurements.

Summary Stats	Larvae		Juvenile		Adult		Av. Habitat	
4/08	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Depth	21.07	7.459	31.40	16.11	48.40	19.74	68.72	26.22
Current Velocity	0.041	0.018	0.049	0.240	0.136	0.125	0.185	0.177
Substrate	7.400	1.056	6.670	1.345	5.470	2.503	5.000	2.680
Cover	2.600	3.869	2.930	4.096	2.130	2.850	1.860	2.352

Summary Stats	Larvae		Juvenile		Adult		Av. Habitat	
7/08	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Depth	28.27	15.57	54.53	12.77	65.67	11.78	35.86	17.51
Current Velocity	0.093	0.237	0.098	0.237	0.108	0.050	0.098	0.105
Substrate	5.470	1.106	6.870	1.302	6.670	1.175	8.260	0.828
Cover	2.670	3.331	0.400	0.632	1.670	3.309	1.780	3.209

Summary Stats	Larvae		Juvenile		Adult		Av. Habitat	
2008	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Depth	24.67	12.54	42.97	18.50	57.03	18.23	52.29	27.65
Current Velocity	0.030	0.023	0.074	0.046	0.122	0.095	0.140	0.152
Substrate	6.430	1.431	6.770	1.035	6.070	2.016	6.630	2.565
Cover	2.630	3.548	1.670	3.155	1.900	3.044	1.820	2.876

Distance to Bank	Larvae		Juvenile		Adult	
2008	Statistic	P	Statistic	P	Statistic	P
	0.121	0.768	0.172	0.339	0.106	0.886

Table 3. Available habitat and use statistic (D = K-S tests) (X^2 = Chi-square tests) and p-values for May 2007.

Depth	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
Larvae	0.545	0.076	----------------		---------------		------------	
Juvenile	0.545	0.076	0.000	1.000	---------------		------------	
Adult	0.727	**0.006**	0.182	0.993	0.182	0.993	------------	

Flow	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
Larvae	0.400	0.400	----------------		---------------		------------	
Juvenile	0.500	0.164	0.300	0.759	---------------		------------	
Adult	0.400	0.400	0.100	1.000	0.200	0.988	------------	

Substrate	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
Larvae	0.400	0.400	----------------		---------------		------------	
Juvenile	0.500	0.164	0.100	1.000	---------------		------------	
Adult	0.100	1.000	0.100	1.000	0.100	1.000	-----------	

Cover	Available		Larvae		Juvenile		Adult	
	(X^2)	P	(X^2)	P	(X^2)	P	(X^2)	P
Larvae	3.182	0.364	----------------		----------------		------------	
Juvenile	6.091	0.107	3.182	0.364	----------------		------------	
Adult	5.364	0.147	5.364	0.147	6.091	0.107	-----------	

Table 4. Available habitat and use statistic (D = K-S tests) (X^2 = Chi-square tests) and p-values for August 2007.

Depth	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
Larvae	0.500	0.164	----------------		---------------		------------	
Juvenile	0.400	0.400	0.300	0.759	---------------		------------	
Adult	0.500	0.164	0.200	0.988	0.300	0.759	------------	

Flow	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
Larvae	0.400	0.400	----------------		---------------		------------	
Juvenile	0.500	0.164	0.300	0.759	---------------		------------	
Adult	0.100	1.000	0.100	1.000	0.100	1.000	------------	

Substrate	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
Larvae	0.200	0.988	----------------		---------------		------------	
Juvenile	0.300	0.759	0.100	1.000	---------------		------------	
Adult	0.200	0.988	0.100	1.000	0.100	1.000	------------	

Cover	Available		Larvae		Juvenile		Adult	
	(X^2)	P	(X^2)	P	(X^2)	P	(X^2)	P
Larvae	9.000	0.029	----------------		----------------		------------	
Juvenile	3.091	0.543	9.000	0.029	----------------		------------	
Adult	8.545	0.074	8.545	0.074	3.091	0.543	------------	

Table 5. Available habitat and use statistic (D = K-S tests) (X^2 = Chi-square tests) and p-values for May and August 2007 combined.

Depth	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
Larvae	0.727	**0.006**	----------------		---------------		------------	
Juvenile	0.727	**0.006**	0.364	0.461	---------------		------------	
Adult	0.727	**0.006**	0.091	1.000	0.273	0.808	------------	

Flow	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
Larvae	0.400	0.400	----------------		---------------		------------	
Juvenile	0.400	0.400	0.300	0.759	---------------		------------	
Adult	0.400	0.400	0.200	0.988	0.100	1.000	------------	

Substrate	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
Larvae	0.500	0.164	----------------		---------------		------------	
Juvenile	0.600	0.055	0.200	0.988	---------------		------------	
Adult	0.500	0.164	0.200	0.988	0.100	1.000	-----------	

Cover	Available		Larvae		Juvenile		Adult	
	(X^2)	P	(X^2)	P	(X^2)	P	(X^2)	P
Larvae	4.909	0.297	---------------		----------------		------------	
Juvenile	1.545	0.908	4.909	0.297	----------------		------------	
Adult	7.000	0.221	7.000	0.221	1.545	0.908	------------	

Table 6. Available habitat and use statistic (D = K-S tests) (X^2 = Chi-square tests) and p-values for April 2008.

Depth	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
Larvae	0.500	0.037	----------------		---------------		------------	
Juvenile	0.313	0.415	0.300	0.759	---------------		------------	
Adult	0.375	0.211	0.250	0.699	0.063	1.000	------------	

Flow	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
Larvae	0.400	0.400	----------------		---------------		------------	
Juvenile	0.400	0.400	0.200	0.988	---------------		------------	
Adult	0.500	0.164	0.300	0.759	0.100	1.000	------------	

Substrate	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
Larvae	0.400	0.400	----------------		---------------		------------	
Juvenile	0.300	0.759	0.100	1.000	---------------		------------	
Adult	0.300	0.759	0.100	1.000	0.100	1.000	------------	

Cover	Available		Larvae		Juvenile		Adult	
	(X^2)	P	(X^2)	P	(X^2)	P	(X^2)	P
Larvae	4.636	0.200	----------------		----------------		------------	
Juvenile	3.182	0.364	4.636	0.200	----------------		------------	
Adult	4.909	0.297	4.909	0.297	3.182	0.364	------------	

Table 7. Available habitat and use statistic (D = K-S tests) (X^2 = Chi-square tests) and p-values for July 2008.

Depth	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
Larvae	0.455	0.206	----------------		---------------		------------	
Juvenile	0.364	0.461	0.182	0.993	---------------		------------	
Adult	0.455	0.206	0.091	1.000	0.182	0.993	------------	

Flow	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
Larvae	0.500	0.164	----------------		---------------		------------	
Juvenile	0.500	0.164	0.300	0.759	---------------		------------	
Adult	0.500	0.164	0.100	1.000	0.200	0.988	------------	

Substrate	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
Larvae	0.300	0.759	----------------		---------------		------------	
Juvenile	0.200	0.988	0.200	0.988	---------------		------------	
Adult	0.200	0.988	0.100	1.000	0.100	1.000	-----------	

Cover	Available		Larvae		Juvenile		Adult	
	(X^2)	P	(X^2)	P	(X^2)	P	(X^2)	P
Larvae	4.909	0.297	----------------		----------------		------------	
Juvenile	13.36	**0.004**	4.909	0.297	----------------		------------	
Adult	3.455	0.178	3.455	0.178	13.36	**0.004**	------------	

Table 8. Available habitat and use statistic (D = K-S tests) (X^2 = Chi-square tests) and p-values for April and July 2008 combined.

Depth	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
Larvae	0.438	0.094	----------------		---------------		------------	
Juvenile	0.438	0.094	0.125	1.000	---------------		------------	
Adult	0.438	0.094	0.125	1.000	0.000	1.000	------------	

Flow	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
Larvae	0.500	0.164	----------------		---------------		------------	
Juvenile	0.400	0.400	0.300	0.759	---------------		------------	
Adult	0.500	0.164	0.300	0.759	0.400	0.400	------------	

Substrate	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
Larvae	0.500	0.164	----------------		---------------		------------	
Juvenile	0.400	0.400	0.100	1.000	---------------		------------	
Adult	0.500	0.164	0.100	1.000	0.100	1.000	-----------	

Cover	Available		Larvae		Juvenile		Adult	
	(X^2)	P	(X^2)	P	(X^2)	P	(X^2)	P
Larvae	0.800	0.999	----------------		----------------		------------	
Juvenile	4.000	0.406	0.800	0.999	----------------		------------	
Adult	2.000	0.849	2.000	0.849	4.000	0.406	-----------	

Table 9. Available and use statistic (D = K-S tests) (X^2 = Chi-square tests) and p-values for combined comparison of habitat variables 2007 & 2008.

Depth	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
	0.415	0.212	0.176	0.988	0.301	0.596	0.119	1.000

Flow	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
	0.400	0.400	0.100	1.000	0.200	0.988	0.200	0.988

Substrate	Available		Larvae		Juvenile		Adult	
	D	P	D	P	D	P	D	P
	0.400	0.400	0.200	0.988	0.100	1.000	0.100	1.000

Cover	Available		Larvae		Juvenile		Adult	
	(X^2)	P	(X^2)	P	(X^2)	P	(X^2)	P
	0.800	0.999	1.545	0.908	5.818	0.213	3.727	0.589

Table 10. Retained component loadings from principal component analyses available habitat variables in 2007 & 2008.

Component loadings from principal components analyses for habitat variables in 2007.

Variable and statistic	PC1	PC2
Depth	0.750	-0.196
Current velocity	0.171	0.902
Substrate	0.664	-0.288
Cover	-0.613	0.300
Eigenvalue	1.410	1.025
Variance explained	35%	61%

Component loadings from principal components analyses for habitat variables in 2008.

Variable and statistic	PC1	PC2
Depth	0.865	0.131
Current velocity	0.218	-0.697
Substrate	-0.862	0.123
Cover	-0.089	0.759
Eigenvalue	1.547	1.095
Variance explained	39%	66%

Table 11. Results of principle components analysis for available habitat and use for larvae, juvenile and adult Cape Fear shiners with habitat variables combined for 2007 & 2008.

Available habitat and use 2007

Total Variance Explained

	Initial Eigenvalues		
Component	Total	% of Variance	Cumulative %
1	2.778	69.452	69.452
2	0.851	21.271	90.723
3	0.283	7.086	97.809
4	0.088	2.191	100

Available habitat and use 2008

Total Variance Explained

	Initial Eigenvalues		
Component	Total	% of Variance	Cumulative %
1	2.625	65.626	65.626
2	1.011	25.286	90.912
3	0.280	6.998	97.909
4	0.084	2.091	100

Table 12. Principle components analysis of habitat use for all variables separated for larvae, juvenile, adult 2007 and 2008.

Total Variance Explained

2007	Initial Eigenvalues		
Component	Total	% of Variance	Cumulative %
1	2.463	20.527	20.527
2	1.953	16.275	36.802
3	1.791	14.928	51.730
4	1.667	13.893	65.623
5	1.040	8.664	74.287
6	0.795	6.626	80.913
7	0.590	4.917	85.830
8	0.519	4.321	90.151
9	0.468	3.903	94.054
10	0.312	2.600	96.654
11	0.213	1.774	98.428
12	0.189	1.572	100

Total Variance Explained

2008	Initial Eigenvalues		
Component	Total	% of Variance	Cumulative %
1	3.151	26.256	26.256
2	1.938	16.150	36.802
3	1.566	13.051	51.730
4	1.371	11.425	65.623
5	1.042	8.652	80.913
7	0.690	5.754	85.830
8	0.437	3.642	90.151
9	0.370	3.087	94.054
10	0.304	2.533	96.654
11	0.236	1.969	98.428
12	0.100	0.800	100

Table 13. Results of three-way MANOVA for experimental tank trials of Cape Fear shiner mesolarvae and metalarvae.

Source	Variable	Sum of Sq.	df	Mean Sq.	F	Sig.
Corrected Model	Flow	5.715	14	0.408	1.676	0.060
	Substrate	6.143	14	0.439	1.812	0.036
	Depth	8.857	14	0.633	2.712	0.001
Intercept	Flow	105.643	1	105.643	433.750	0.000
	Substrate	75.913	1	75.913	313.490	0.000
	Depth	78.569	1	78.569	336.835	0.000
Size	Flow	0.156	1	0.156	0.642	0.424
	Substrate	0.002	1	0.002	0.010	0.922
	Depth	0.990	1	0.990	4.242	**0.040**
Time	Flow	4.825	8	0.603	2.477	**0.013**
	Substrate	3.721	8	0.465	1.921	0.057
	Depth	6.613	8	0.827	3.544	**0.001**
Size*Time	Flow	0.769	5	0.154	0.631	0.676
	Substrate	1.536	5	0.307	1.269	0.277
	Depth	2.48	5	0.496	2.127	0.062
Error	Flow	74.285	305	0.244		
	Substrate	73.857	305	0.242		
	Depth	71.143	305	0.233		
Total	Flow	800	320			
	Substrate	800	320			
	Depth	800	320			
Corrected Total	Flow	80	319			
	Substrate	80	319			
	Depth	80	319			

a $r^2 = 0.071$ (adj. = 0.029), b $r^2 = 0.077$ (adj. = 0.034), c $r^2 = 0.111$ (adj. = 0.070)

Table 14. Results of three-way MANOVA for tank trials of Cape Fear shiner early, middle, and late juveniles and maximum distance moved (D statistic and P-values) by juveniles in test tank.

Source	Variable	Sum of Sq.	df	Mean Sq.	F	Sig.
Corrected Model	Flow	22.476	22	1.022	1.556	0.050
	Substrate	4.585	22	0.208	0.828	0.691
	Depth	17.577	22	0.799	3.428	0.000
Intercept	Flow	200.161	1	200.161	304.928	0.000
	Substrate	115.744	1	115.744	459.901	0.000
	Depth	135.379	1	135.379	580.946	0.000
Size	Flow	0.158	2	0.259	0.395	0.674
	Substrate	0.014	2	0.007	0.028	0.973
	Depth	0.917	2	0.458	1.967	0.141
Time	Flow	12.595	8	1.574	2.398	**0.015**
	Substrate	3.011	8	0.376	1.496	0.155
	Depth	12.659	8	1.582	6.79	**< 0.001**
Size*Time	Flow	11.128	12	0.927	1.413	0.155
	Substrate	1.564	12	0.130	0.518	0.904
	Depth	3.562	12	0.297	1.274	0.229
Error	Flow	457.524	697	0.656		
	Substrate	175.415	697	0.252		
	Depth	162.423	697	0.233		
Total	Flow	3360	720			
	Substrate	1800	720			
	Depth	1800	720			
Corrected Total	Flow	480	719			
	Substrate	180	719			
	Depth	180	719			

a $r^2 = 0.047$ (adj. = 0.017), b $r^2 = 0.025$ (adj. = -0.005), c $r^2 = 0.098$ (adj. = 0.069)

Maximum Distance Moved

Size	Age	Stage	D statistic	P value
20-24 mm TL	~70 days	Early Juvenile	0.173	0.585
23-32 mm TL	~90 days	Mid Juvenile	0.143	0.810
31-36 mm TL	~125 days	Late Juvenile	0.176	0.564

Table 15. Categories used to describe Rocky River substrate composition based on a modified Wentworth particle size scale. Classifications were used for substrate frequency histograms.

1 - bedrock

2 - gravel

3- cobble

4 - boulder

5 - bedrock/gravel

6 - bedrock/cobble

7 - bedrock/boulder

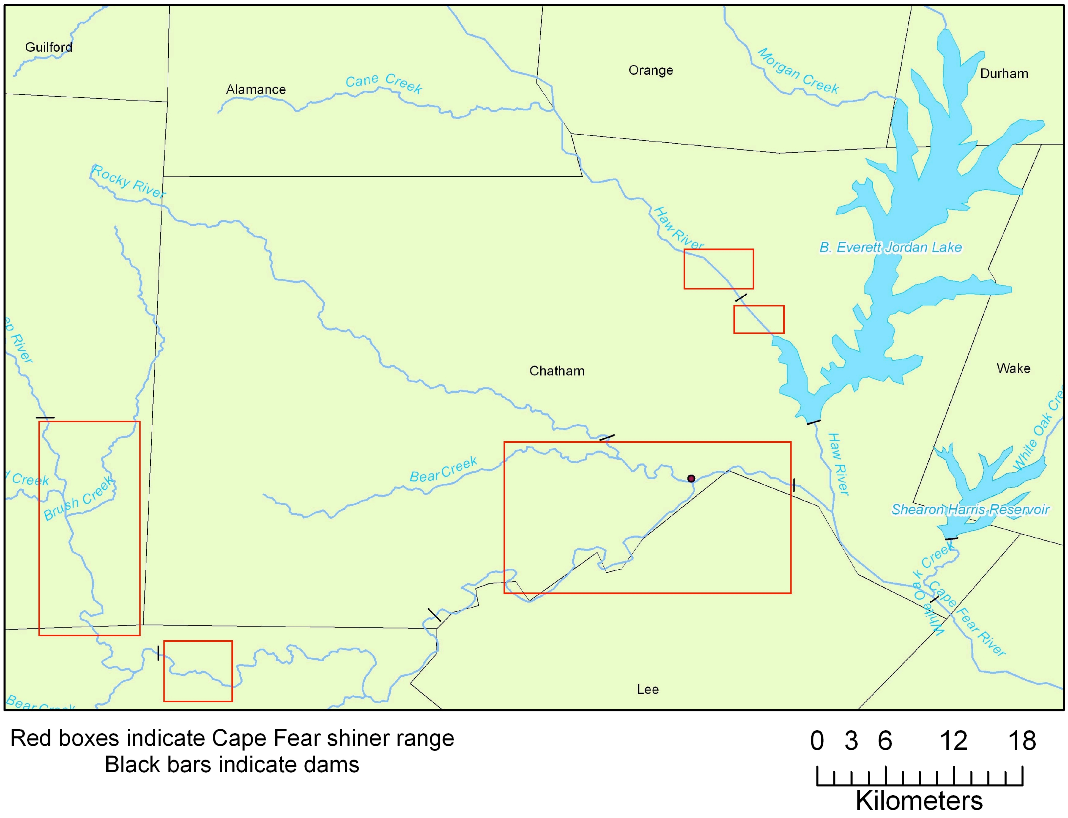
8 - bedrock/gravel/cobble

9 - bedrock/gravel/boulder

10 - bedrock/cobble/boulder

Figure 1.

Map of Cape Fear shiner distribution and location of sampling sites on the Rocky River, NC



Distribution of Cape Fear Shiner

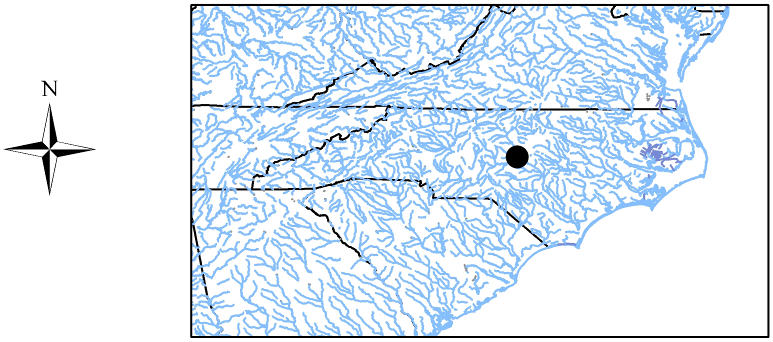


Figure 2. Tank diagram for mesolarval and metalarval experimental trials.

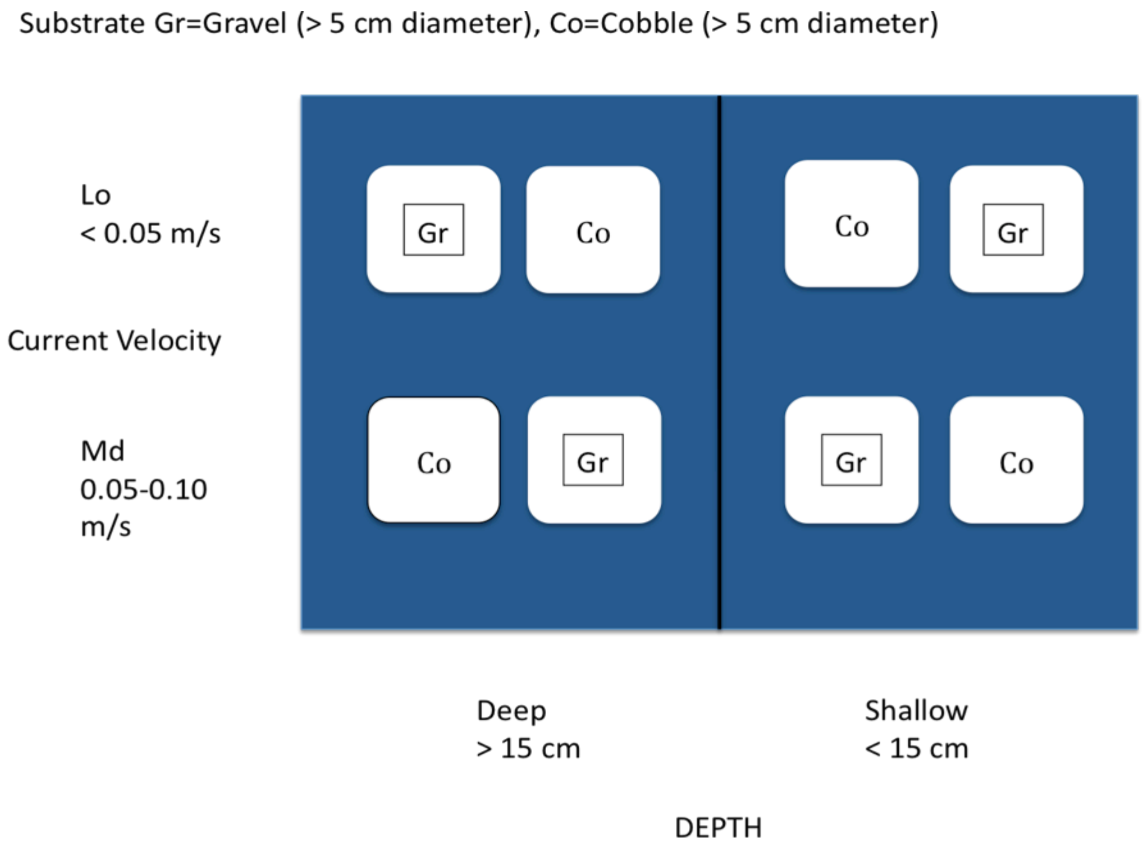


Figure 3. Tank diagram for early, mid, and late juvenile experimental trials.

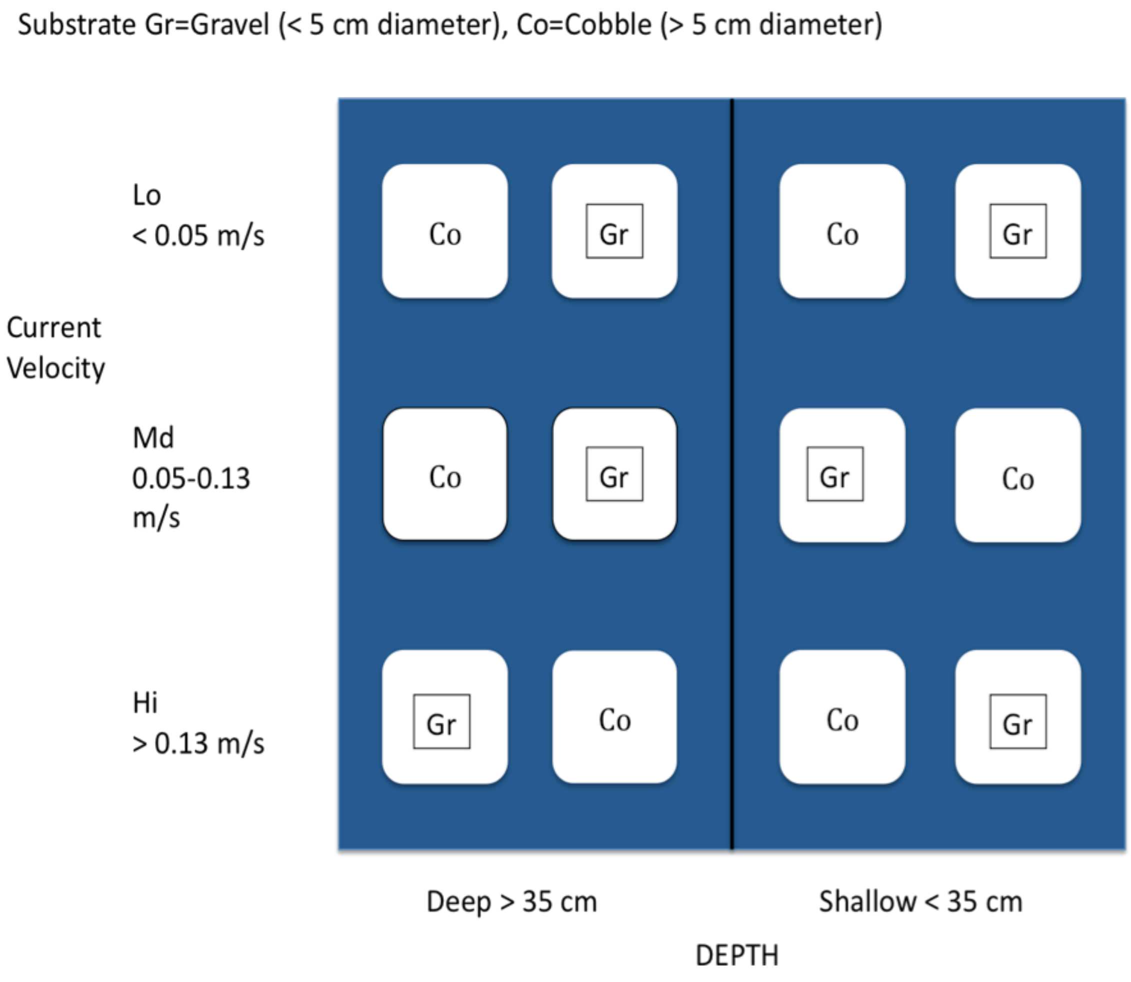


Figure 4. Frequency histogram of available depth and use May 2007.

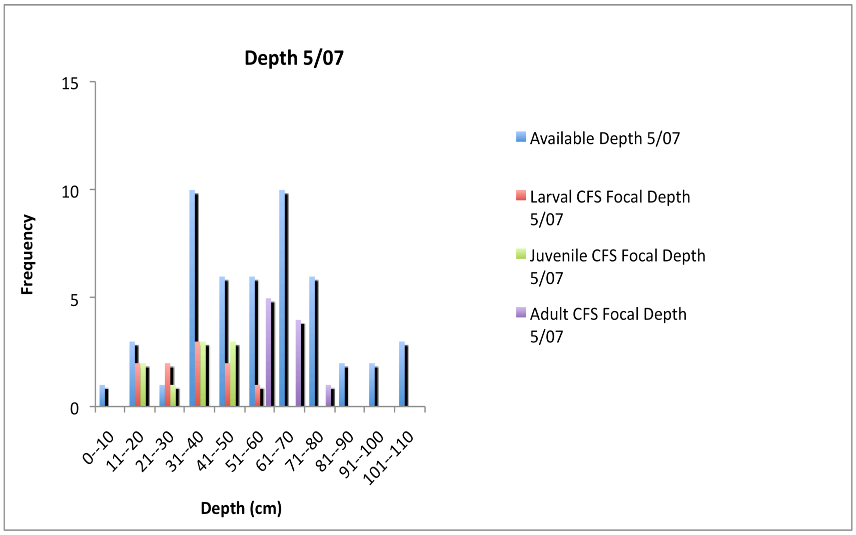


Figure 5. Frequency histogram of available depth and use August 2007.

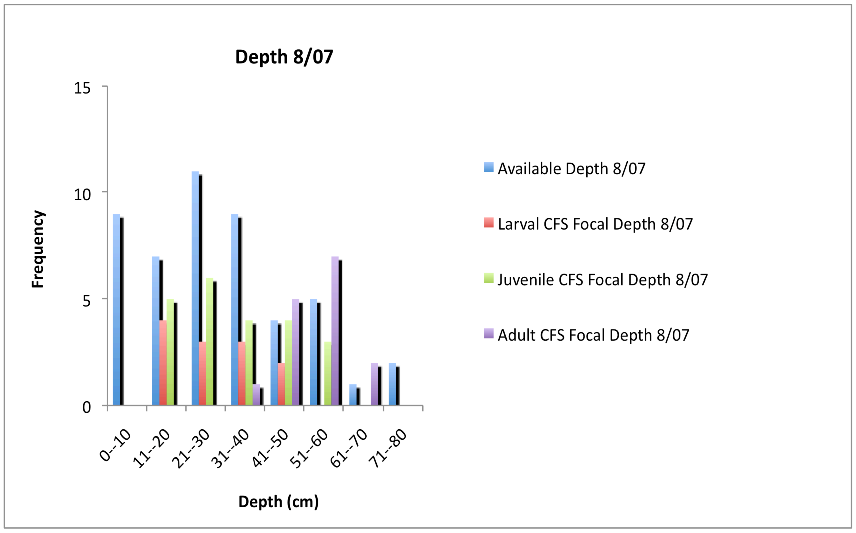


Figure 6. Frequency histogram of available and use depth April 2008.

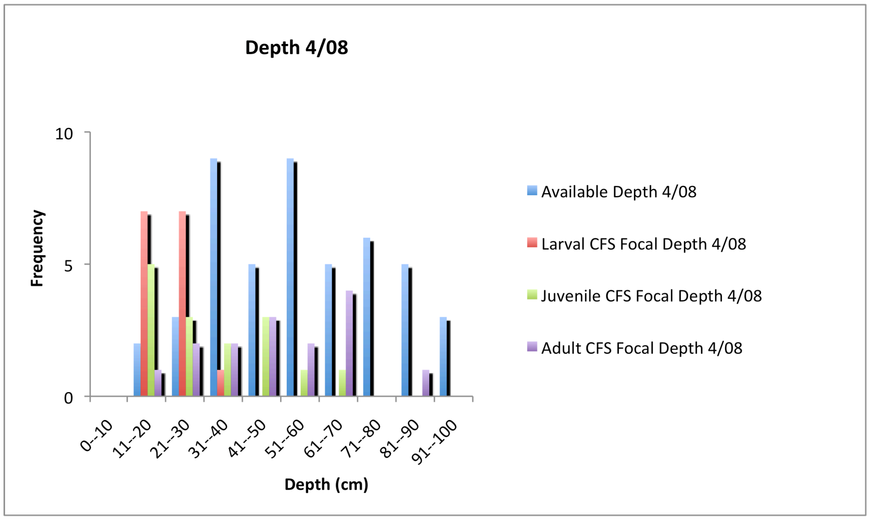


Figure 7. Frequency histogram of available depth and use July 2008.

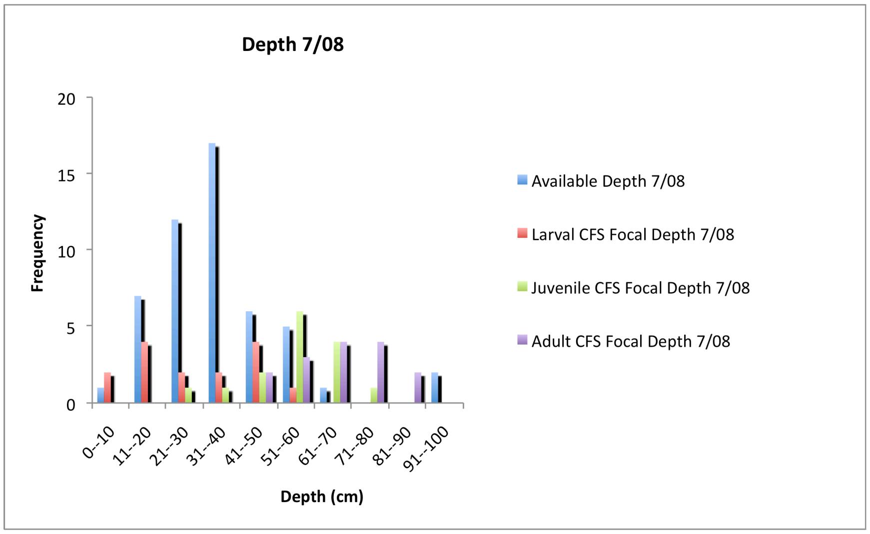


Figure 8. Frequency histogram of available depth and use 2007.

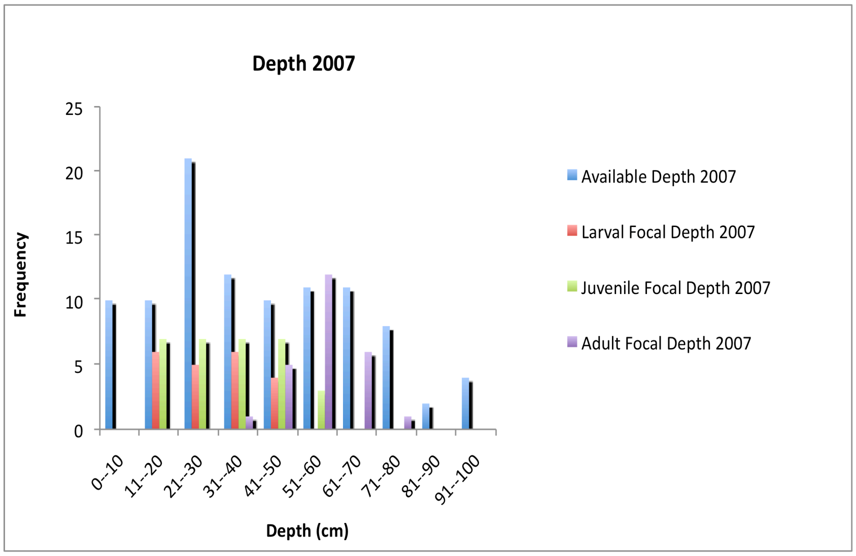


Figure 9. Frequency histogram of available depth and use 2008.

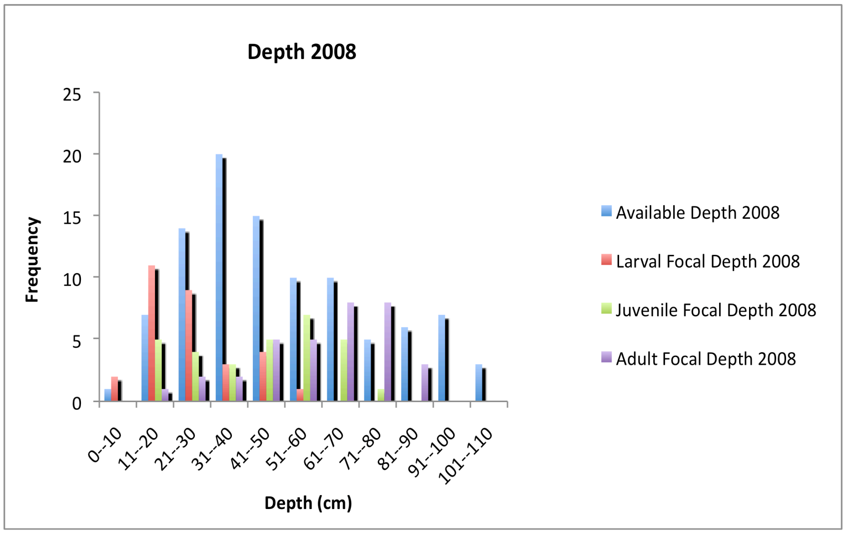


Figure 10. Frequency histogram of available current velocity and use May 2007.

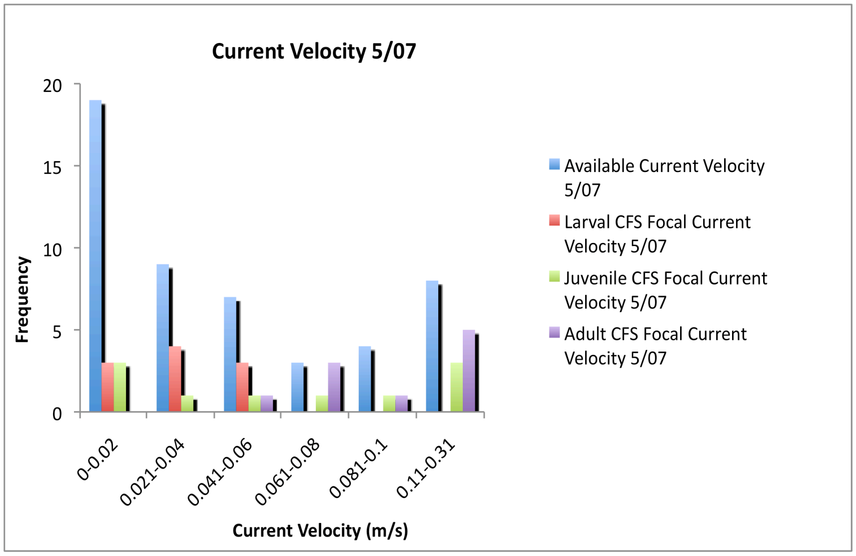


Figure 11. Frequency histogram of available current velocity and use August 2007.

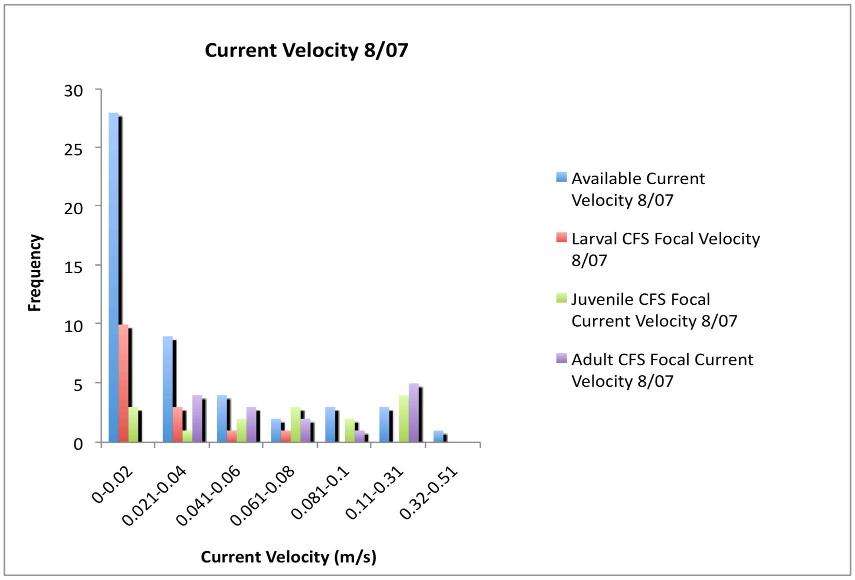


Figure 12. Frequency histogram of available current velocity and use April 2008.

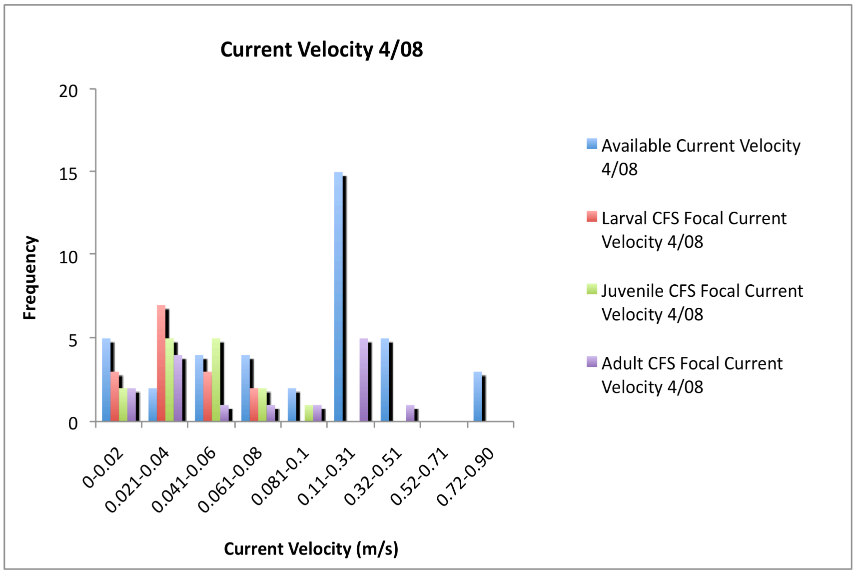


Figure 13. Frequency histogram of available current velocity and use July 2008.

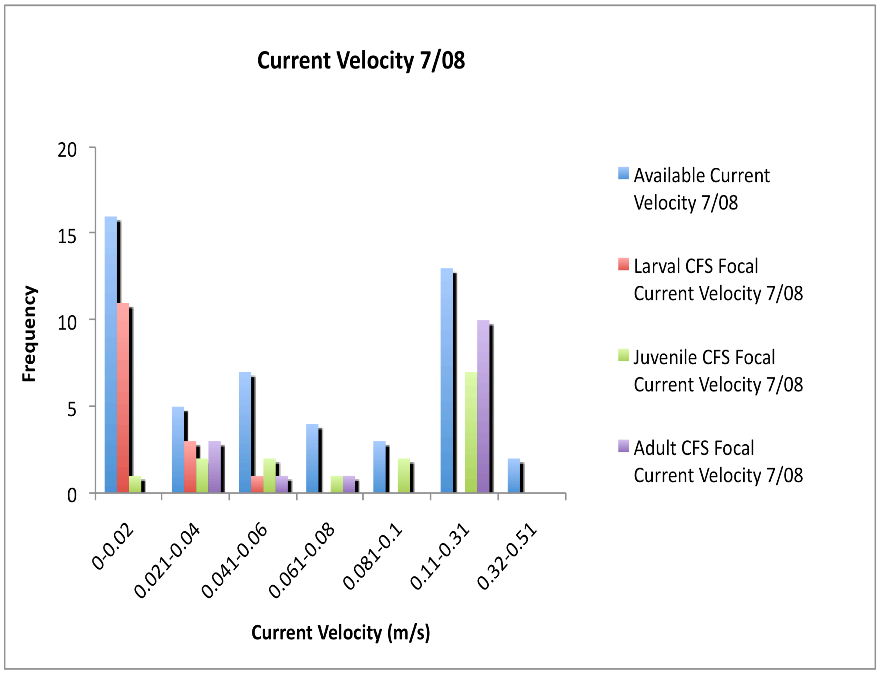


Figure 14. Frequency histogram of available current velocity and use 2007.

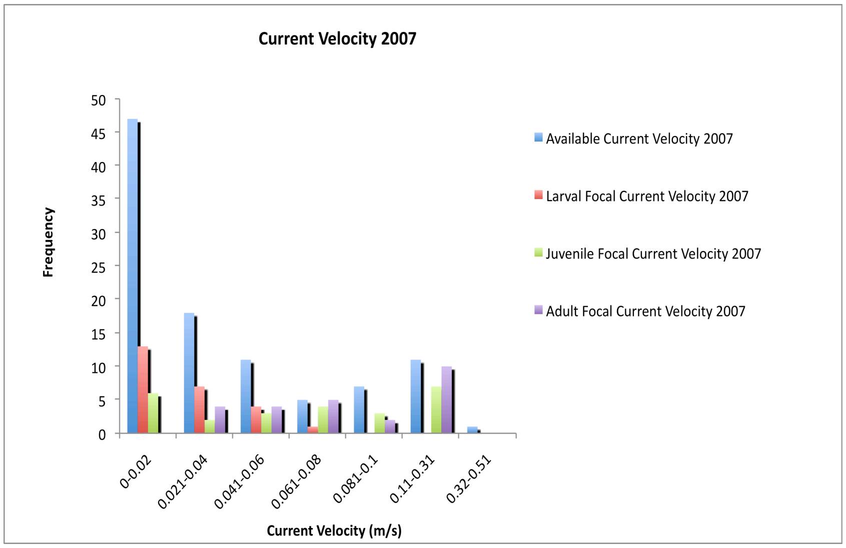


Figure 15. Frequency histogram of available current velocity and use 2008.

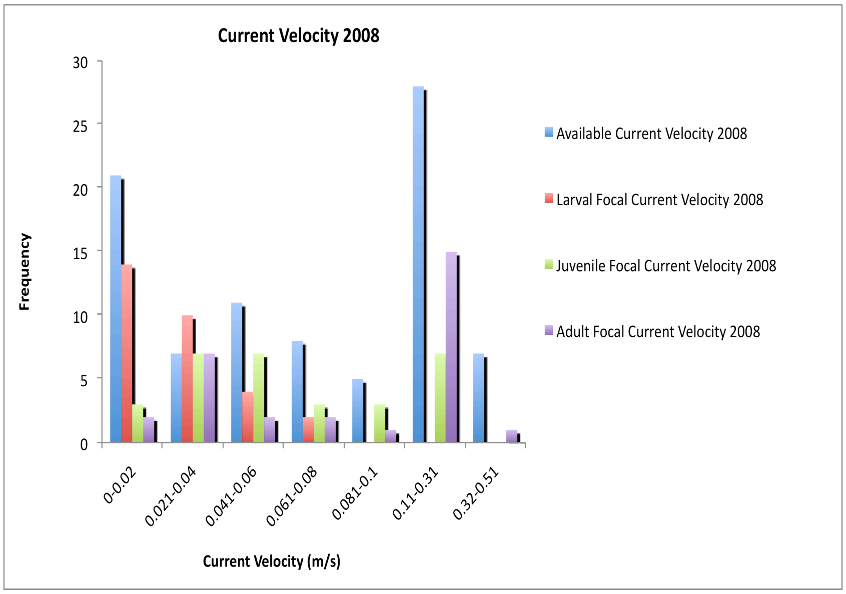


Figure 16. Frequency histogram of available substrate and use May 2005.

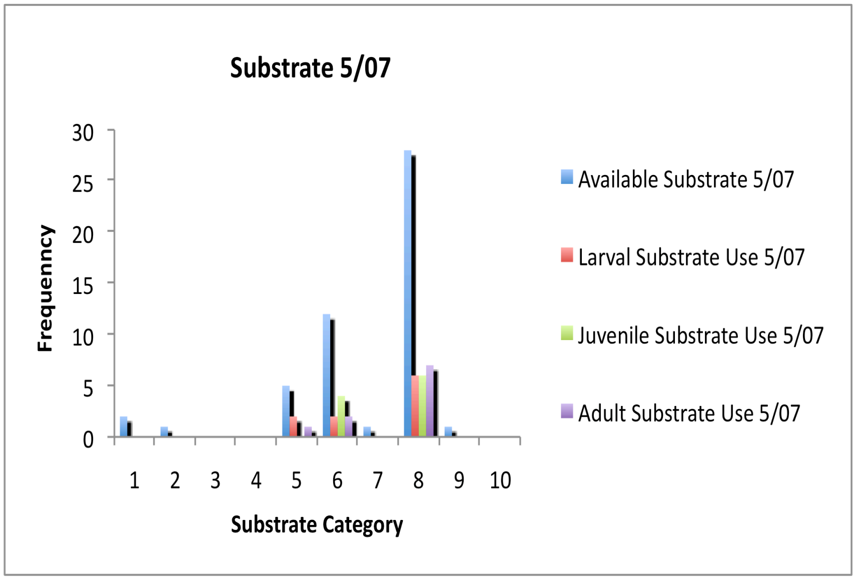


Figure 17. Frequency histogram of available substrate and use August 2007.

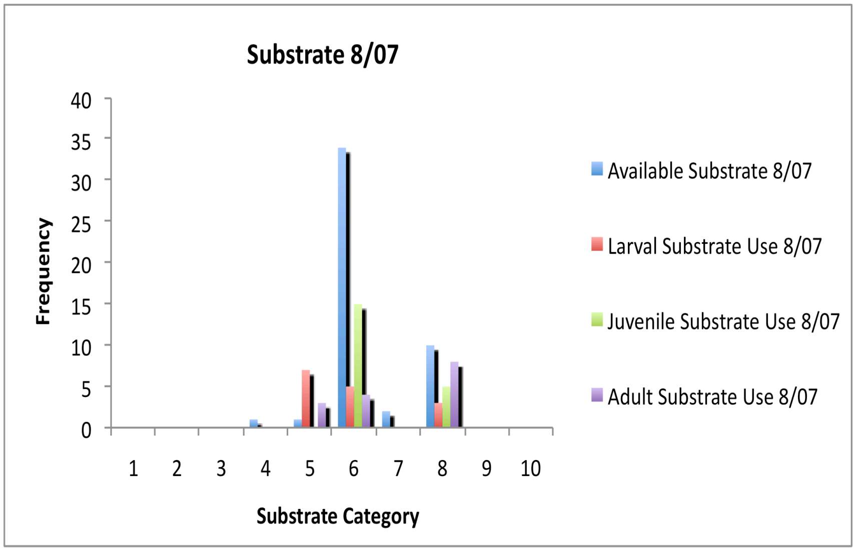


Figure 18. Frequency histogram of available substrate and use April 2008.

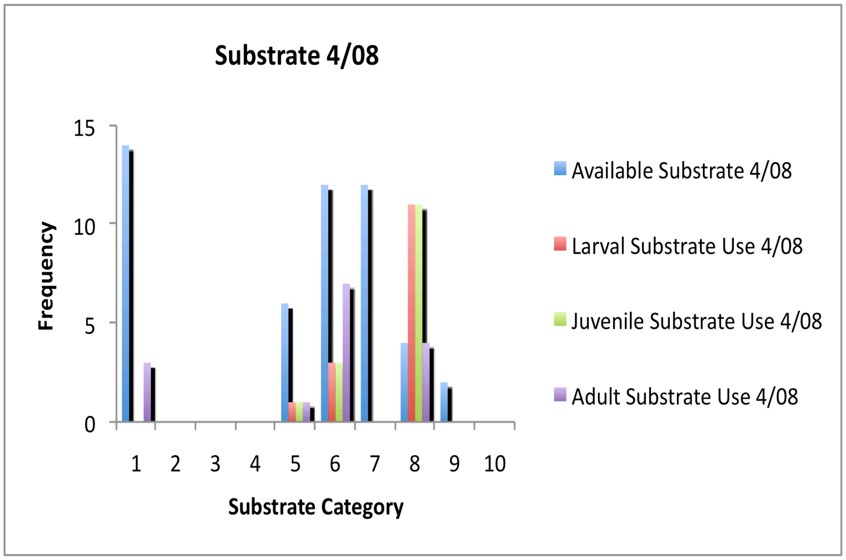


Figure 19. Frequency histogram of available substrate and use July 2008.

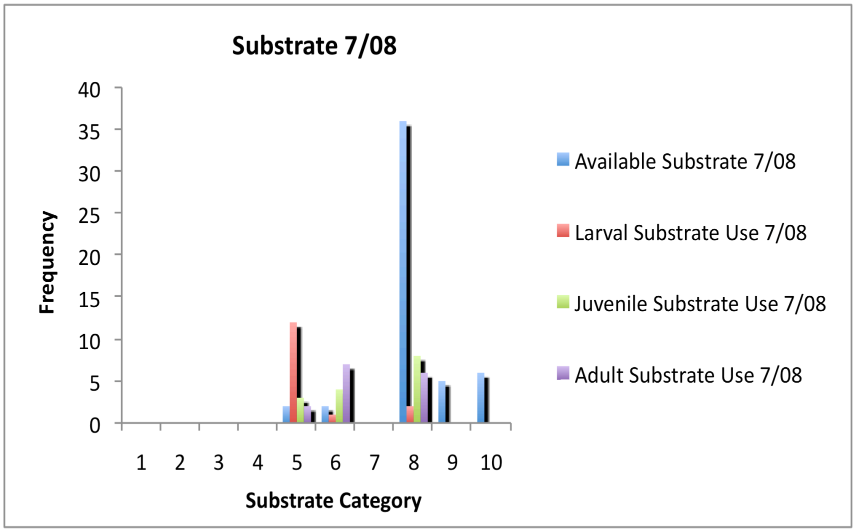


Figure 20. Frequency histogram of available substrate and use 2007.

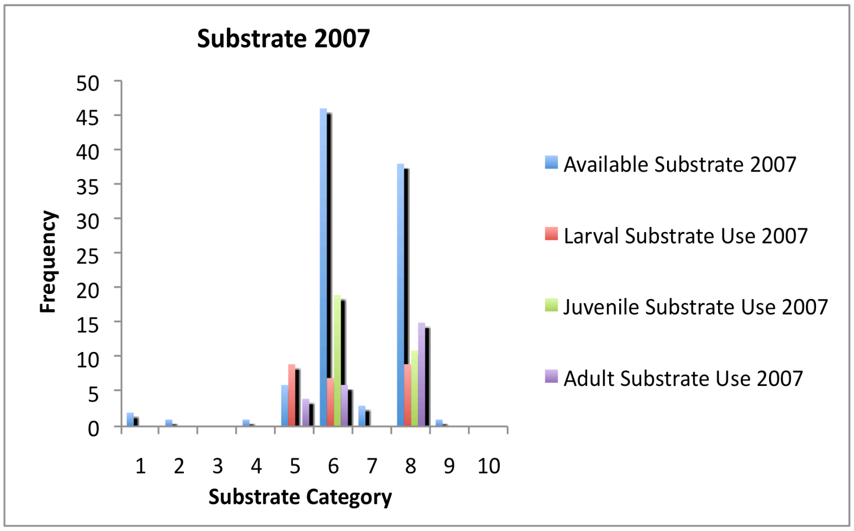


Figure 21. Frequency histogram of available substrate and use 2008.

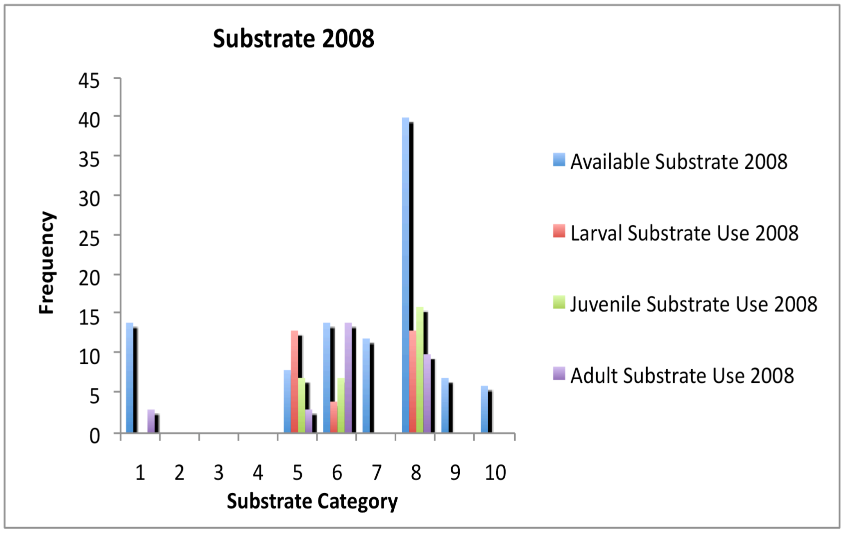


Figure 22. Frequency histogram of available cover and use May 2007.

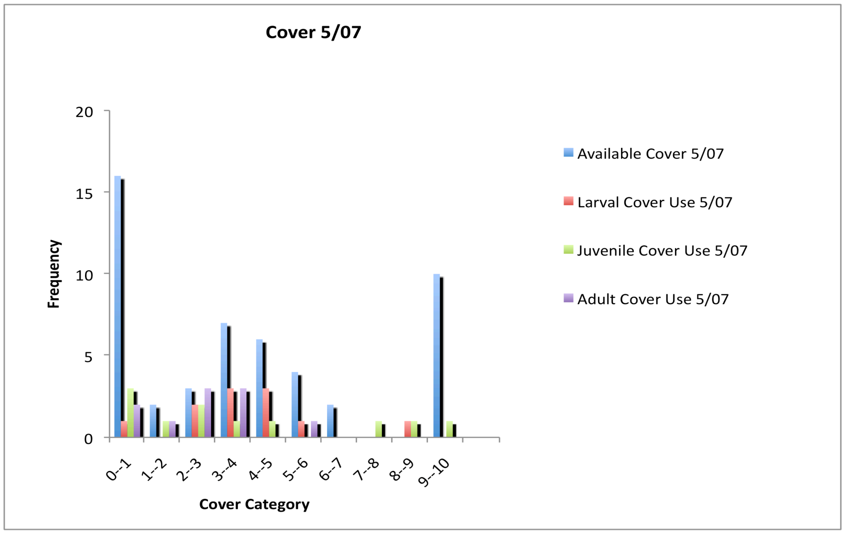


Figure 23. Frequency histogram of available cover and use August 2007.

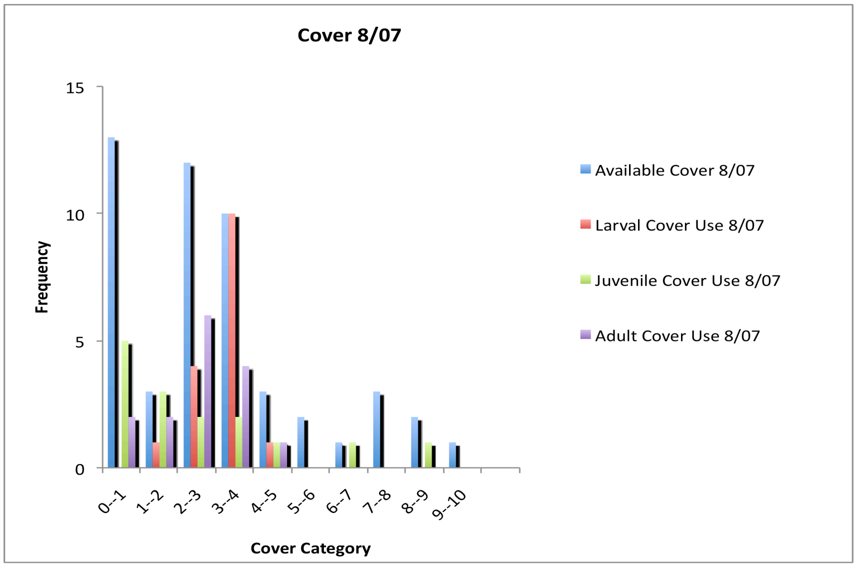


Figure 24. Frequency histogram of available cover and use April 2008.

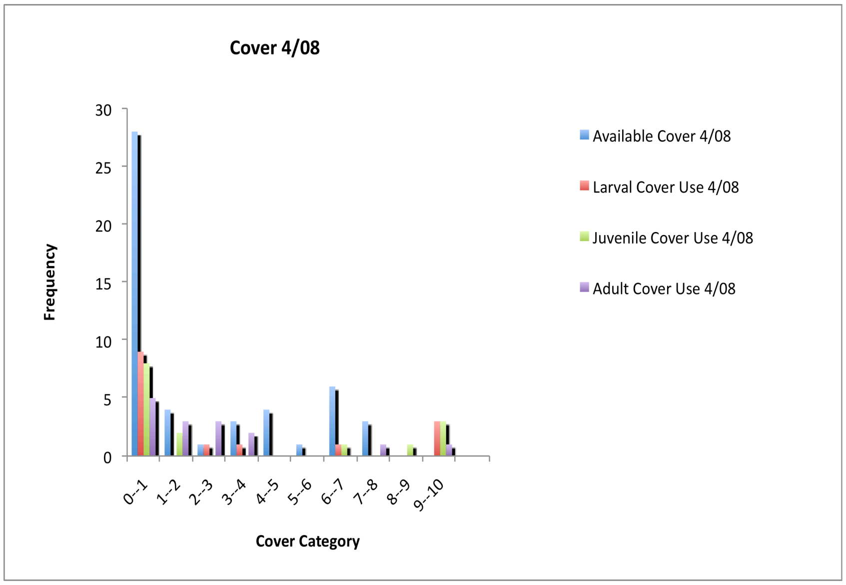


Figure 25. Frequency histogram of available cover and use July 2008.

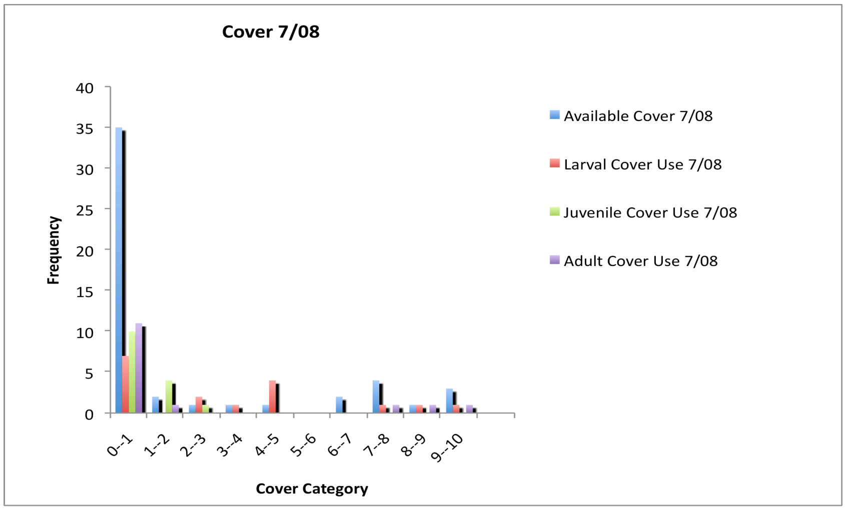


Figure 26. Frequency histogram of available cover and use 2007.

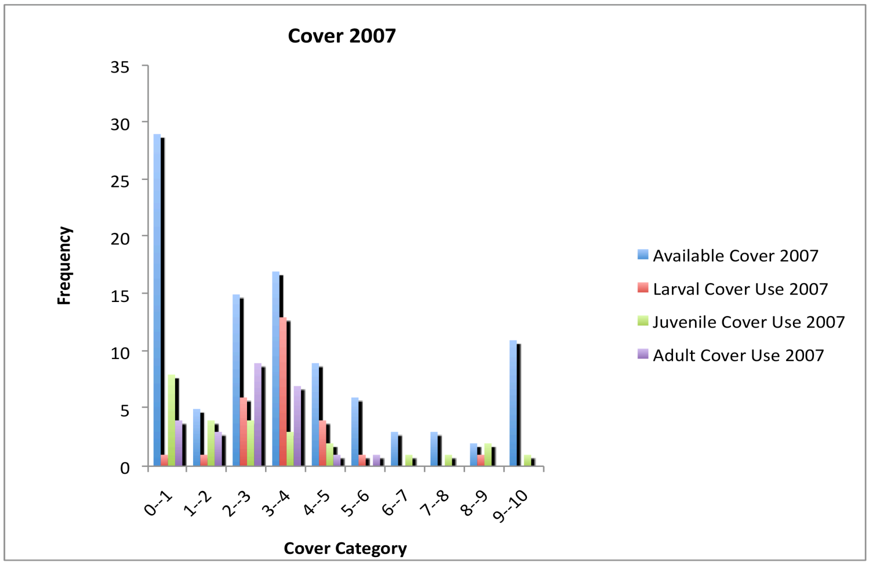


Figure 27. Frequency histogram of available cover and use 2008.

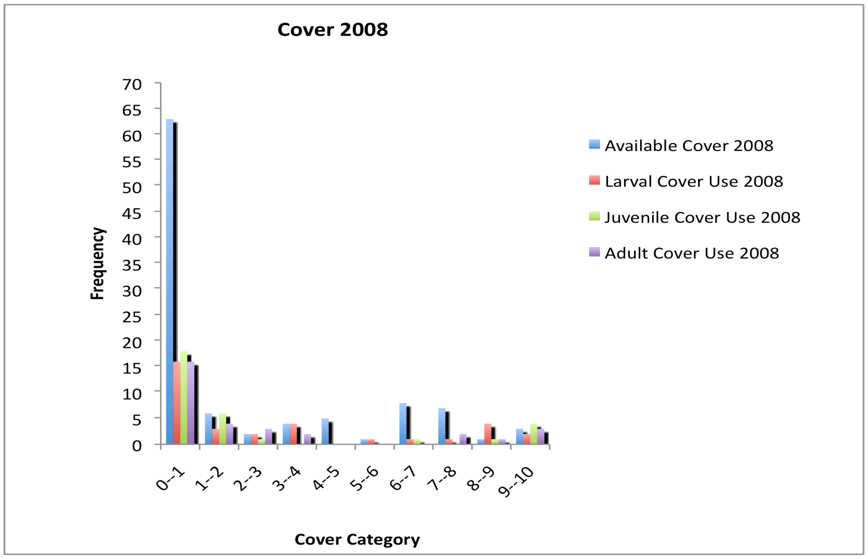


Figure 28. PCA component plot for larvae, juvenile, adult size classes habitat use and available combined 2007.

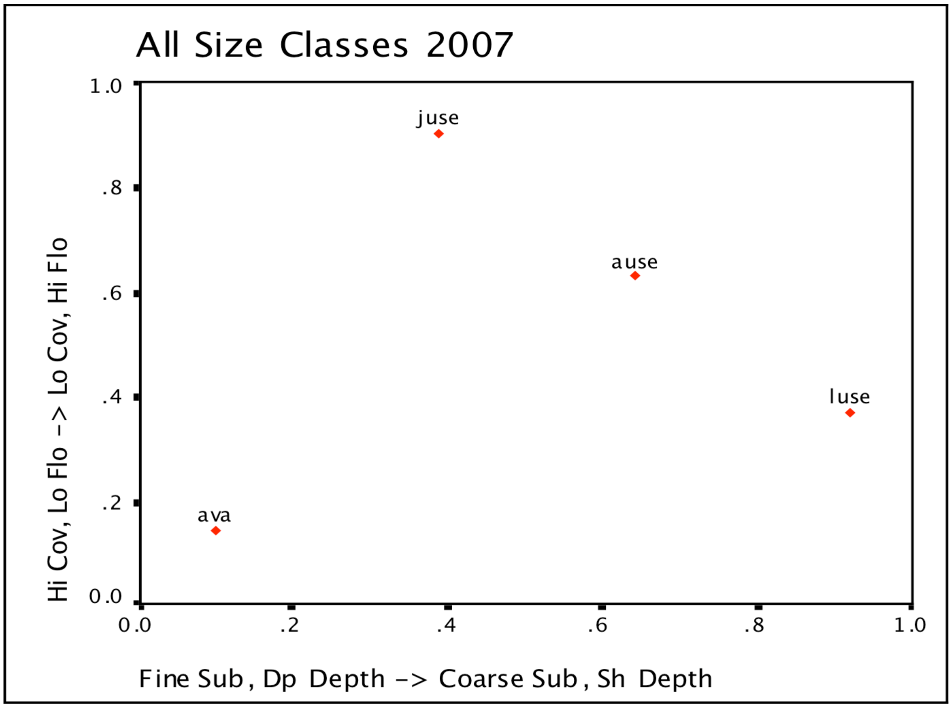


Figure 29. PCA scatterplot for all habitat variables and size classes 2007.

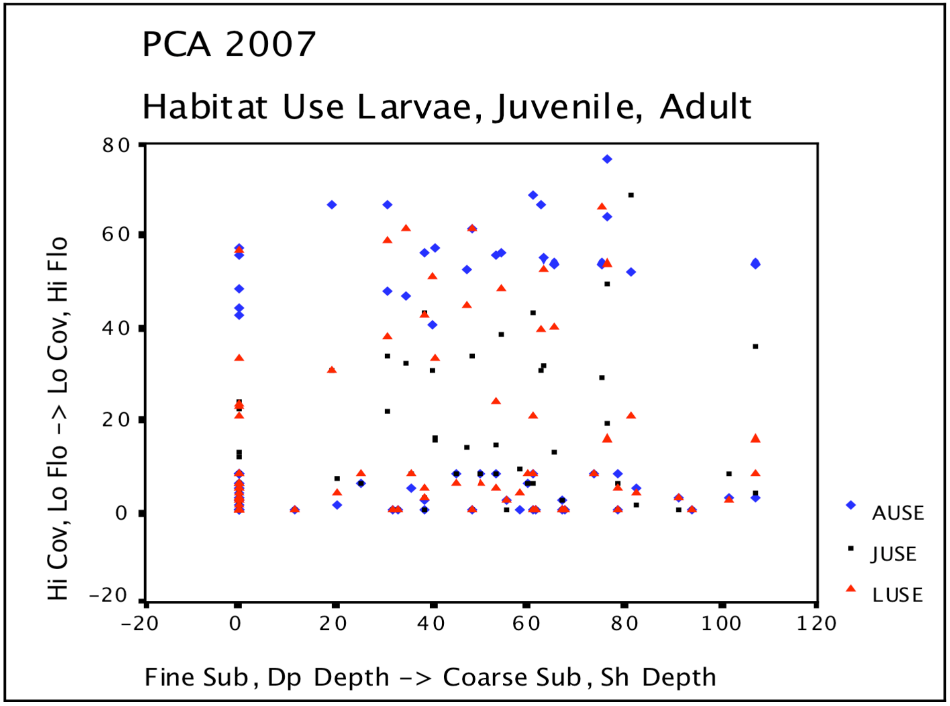


Figure 30. PCA component plot for larvae, juvenile, adult size classes habitat use and available combined 2008.

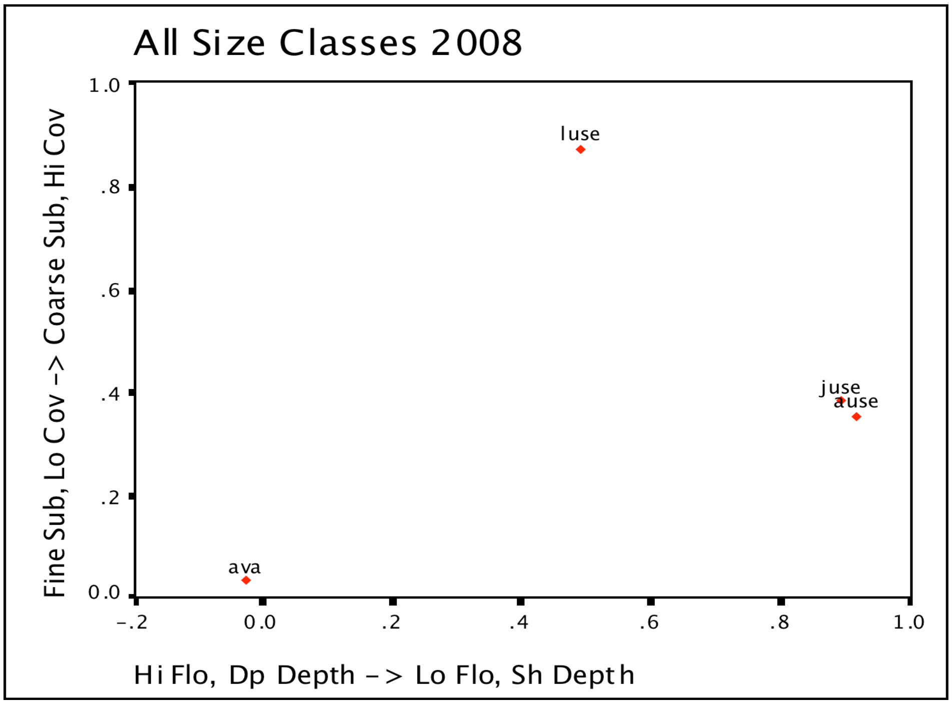


Figure 31. PCA scatterplot for all habitat variables and size classes 2008.

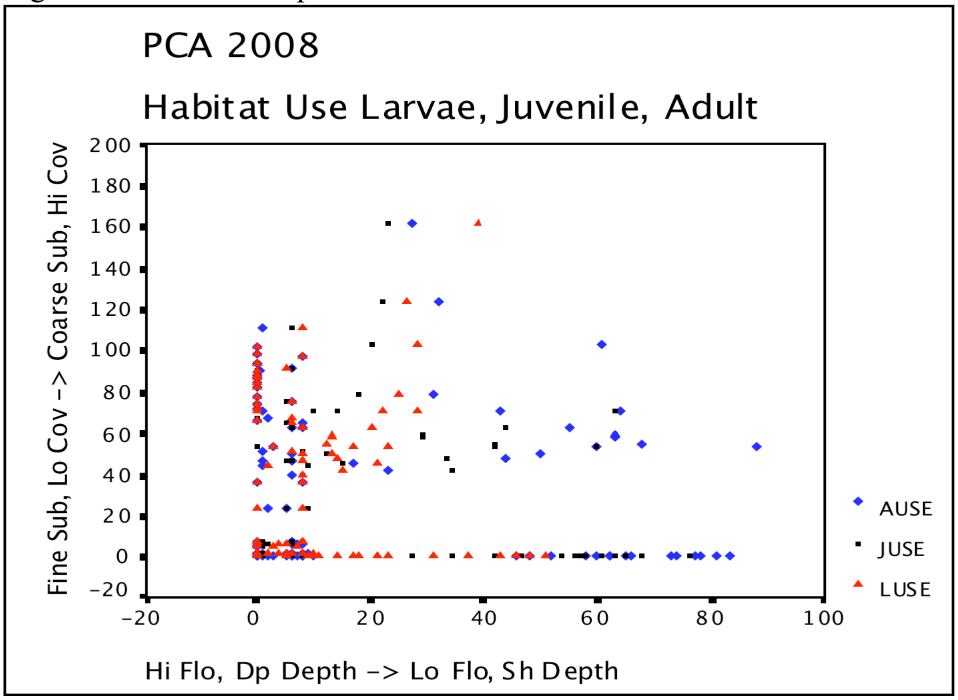


Figure 32. PCA component plot habitat variables separate for Cape Fear shiner larvae (l), juvenile (j), and adult (a) 2007.

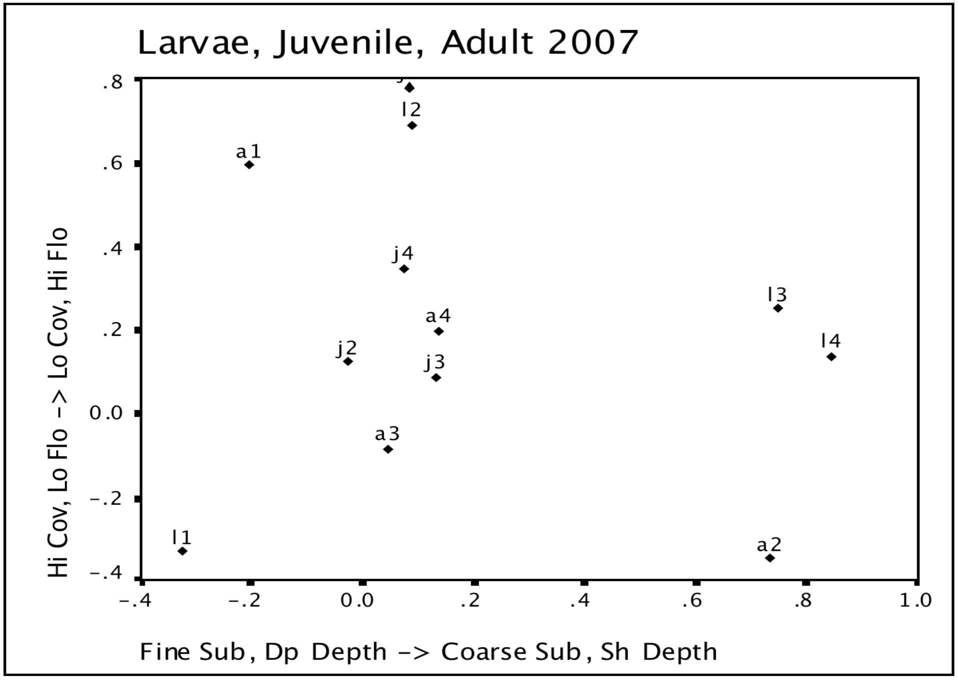


Figure 33. PCA component plot habitat variables separate for Cape Fear shiner larvae (l), juvenile (j), and adult (a) 2008.