ABSTRACT

HOWARD, AMANDA KELLY. Influence of instream physical habitat and water quality on the survival and occurrence of the endangered Cape Fear shiner. (Under the direction of Thomas J. Kwak and W. Gregory Cope)

The Cape Fear shiner *Notropis mekistocholas* is a recently described cyprinid fish endemic to the Cape Fear River Basin of North Carolina. Only five declining populations of the fish remain, and therefore, it has been listed as endangered by the U.S. Government. Determining habitat requirements of the Cape Fear shiner, including physical habitat and water quality, is critical to the species' survival and future restoration. This study integrated the sciences of toxicology and conservation biology, and simultaneously assessed ecosystem-level influences of habitat (water and physical environments) on survival, growth, occurrence, and distribution of the Cape Fear shiner. I conducted an instream microhabitat suitability analysis among five sites on the Rocky and Deep rivers to (1) quantify Cape Fear shiner microhabitat use, availability, and suitability in extant habitats, (2) determine if physical habitat alterations are a likely cause of extirpation of the Cape Fear shiner at historical locations and if instream habitat is a limiting factor to occurrence and survival of the species in extant habitats and at potential reintroduction sites, and (3) estimate population density at selected extant sites. I used an *in situ* 28-day bioassay with captively propagated Cape Fear shiners to (1) determine if water quality is a limiting factor to the occurrence, growth, and survival of the Cape Fear shiner, (2) document habitat suitability by assessing inorganic and organic contaminants through chemical analyses and review of existing data, and (3) assess the protectiveness of water quality standards for primary pollutants based on comparisons of laboratory, field toxicity, and water chemistry data.

Cape Fear shiners most frequently occupied riffles and velocity breaks (i.e., areas of swift water adjacent to slow water), moderate depths, and gravel substrates. They used habitat non-randomly with respect to available habitat, and habitat use was similar between post-spawning and spawning seasons. However, Cape Fear shiners shifted to shallower depths during the spawning season, suggesting that adequate depth distribution may be an important element of Cape Fear shiner habitat. Comparisons of suitable microhabitat among river reaches where the Cape Fear shiner is extant, rare, or extirpated suggest that suitable substrate (gravel) may be lacking where the fish is rare, and that suitable microhabitat combinations, especially for water velocity, are rare at all sites. Cape Fear shiner density was too low to be estimated in upstream reaches of the Deep River where gravel substrate is limited. Population density ranged from 795 fish/ha to 1,393 fish/ha at three sites surveyed. Potential reintroduction sites had shallower mean depths than those at extant sites, and the extirpated site on the Rocky River contained the most suitable physical habitat, but lacked adequate water quality. A site on the Deep River where the species persists, but is rare, is a candidate reach for habitat restoration, but would require substrate alteration to improve conditions for the Cape Fear shiner.

After conclusion of the 28-day *in situ* test, I measured fish survival, growth (an increase in total length), and contaminant accumulation. Survival of caged fish averaged 76% and ranged from 53% to 100%. Sites with the greatest mean survival were on the Deep River (87%), followed by those on the Rocky River (74%), and were lowest on the Haw River (66%). Fish survival was significantly lower at five sites, two in the Haw River, two in the Rocky River, and one in the Deep River. Caged fish grew significantly at four of the 10 sites, and all fish accumulated quantities of Cd, Hg, PCBs, DDTs, and other contaminants

over the test duration. Results from the *in situ* exposures indicate that a reintroduction site on the Rocky River does not have adequate water quality to support reintroduction, yet results from the instream habitat assessment indicate that physical habitat is similar to extant Cape Fear shiner locations.

Finally, the survival and recovery of the Cape Fear shiner is dependent upon the successful protection of remaining suitable physical habitat and water quality that will require broad-scale examination and approaches considering physical instream habitat, water quality and contaminants, biotic interactions with other organisms, as well as human uses and alterations of the river, riparian zone, and watershed.

INFLUENCE OF INSTREAM PHYSICAL HABITAT AND WATER QUALITY ON THE SURVIVAL AND OCCURRENCE OF THE ENDANGERED CAPE FEAR SHINER

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BIOGRAPHY

Amanda Kelly Howard (she prefers Mandy, always has) was born in Tucson, Arizona, on 29 April 1976 to Carl and Carol Howard, both of southern origins. A few months after her birth, they packed her up and moved back to Alabama, to the town of Moody, where her maternal great-grandfather had been the first mayor. She graduated from Moody High School in 1994 and entered Auburn University that fall to study marine biology. At the time, she had no idea what "marine biology" meant, but she knew it wasn't business or finance, and that it probably had to do with science and nature, which she loved.

The turning point in her scientific direction came when she took an ichthyology class from Dr. Carol Johnston in the winter of 1998, and was completely amazed by the diversity of fishes in those small Alabama streams. Her interest in fishes continued to grow while she volunteered and worked with Dr. Johnston in the ichthyology lab.

After graduating from Auburn in March 1999, she began work as a technician for the Alabama Cooperative Fish and Wildlife Research Unit. Looking to broaden her horizons and fulfill a lifelong dream of working in the Florida Everglades, she moved to Miami in August 1999 to work as a research technician at Florida International University. While there, she drove airboats, conducted experimental research in Everglades National Park, and made some terrific friends. But, she really missed the streams and fishes of the southeast and decided to return to the south (the real south, not south Florida) to pursue a Master's degree at North Carolina State University in 2000. The content of this thesis dominated the following two years of her life.

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Drew Dutterer, Nick Jeffers, Ed Malindzak, Ryan Speckman, and Stephen Wilkes assisted with data collection under harsh field conditions. Peter Lazaro helped with the water quality analyses and also provided field assistance. A special thanks goes to Bill Pine and Dave Hewitt for friendly support whenever I needed it and for dealing with me on a daily basis.

The constant support of my parents has been a blessing all my life, and I am sincerely grateful for all they have done and said throughout these two years. My experience at NC

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Chapter 1
INFLUENCE OF INSTREAM PHYSICAL HABITAT ON THE SURVIVAL AND OCCURRENCE OF THE ENDANGERED CAPE FEAR SHINER

Introduction

Worldwide, hydrological alterations, such as dam construction and stream channelization, and degraded water quality are producing global-scale negative effects on the environment (Rosenberg et al. 2000). Consequently, 50% of the species that are federally threatened or endangered in the United States are dependent upon life in water at some time in their life cycle (USFWS 2002). Freshwater fishes are the most diverse of all vertebrate groups, but they are also one of the most vulnerable due to ubiquitous degradation of aquatic ecosystems (Angermeier 1995; Warren et al. 2000; Duncan and Lockwood 2001).

The drainage basins of the southern United States contain the greatest diversity and number of endemic freshwater fishes in North America, north of Mexico, yet many populations are declining; 28% (187 taxa) are recognized as extinct, endangered, threatened, or vulnerable to extinction (Burr and Mayden 1992; Warren et al. 1997, 2000). The growing imperilment of fishes and other aquatic faunas is predominantly due to human mediated changes within watersheds including construction of large and small impoundments, water withdrawals, urbanization and other land-use alterations, and environmental pollution (Moyle and Leidy 1992; Burkhead et al. 1997; Burkhead and Jelks 2001).

Habitat loss and increasing insularization of populations are factors that have been related to the extinction of species (Angermeier 1995). Cataclysmic loss of diversity via extinction is not the norm (Warren et al. 1997). Instead, regional extirpations generally precede extinction and indicate a population's sensitivity to habitat degradation and insularization (Angermeier 1995). Furthermore, isolated endemics and other geographically restricted species are more vulnerable to catastrophic events such as droughts, floods, or chemical spills, and localized degradation of physical habitat and water quality, and

therefore, have a greater risk of extirpation and extinction (Warren and Burr 1994; Burkhead et al. 1997). Information relating to the ecology of rare native fishes, including habitat needs and natural history, is critical to help explain reasons for decline and to help improve recovery efforts (Warren et al. 1997).

The Cape Fear shiner, *Notropis mekistocholas* (Cyprinidae), a federally endangered, restricted-range endemic of the Cape Fear River drainage, North Carolina, is among the Southeast's declining fish species (USFWS 1987). This species is a relatively recent discovery, having first been collected in the early 1960's by Snelson and later described by him (Snelson 1971). Since the time of its initial discovery, it has been extirpated from much of its historic range and is currently known from only five remaining populations in the Cape Fear River basin (Pottern and Huish 1985, 1986, 1987; NCWRC 1995, 1996).

Important elements of physical instream habitat that are necessary to support Cape Fear shiner populations are medium-sized rivers and streams with adequate flow and substrate compositions, which create suitable combinations of water depth and velocity over substrates that support physical cover, such as woody debris and plant material. The species is most frequently associated with habitats of gravel, cobble, and boulder substrates (Pottern and Huish 1985; USFWS 1988). Adults have been collected in riffles, shallow runs, and slow pools with these substrates, while both juveniles and adults occur in slackwater and flooded side-channels of good water quality and relatively low silt loads. Emersed aquatic vegetation, specifically American water-willow *Justicia americana*, or conditions associated with such vegetation create highly suitable habitat for the Cape Fear shiner, especially during spawning (USFWS 1988; NCWRC 1995). Primary proximate stressors negatively affecting the Cape Fear shiner may be degraded physical instream habitat or changes in water quality

(Pottern and Huish 1985). Pervasive and complex changes to the landscape have led to degraded water quality, habitat loss, and the fragmentation and isolation of Cape Fear shiner populations that we observe today. Prior to the initiation of this research, there was no quantitative information on the habitat ecology of the Cape Fear shiner.

The influence of dams is a critical detriment to the physical habitat of the Cape Fear shiner. The impact of dams and associated impoundments on aquatic ecosystems is pervasive, and they harm instream physical habitat by altering flows and changing the biological and physical characteristics of river channels (Bednarek 2001); further, they disrupt metapopulation dynamics and prevent dispersal of individuals (Winston et al. 1991; Schrank et al. 2001). Construction of dams has greatly altered the Cape Fear River ecosystem, fragmenting what was once a continuous Cape Fear shiner population into several remnant declining ones. Stream reaches that once provided continuous, highly suitable riffle–pool sequences and emersed aquatic vegetation were impounded to create unsuitable lentic surroundings and fragmenting remaining habitat patches.

Sediment transport is a natural part of the fluvial process (Waters 1995), but excessive sedimentation from soil erosion and agricultural runoff can threaten aquatic organisms (Pimentel et al. 1995). Sedimentation is the most widespread cause of stream impairment in the Cape Fear Basin (NCDWQ 1996). The Cape Fear shiner is vulnerable to excessive sedimentation, owing to its feeding habits utilizing benthic algae and spawning habitat over coarse substrate materials (Snelson 1971; Pottern and Huish 1985; personal observation).

Quantitatively determining specific habitat requirements of this species among the remaining populations is critical to its survival. My study contributes components of

information necessary to conserve and protect remaining habitats as well as to restore habitats that have been degraded. It is my hope that this information will prove useful in the strategic planning and broad restoration efforts as laid out in the Cape Fear shiner recovery plan (USFWS 1988).

Objectives

Cape Fear shiner populations have steadily declined since the species' discovery in 1962, and physical habitat degradation and poor water quality are likely causes. In Chapter 1, I focus on quantifying physical habitat suitability of the Cape Fear shiner and relate it to historical and extant locations in order to assess the habitat quality of potential reintroduction or population augmentation sites. This chapter complements Chapter 2 on water quality and toxicology of Cape Fear shiner habitat to improve our overall understanding of the fish's ecology and assist federal and state resource management agencies in recovery and restoration of this endangered species.

The objectives of research presented in Chapter 1 were to (1) quantify Cape Fear shiner microhabitat use, availability, and suitability in extant habitats during the spring spawning season and summer post-spawning season (2) determine if physical habitat alterations were a likely cause of extirpation of the Cape Fear shiner at historical locations and if instream habitat is a limiting factor to occurrence and survival of the species in extant habitats and potential reintroduction and population augmentation sites and (3) to quantify population density of the Cape Fear shiner.

Methods

Study area

The Cape Fear River rises in the north-central Piedmont region of North Carolina, near the cities of Greensboro and High Point and flows southeasterly to the Atlantic Ocean. It is one of only four basins located entirely within the state and is the largest among those, spanning a 15,000-km^2 watershed and 9,735 km of freshwater streams and rivers. The basin supports approximately 22.1% of the state's human population, including 116 municipalities and all or portions of 26 counties (NCDWQ 2000). Land use in the Cape Fear Basin is 26% agriculture, 59% forest, 6% urban, and 9% other uses (NCDWQ 1996). From 1982 to 1992 there was a 43% increase in the amount of developed land in the basin. The basin contains 54% of the state's swine operations, and its swine populations increased 90% from 1994 to 1998 (NCDWQ 2000).

The extant populations of the Cape Fear shiner are located in the Deep, Haw, and Rocky rivers in Randolph, Moore, Lee and Chatham counties, North Carolina (USFWS 1988; NCWRC 1996). I selected six primary study sites to collect data on Cape Fear shiner microhabitat use, availability, and suitability, and to estimate population density estimates (Figure 1). These included four river reaches where the Cape Fear shiner is extant and common (use, availability, and density data collected, sites 1–2, 4–5 below), one where the fish is extant, but rare (availability data only, site 6), and one where the fish has been extirpated (availability data only, site 3); these locations are described below.

(1) Rocky River, 500 m upstream and 500 m downstream of U.S. Highway 15-501, Chatham County. Cape Fear shiner is extant; microhabitat use, availability data, summer 2001 and spring 2002; density estimate summer 2002.

(2) Rocky River, 200 m upstream of the confluence with the Deep River, Chatham County. Cape Fear shiner is extant; microhabitat use and availability data, summer 2001 and spring 2002.

(3) Rocky River, at the NC Highway 902 bridge crossing, Chatham County,. Cape Fear shiner is extirpated; microhabitat availability data only, summer 2001.

(4) Deep River, 100 m downstream of confluence with Rocky River, Chatham County. Cape Fear shiner is extant; density estimate summer 2002.

(5) Deep River, approximately 1 km downstream of Highfalls Dam, Moore County. Cape Fear shiner is extant; microhabitat use and availability data, summer 2001 and spring 2002; density estimate summer 2002.

(6) Deep River, downstream of Coleridge Dam, Randolph County. Cape Fear shiner is extant, but rare; microhabitat availability data only, summer 2001.

Microhabitat use, availability, and suitability

Fish microhabitat use. From 29 July 2001 to 5 October 2001 and from 29 April 2002 to 5 June 2002, Cape Fear shiners were observed, microhabitats were identified, and characteristics were quantified for summer–fall post-spawning (2001) and spring spawning (2002) periods at sites 1, 2, and 5. At each site, on multiple occasions, I snorkeled in an upstream direction to locate fish with minimal disturbance. When an individual or group of

Cape Fear shiners was observed, I dropped a colored weight to mark the precise location of the fish, and I immediately measured and recorded focal depth and focal velocity, which are defined as the distance between the fish's snout and the substrate, and the velocity at the fish's snout, respectively. For a group of Cape Fear shiners, I estimated the average focal depth and focal velocity of the group. Reaches were snorkeled in approximately 50-m sections. Distance to cover was also recorded at each Cape Fear shiner location. After thoroughly searching a section, we returned to each colored weight and measured physical habitat characteristics. Water depth, mean column velocity, focal velocity, focal depth, substrate composition, and associated physical cover were measured at 99 (2001) and 66 (2002) specific Cape Fear shiner locations (totals for three sites). General observations relating to feeding behavior were also recorded in 2001 and 2002. The spring 2002 microhabitat data were collected during the spawning period for this species. Although spawning related activities were observed, the data are meant to represent general microhabitat use during the spawning season, rather than precise spawning measurements.

Water depth was measured with a top-set wading rod to the nearest centimeter, and velocity was measured with a Model 2000, Marsh McBirney, portable flow meter. Mean velocity was measured at 0.6 of total depth from the water surface (depths less than 0.76 m) or was calculated as the average of measurements at 0.2 and 0.8 of total depth (depths greater than or equal to 0.76 m). Substrate was categorically determined visually, and the dominant substrate was classified according to a modified Wentworth particle size scale (Table 1). Categories used in the analysis for substrate were silt, sand, gravel, cobble, small boulder, large boulder, and mammoth boulder. The categories represent dominant substrate on an increasing particle size scale, and therefore substrate was considered a continuous variable

for my analysis. Associated physical cover categories were algae, American water-willow, other aquatic macrophytes, rock overhang, roots, terrestrial vegetation, and woody debris.

Available Microhabitat Surveys. During August through September 2001, coinciding with the post-spawning period in which the microhabitat use data were gathered, available microhabitat surveys were conducted at the three sites with extant Cape Fear shiner populations (sites 1, 2, and 5) and at the two sites where the fish is rare or extirpated (sites 3 and 6). We utilized the transect and point-intercept method to quantify available microhabitat under typical base-flow conditions (Simonson et al. 1994). At each site, we took 15 measurements of stream width to obtain a mean stream width (MSW), which we used to determine the appropriate length of the reach and distance between transects to be sampled. The location of the first transect was selected randomly, and a minimum of 10 equally-spaced transects were sampled within the reach. A minimum of 10, regularly spaced points were sampled along each transect; thus at least 100 points were sampled per reach. This is greater sampling intensity than that recommended by Simonson et al. (1994). Data collected at points sampled along each transect included all variables quantified for microhabitat use.

During May through June 2002, coinciding with the spawning period in which microhabitat use data were gathered, I repeated microhabitat surveys at the three sites with extant Cape Fear shiner populations (sites 1, 2, and 5). In these surveys, data were collected for the same physical variables and following the same transect selection procedure, as described previously, but only five transects were sampled at each site. Measuring fewer transects was justified by taking a stratified sample from the transects sampled in 2001 and testing for differences in the distribution of continuous variables using a Kolmogorov-

Smirnov (K-S) two-sample test for depth, mean column velocity, and substrate, and a chi-square test on categorical cover data. All tests yielded *P*-values greater than 0.05.

Statistical analyses on microhabitat use and availability. Post-spawning (summer-fall 2001) and spawning (spring 2002) season microhabitat data were analyzed separately. I used principal components analysis (PCA) on habitat availability data for continuous variables (depth, mean column velocity, and substrate) to quantify habitat characteristics with fewer variables. Cover was omitted from this analysis. PCA was preformed separately for habitat availability data by river and period (spawning, post-spawning) for a total of four analyses. The PCA extracted linear combinations of the original variables that explained the maximum amount of variation in the data. Components with an eigenvalue near one (i.e., greater than 0.90) were retained (Stevens 2002). Microhabitat-use component scores were calculated from the scoring coefficients generated by the habitat-available PCA, stratified by river and period. Comparing microhabitat-use and availability component scores with a K-S two-sample test (Sokal and Rohlf 1981) tested for non-random habitat use. To determine which variables were responsible for component score differences, K-S two-sample tests were performed on univariate distributions of microhabitat use and availability for water depth, mean column velocity, and substrate composition. A chi-square test was performed on cover data to test for non-random cover use. All statistical analyses were performed using PC SAS v8.1 (1999-2000).

Microhabitat Suitability. Microhabitat suitability was quantified as microhabitat use divided by availability. This parameter expresses the relative importance of microhabitats based on the intensity of use relative to the amount available (Bovee 1986). Suitability was calculated for ranges or category of each variable (depth, mean velocity, substrate

composition, and cover), according to river (Rocky River sites combined), and then results for each variable were standardized to a maximum of 1.0, with a value of 1.0 designating the most suitable range or categories, with suitability of other ranges or categories decreasing toward zero. To determine overall species suitability for each variable, suitability values for each range or category of a variable in the two rivers were summed, and those results were standardized to 1.0 again. This analysis was preformed separately for data from each period (spawning, post-spawning).

Cape Fear shiner population density

Cape Fear shiner population density was estimated using the strip transect method (Buckland et al. 2001), that is, snorkeling through a measured strip transect and visually counting all individuals in the transect. Populations were estimated at two sites in the Deep River and one in the Rocky River (sites 1, 4, and 5) during summer 2002. Surveying was also attempted at two other sites where the Cape Fear shiner is considered extant, just below the Coleridge Dam and at SR 1456 (Howard's Mill Road), both in the Deep River. Only two individual Cape Fear shiners were observed at SR 1456 after intensive snorkeling, and no Cape Fear shiners were observed below the Coleridge Dam. Due to the low density of the Cape Fear shiner at these two sites, it was not possible to generate reasonable density estimates for either site.

Ten strip transects were surveyed to estimate population density at each site surveyed (sites 1, 4, 5). The first transect was chosen randomly, and the following nine transects were spaced every 50 meters in an upstream direction. The width of each transect was based on the visibility in water on the day of sampling. The length of each transect varied based on how far I could snorkel without an obstruction (i.e., mammoth boulder or woody debris). To

reduce bias associated with the visual assessment of strip width, I used weights attached to flagging tape and a float to mark the boundary of the strip being sampled. Cape Fear shiners are often found in clusters. To account for this clumped distribution, I counted all fish in a cluster if more than 50% of the individuals were within the strip boundary, and conversely I did not count individuals in the cluster if more than 50% were outside the strip boundary (Buckland et al. 2001). The width and length of each transect, and the number of Cape Fear shiners in each was recorded to approximate Cape Fear shiner density (fish/hectare). Mean stream width was incorporated to calculate number of fish per linear kilometer of river. I generated mean Cape Fear shiner density for each site and associated confidence intervals among the 10 transects using standard statistical methods (Sokal and Rohlf 1981). These estimates represent minimum densities due to the possibility that individual fish in the strip were not detected.

Results

Microhabitat use, availability, and suitability

Post-spawning season. Available habitat in the Rocky River (sites 1 and 2) during the post-spawning season (summer 2001) was described by gradients from riffle to pool (component 1) and from bank to thalweg (i.e., the swiftest, deepest part of the channel; component 2; Table 2). Two principal components explained a combined 77% of the variance in the available habitat in the Rocky River during summer 2001(Table 2). All three variables were significantly correlated with component 1, and substrate and mean velocity were significantly correlated with component 2 (Table 2). Component 1 (riffle-pool) was interpreted as describing a gradient from riffles to pools because it was positively loaded on depth and substrate, and negatively loaded on velocity (Figure 2a). Pools in the Rocky River

were deep, with lower velocities, and coarser substrate (i.e., boulder or bedrock), relative to riffles that were shallow with higher velocities and finer substrates (i.e., gravel or cobble). Component 2 (bank-thalweg) was interpreted as describing a gradient from near-bank to mid-channel areas, because it was positively loaded on substrate and mean velocity (Figure 2a). Near-bank areas in the Rocky River had fine substrates (i.e., silt and sand) and lower velocities, compared to mid-channel areas (thalweg) with higher velocities and coarse substrates.

Cape Fear shiners occupied microhabitats in the Rocky River during the post-spawning season that were most often associated with riffle habitat (Figure 2a). I found the Cape Fear shiner most frequently associated with moderate depths (40-49 cm), water velocity breaks (i.e., areas of swift water adjacent to slow water), cobble substrates, and emersed vegetation (American water-willow) (Figures 4-5), which are all characteristics associated with riffles. This result was supported by K-S two-sample comparisons between habitat available PCA scores and Cape Fear shiner microhabitat use scores, indicating that Cape Fear shiners occupied microhabitats in a non-random manner with respect to component 1 (riffle-pool) ($P = 0.003$; Table 3). Distributions of component 2 (bank-thalweg) scores of microhabitat use and availability were not significantly different ($P = 0.65$; Table 3), indicating that the Cape Fear shiner used habitat in a random manner with respect to near-bank or mid-channel.

The non-random habitat use revealed by component 1 scores is further supported by the univariate analysis of Cape Fear shiner microhabitat use and availability frequency distributions. Frequency distributions of microhabitat use were significantly different than those corresponding distributions of microhabitat availability for all four variables ($P < 0.05$;

Figures 3-4), indicating non-random habitat use in the Rocky River by this species. Mean values of depth varied moderately between those for fish use and availability (37.5 cm versus 43.0 cm; Table 4), as did means of use and availability for mean velocity (0.037 m/s versus 0.031m/s). Cobble was the most frequently encountered substrate at Cape Fear shiner locations (Figure 4a). Cape Fear shiners were not associated with physical cover at a majority of microhabitat locations in the Rocky River, but among cover objects with which the Cape Fear shiner associated, American water-willow was the most common (Figure 4b).

Available post-spawning habitat in the Deep River (site 5) was described by gradients from bank to thalweg (component 1) and from pool to riffle (component 2; Table 2). Two principal components explained a combined 73% of the variance in the Deep River (Table 2). All three variables were significantly correlated with component 1, and depth and substrate were significantly correlated with component 2 (Table 2). Component 1 (bank-thalweg) was interpreted as describing areas from near-bank to the thalweg, because it was positively loaded on depth, mean velocity, and substrate (Figure 2b). Bank areas in the Deep River have fine substrates (i.e. silt and sand), relative to the thalweg which was deep, with the highest velocities, and coarse substrates (i.e. boulder or bedrock). Component 2 (pool-riffle) was interpreted as describing the gradient from pool to riffle because it was negatively loaded on depth and positively loaded on substrate (Figure 2b). Pools in the Deep River had finer substrates, such as silt and sand, as compared to pools in the Rocky, which had coarser substrates, and riffles in the Deep River had coarse substrates such as gravel, cobble, and boulders (Figure 2a and b).

In the Deep River, Cape Fear shiners were most frequently associated with moderate depths (40-49 cm), velocity breaks, and gravel substrate (Figure 5 and 6a). Cape Fear shiners

were not associated with physical cover at a majority of locations, but when they associated with cover, it was most commonly American water-willow (Figure 6b). Their habitat use with respect to riffle-pool and bank-thalweg gradients was non-random as indicated by distributions of component 1 ($P = 0.033$) and component 2 ($P = 0.022$) scores for habitat availability and microhabitat use (Table 3). Univariate comparisons of the frequency distributions of available habitat and microhabitat use for depth and cover were also significantly different (Figures 5a, 6b), and the use and availability means for depth differed moderately (41.2 cm versus 35.3 cm; Table 4), similar to those in the Rocky River. However, mean velocity distributions were not significantly different in the Deep River ($P = 0.25$; Figure 3b) even though a substantial difference in mean values occurred (0.048 m/s versus 0.106 m/s; Table 4), and the comparison for substrate composition was marginally significant ($P = 0.065$; Figure 6a), suggesting that Cape Fear shiners occupied microhabitats randomly with respect to velocity and substrate in the Deep River. This result may be due to the greater proportion of finer substrates (i.e., gravel and cobble) and the range of mean velocities available in the Deep River. Because the range of depths occupied by the Cape Fear shiner in the Deep River was very narrow (30-60 cm; Figure 5a) and the frequency distributions are significantly different for depth ($P = 0.001$), the Cape Fear shiner is selective for depth in the Deep River. This conclusion is further supported by the cluster of habitat use components scores, which correspond to moderate depths between riffles and pools and between the bank and thalweg (Figure 2b).

Cape Fear shiners occupied similar focal depths and focal velocities in the Rocky and Deep rivers during the post-spawning season. In the Rocky River, mean focal depth was 11.9 cm and mean focal velocity was 0.026 m/s (Table 4). Mean focal depth in the Deep

River was 10.4 cm and mean focal velocity was 0.022 m/s (Table 4). Cape Fear shiners were most frequently located at focal depths of 10–15 cm and focal velocities from 0-0.02 m/s in both rivers (Figure 7). Mean focal depth in both rivers is one-third or less than mean total depth, and this is expected for an epi-benthic species whose primary food source is detritus and plant material located on or in the substrate (Snelson 1971; personal communication, John Groves, North Carolina Zoological Park, Asheboro). Mean focal velocities were lower than mean column velocities of Cape Fear shiner locations in both rivers (Table 4).

Cape Fear shiners were more frequently located farther from cover in the Deep River than in the Rocky River during the post-spawning season. Cape Fear shiners were located within 25 cm of cover at 70% of locations in the Rocky River (Figure 8a). In contrast, only 24% of locations in the Deep River during the post-spawning season were within 25 cm of cover, and 66% of locations were greater than 50 cm from cover (Figure 8b). Cover was available in the Rocky and Deep Rivers at 45% and 52% of the availability points, respectively, with similar proportions of available American water-willow in both rivers (Figures 4b, 6b). American water-willow occupies areas with similar depths, velocity, and substrate, as does the Cape Fear shiner. Therefore, the more distance from cover of microhabitat use locations in the Deep River may be explained by the greater availability of optimal substrates and velocities that were not associated with American water-willow beds. And conversely, available microhabitats that were most suitable for Cape Fear shiner and American water-willow were in shorter supply in the Rocky River, which may have lead to a closer association with American water-willow.

Disproportionate use of microhabitats relative to their availability (non-random or selective habitat use) led to the identification of most suitable microhabitats, which differed

in some characteristics from those most frequently occupied by Cape Fear shiners. Most suitable microhabitats had similar depth, higher mean velocity, similar substrate, and a greater cover association with the aquatic macrophyte American water-willow (Figures 3–6 and 9–10). The most suitable Cape Fear shiner habitat, based on relative proportions of microhabitat use and availability from both rivers (three sites total) was 40–49 cm deep, with mean water velocity of 0.16–0.19 m/s, over gravel substrate, and associated with beds of American water-willow (Figures 9–10).

Microhabitat comparison among extant, extirpated, and rare sites. Comparisons of mean values of physical variables describing use and availability among river reaches where the Cape Fear shiner is extant, rare, or extirpated revealed shallower mean water depths at rare or extirpated sites, relative to those extant, and mean velocities that were similar among sites. Mean depth of the reach sampled in the Rocky River where the Cape Fear shiner has been extirpated (site 3) was 16 cm (37%) lower than that of two reaches where the Cape Fear shiner is extant (sites 1 and 2) and 10.5 cm (28%) lower than mean depth of microhabitats occupied by the fish in that river (Table 2). The same trend occurred in the Deep River where the mean depth of a reach where the Cape Fear shiner is extant, but rare (site 6) was 7.8 cm (22%) lower than that of a site where the fish is extant and common (site 5) and 13.7 cm (33%) lower than occupied microhabitats in that river (Table 4). Mean depths of extirpated and rare sites on these rivers were b below the most suitable range for Cape Fear shiners (40–49 cm), as was the mean depth at the Deep River extant site; however, mean depth of Rocky River extant sites fell within the suitable range of depths (Table 4, Figure 9a). Mean velocities of reaches on the Rocky River where the Cape Fear shiner is extant and extirpated were both similar to that of microhabitats occupied by the fish, and while mean

velocity of the Deep River reach where the fish is rare was lower than that of the extant reach, both were greater than that of occupied microhabitats (Table 4).

Comparing of proportions of suitable microhabitat available among river reaches where the Cape Fear shiner is extant, rare, or extirpated suggests that habitat similar to extant sites is available at rare or extirpated sites in both rivers, with the exception of a lack of suitable substrate at the rare site in the Deep River. In the Rocky River, the site where the fish is extirpated contained proportions of suitable microhabitats for depth (40–49 cm, 11.2% versus 12.4%), mean velocity (0.16–0.19 m/s, 0.7% versus 0.8%), substrate (gravel, 14.4% versus 11.6%), and cover (American water-willow, 23.9% versus 13.2%) that were equivalent or exceeded those proportions of extant sites (Table 5). Similarly at Deep River sites, the proportion of suitable depth (40–49 cm, 17.8% versus 13.6%), mean velocity (0.16–0.19 m/s, 4.1% versus 1.7%), and cover (American water-willow, 17.2% versus 9.3%) at the rare site exceeded those corresponding proportions at the extant site. Conversely, there was a much lower percentage of suitable substrate (gravel, 4.7% versus 26.3%) at the rare site versus the extant site of the Deep River (Table 5). The low proportion of gravel substrate available at the Deep River site where the Cape Fear shiner is rare (site 6) is not likely due to embeddedness by fine sediments (i.e., silt and sand), as only 5% of the substrate at the rare site was composed of fine sediment, while 32% of the substrate at the extant site (site 5) was fine particles.

Suitable microhabitats composed 26.3% or less of the total available habitat for all variables examined, and suitable velocities were available at no more than 1.7% of any extant site, which suggests that suitable Cape Fear shiner habitat may be scarce, even at sites where the fish is common. The proportion of suitable microhabitat at a site ranged from 0.8% for

suitable mean velocity at the extant site of the Rocky River to 26.3% for suitable substrate at the extant site on the Deep River (Table 5). Considering that suitable microhabitat for a fish species must provide the proper combination of suitable characteristics for depth, velocity, substrate, and cover, it is reasonable to conclude that the occurrence of those conditions in the river reaches surveyed is very rare.

Spawning season. Available habitat in the Rocky River (sites 1 and 2) during spawning season (spring 2002) was described by gradients from riffle to pool (component 1) and from thalweg to bank (component 2; Table 2; Figure 11a). Two principal components explained a combined 71% of the variance in the data (Table 2). All three variables were significantly correlated with component 1, and substrate and mean velocity were significantly correlated with component 2 (Table 2). The interpretation of the axes from the PCA on habitat available data from the Rocky River during summer 2001 and spring 2002 are identical, and therefore the description will not be repeated here (refer to section on Post-spawning season).

Cape Fear shiners used microhabitats in the Rocky River during the spawning season (spring 2002) that were associated with riffle habitat (Figure 11a). Cape Fear shiners were most frequently associated with shallower depths than in the post-spawning season (20-29 cm versus 40–49 cm) and the distributions of available depths during the post-spawning and spawning season were not significantly different ($P > 0.72$) in a K-S two-sample test. However, Cape Fear shiners were similarly associated with low velocities (0–0.03 m/s) and velocity breaks, gravel substrate, and when cover was used, it was most frequently American water-willow (Figures 12–13). With the exception of mean velocity, all most frequently used categories are characteristics of riffles, as in the post-spawning season. Higher velocities

were very rare in the Rocky River in the spring (Figure 12b), possibly due to drought conditions. The K-S two-sample comparisons between habitat available component scores and microhabitat use scores for PC 1 and PC 2 were both significantly different ($P = 0.0001$ and $P = 0.0012$, respectively; Table 3). This result indicates that Cape Fear shiners occupied microhabitat selectively or non-randomly with respect to both axes. Faster velocities were in short supply (Figures 11a and 12b), and Cape Fear shiner microhabitat use scores are clustered nearer the riffle end of the component 1 axis. Deeper water was available with coarse substrates (i.e., boulders), but Cape Fear shiners were selecting for microhabitats with substrates finer than boulders (i.e., gravel and cobble) with shallower depth in or near (i.e., velocity breaks) moderate mean velocities.

The non-random habitat use revealed by component score results is further supported by the univariate analysis of microhabitat use and availability frequency distributions. Frequency distributions of microhabitat use were significantly different than those corresponding distributions of microhabitat availability for all four variables ($P < 0.05$; Figure 12-13; Table 3), indicating non-random use in the Rocky River during the spawning season. Mean values of depth varied greatly between those for fish use and availability (32.5 cm versus 47.7 cm; Table 6), as did mean values for mean velocity (0.026 m/s versus 0.016 m/s). Cobble was the most frequently encountered substrate at Cape Fear shiner locations (Figure 13a). Cape Fear shiners were not associated with cover at a majority of locations, but among cover types with which the fish was associated, American water-willow was the most common (Figure 13b). Cape Fear shiners occupied similar microhabitats in the Rocky River during the spawning season relative to those occupied during the post-spawning season, with the exception that depths occupied in the spawning season were shallower.

Available habitat in the Deep River (site 5) during the spawning season was described by gradients from riffle to pool (component 1) and from bank to thalweg (component 2; Table 2). Two principal components explained a combined 73% of the variance in the data (Table 2). All three variables were significantly correlated with component 1 (a consistent trend in the analyses) and depth and mean velocity were significantly correlated with component 2 (Table 2). Component 1 (riffle-pool) was interpreted as describing the gradient from riffles to pools because it was positively loaded on depth and substrate, and negatively loaded on mean velocity (Figure 11b). Pools in the Deep River were deep, with coarse substrate and slower velocities. Component 2 (bank-thalweg) was interpreted as describing the gradient between near-bank areas and the thalweg because it was positively loaded on substrate and mean velocity (Figure 11b). Near-bank areas in the Deep River had fine substrates (i.e., silt and sand), whereas the thalweg had higher velocities and coarser substrates.

In the Deep River during the spawning season, Cape Fear shiners were most frequently associated with shallower depths (30–39 cm versus 40–49; Figures 6a and 15a) than during the post-spawning season, and the distributions of available depth during the post-spawning and spawning season were not significantly different ($P > 0.20$) in a two-sample K-S test. Cape Fear shiners were most frequently associated with velocities of 0–0.03 m/s, gravel substrate, and did not associate with cover at a majority of locations, but American water-willow was the most common cover type used (Figures 14a and 15). The distributions of component 1 scores for microhabitat use and habitat available were significantly different ($P < 0.0001$), but the distributions of component 2 scores were not significantly different ($P = 0.16$). These results indicate that fish occupied habitat selectively

with respect to component 1 (riffle-pool), and randomly with respect to component 2 (bank-thalweg). Cape Fear shiners occupied microhabitats that were relatively shallow with higher velocities than those habitats available, and this result is consistent for all principal components analyses (Figure 11b).

Univariate comparisons of microhabitat use and availability frequency distributions were significantly different for all four variables (Figures 14–15; Table 3), again indicating non-random habitat use during the spawning season. Categories used most frequently are those associated with riffles, with the exception of mean velocity; however, higher velocities are in short supply (Figure 14b). Mean values of depth varied moderately between those for fish use and availability (34.6 cm versus 43.9 cm), as did means of use and availability for mean velocity (0.035 m/s versus 0.046 m/s). Cape Fear shiners occupied microhabitats during the spawning season that were shallower than those occupied during the post-spawning season.

Cape Fear shiners occupied similar focal depths in both rivers during the spawning season, but mean focal velocity occupied was greater in the Deep River. Mean focal depth of Cape Fear shiners in the Rocky River was 13.0 cm and 13.4 cm in the Deep River (Table 6). Mean focal velocity in the Rocky River was 0.016 m/s and 0.035 m/s in the Deep River. Cape Fear shiners most frequently occupied focal depths of 10–15 cm and focal velocities of 0–0.02 m/s in both rivers (Figure 16), which was the same result as the post-spawning season. Mean focal depths used in both rivers during the spawning season were slightly greater than those occupied during post-spawning (Tables 4 and 6), however, mean focal velocity used in the Deep River during the spawning season was slightly greater than the mean focal velocity used during the post-spawning season (Tables 4 and 6). Mean focal

velocity used in the Rocky River during the spawning season was slightly less than that occupied during the post-spawning season.

Cape Fear shiners were found near cover more frequently in the Deep River than in the Rocky River during the spawning season, and they were most frequently found within 25 cm of cover in both rivers during both seasons (Figure 8a and b). American water-willow is used in greater frequency than its availability (Figures 13a and 15b), and it is the most common cover object available in both rivers. For reasons discussed in the previous section, Cape Fear shiners closely associate with characteristics of habitat that also favor American water-willow, and while these areas of vegetation may be optimal habitat, it is likely that with the appropriate combination of depth, velocity, and substrate, Cape Fear shiners will occupy areas without cover. This is supported by the occurance of fish as far as 275 cm from a cover object (Figure 8a).

The Cape Fear shiner's distance to cover varied among rivers and seasons. They occupied microhabitats closer to cover objects during the post-spawning season than during the spawning season in the Rocky River (Figure 8a). The frequency distributions of distance to cover in the Rocky River between seasons were significantly different ($P = 0.0228$; Figure 8a). The opposite result was found in the Deep River where Cape Fear shiners were more closely associated with cover objects during the spawning season than during the post-spawning season (Figure 8b). However, the frequency distributions of distance to cover in the Deep River between seasons were only marginally significantly different ($P = 0.0730$), suggesting that the association with cover was similar between the seasons.

Disproportionate use of microhabitats relative to their availability (non-random or selective habitat use) led to the identification of most suitable microhabitats during the

spawning season, and these differed in some characteristics from those most frequently occupied by the Cape Fear shiner during the post-spawning season. Most suitable microhabitats had shallower depth, higher mean velocities, similar substrate, and a similar association with American water-willow (Figures 12–15 and 17–18), relative to the post-spawning season results (Figures 9–10). Thus, the most suitable Cape Fear shiner habitat, based on relative proportions of microhabitat use and availability from both rivers (three sites total) was 20–29 cm deep, with mean velocity of 0.16–0.19 m/s, over gravel substrate, and associated with beds of American water-willow (Figures 17–18). Suitable depth was shallower during the spawning season, relative to the post-spawning season (20–29 cm versus 40–49 cm). Suitable velocity was slower during the spawning season (0.16–0.19 m/s versus 0.08–0.11 m/s; Figures 9–10 and 17–18).

Summary. Cape Fear shiners occupied riffle-type habitat, as interpreted from the PCA, and occupied habitats selectively during both seasons. Microhabitat use was similar between rivers during both seasons, and differed between seasons with respect to depth and mean velocity occupied. Cape Fear shiners occupied habitats with shallower depths and slower velocity in both rivers during the spawning season relative to the post-spawning season. Cape Fear shiners were not associated with cover at the majority of locations. When associated with a cover object, it was most frequently American water-willow. The Cape Fear shiner was more closely associated with American water-willow during the post-spawning season in the Rocky River; however, a closer association with cover, relative to the post-spawning season, was only marginally significant during the spawning season in the Deep River.

Cape Fear shiner population density

Mean population density varied from 795 fish/ha to 1,393 fish/a during summer 2002. Cape Fear shiner mean population density in the Rocky River (site 1) was 1,393 fish/hectare with a 95% confidence interval of 97–2,690 fish/hectare (Table 7). A mean of 19.2 Cape Fear shiners were observed in the ten strip transects sampled, and the mean area surveyed per strip transect was 0.012 ha. Mean population density in the Deep River at site 4 was 795 fish/ha with a 95% confidence interval of 0–1,773 fish/ha, and at site 5 mean population density was 1,056 fish/ha with a 95% confidence interval of 179–1,933 fish/ha (Table 7). A mean of 6.4 fish were observed at the ten strip transects sampled at site 4, and a mean of 10.1 fish were observed at site 5. The mean area surveyed was 0.0084 ha at site 4 and 0.0079 ha at site 5 (Table 7). Mean fish density per kilometer was 6,270 fish/km at site 1, 4,768 fish/km at site 4, and 7,392 fish/km at site 5 (Table 7).

General behavioral and feeding observations

While snorkeling to collect microhabitat data, I also recorded anecdotal observations about general behavior and feeding behavior. During the post-spawning season (summer 2001), 50% of Cape Fear shiners observations were of groups of approximately 10 or more adult individuals (>3 cm). When solitary fish were observed, they were frequently feeding benthically or epibenthically on or in gravel and boulders, and would move between feeding and a school containing multiple minnow species. During the spawning season (spring 2002), Cape Fear shiners were observed most frequently in pairs, assumed to be a male and a female. No direct observations of spawning events (i.e., release of gametes) were observed, but behaviors associated with spawning, such as male-female chasing and males bumping a female's vent with the snout, were observed.

Cape Fear shiners were generally found closer to the substrate than to the surface, and were observed using velocity breaks (i.e., areas of fast water adjacent to slow water). Qualitatively from observations, it appears that this species feeds on macrophytes, periphyton, and detritus that blanket the cobble and boulders in swifter water. Snelson (1971), also observed this in the original species description. Cape Fear shiners also feed on what appeared to be detritus in and around the gravel substrate. Embeddedness was not directly measured. However, Cape Fear shiners were never observed feeding from gravel with a high degree of embeddedness or from boulders that were in heavily silted areas. Cape Fear shiners were only found in reaches with low silt deposition and relatively clean substrates covered with periphyton and detritus.

There is a common assumption that Cape Fear shiners are closely associated with American water-willow. Because of this, most biologists attempting to collect them may do so using the presence of this vegetation as a guide. Cape Fear shiners are easily captured in and around American water-willow beds, but I also located them as far as 60 m from the nearest American water-willow bed while conducting the population density estimates in July 2002. At that specific location, which would be considered riffle habitat, water depth was 35 cm, over cobble and gravel substrates and mean column velocity was 0.11 m/s. American water-willow was not growing in this particular riffle, but Cape Fear shiners were present.

Discussion

Microhabitat use, availability, and suitability

The multivariate analyses on microhabitat use and availability indicate that Cape Fear shiners are associated with riffle-type habitat at the majority of observations, and are using

microhabitats non-randomly. In general, univariate analyses of continuous variables (depth, mean velocity, and substrate) were significantly different for use and availability in both rivers and both seasons, with the exception of the Deep River during the post-spawning season. In this case, depth was the only significantly different continuous distribution (mean velocity and substrate distributions were not significantly different), and indicates that this species was selective for depth in the Deep River during post-spawning. Depth, velocity, and substrate composition are highly correlated variables in rivers, and therefore, it is not unusual that all three paired distributions for use and availability would be different in comparisons between use and availability when a species has a high degree of habitat specificity, as I found for this species. If this species is selecting microhabitats based on one particular variable, such as substrate, it is likely that the depth and mean velocity associated with the substrate will be similar for most observations. Other studies have shown strong interactions between depth, velocity, and substrate for riffle-run guilds of fishes, making it difficult to discern if the fishes are selective for a specific variable (Vadas and Orth 2001). Cape Fear shiners show obvious habitat specificity on a broad scale (i.e., lentic versus lotic habitats), and on a finer scale within the riffle-pool sequence by being selective for specific combinations of depth, velocity, and substrate.

Microhabitat characteristics most suitable for the Cape Fear shiner were waters of moderate depth and velocity over gravel substrate associated with American water-willow, which are general characteristics of riffles in this system. Suitable categories for depth shifted to shallower water suitable velocity was slower in the spawning season. Actual depths occupied during the spawning season were also slightly less in comparisons between seasons, but the reduction was not as dramatic as the shift in suitability. This may be

partially explained by including microhabitat measurements during the spawning season that were not directly related to spawning behaviors. Cape Fear shiners may use deeper water when not directly engaged in reproductive behavior. Distributions of available depths were not significantly different in comparisons between seasons for each river, indicating a real shift in Cape Fear shiner habitat suitability (shallower depths) during the spawning season. However, focal position above the substrate was very similar between rivers and seasons.

Cape Fear shiners were not observed directly in cover at a majority of the microhabitat locations in either river or season; however, they were usually observed within 50 cm of cover (i.e., American water willow), and this may be owing to the similarity between areas of habitat where American water-willow grows and the optimal habitat of this fish, rather than a direct dependence on the plant.

It has been suggested that American water-willow is essential spawning habitat for the Cape Fear shiner (NCWRC 1995). In captivity, Cape Fear shiners have spawned in tanks with artificial cover (cotton mops) and gravel substrate, and eggs have been collected from the gravel (personal communication, Patrick Rakes, Conservation Fisheries, Inc., Knoxville, Tennessee). In contrast, Cape Fear shiners in captivity at the North Carolina Zoological Park in Asheboro spawned in tanks with no cover items, with gravel as the substrate, and eggs were also collected from the gravel (John Groves, NC Zoological Park, Asheboro, NC, personal communication). These observations indicate that under laboratory conditions, Cape Fear shiners can spawn in the presence or absence of cover.

My results do not support a strong association with American water-willow during the spawning season, however, it may provide benefits to the Cape Fear shiner in all seasons. The Cape Fear shiner was more closely associated with American water-willow during the

post-spawning season in the Rocky River, and the evidence for a closer association during spawning in the Deep River is weak. This implies that the presence of American water-willow (or other available cover) may not be a requirement for successful spawning, but it does not discount the beneficial function American water-willow may serve in the field. American water-willow may provide protection from predators and could also serve as velocity refugia when depositing eggs onto the substrate or for egg incubation. Catch per unit effort increases when seining for Cape Fear shiners near American water-willow beds during mid-May (NCWRC 1995), and this may indicate that they migrate to the plant beds during the spring. However, while conducting the population density estimates in July 2002, I observed Cape Fear shiners as far as 60 m from the nearest American water-willow bed. This was an observation after spawning activities had ceased, and therefore, Cape Fear shiners may disperse later in the summer in order to find optimal habitats after the spawning season.

Results of the habitat availability analysis among extant, rare, and extirpated Cape Fear shiner sites support a number of ecological conclusions. First, no one of these microhabitat suitability criteria was abundant within river reaches, and the specific combination of these four factors that would constitute optimal Cape Fear shiner habitat is very rare. In particular, during the post-spawning season, the most suitable range of mean water velocity was extremely rare, occurring in only 0.8% of the area of extant sites on the Rocky River and 1.7% of the extant Deep River site (Table 5). Cape Fear shiners were associated with velocity breaks, where swift water joins slow water, which may partially explain the bimodal distribution of mean velocity suitability (Figure 9b). Comparisons of the availability during summer 2001 of suitable depth among sites where the Cape Fear shiner is

extant and where it is rare or extirpated revealed that similar proportions of suitable depths are available in the reaches sampled, but shallower mean depths were found in reaches where the species is rare or extirpated (Tables 4 and 5). The reduced mean depths may be ecologically relevant to this species, as depth has been considered the most important factor in stream fish habitat selection (Vadas and Orth 2001). Finally, my comparisons between rare and extant Cape Fear shiner habitats in the Deep River suggest that the scarcity of suitable substrate materials in the upper Deep River, which are important for feeding and spawning activities may have contributed to localized decimation of the species and may limit its ecological success where it is extant.

Cape Fear shiner population density

Cape Fear shiner population sizes were proposed to be estimated using a three-pass removal, maximum-likelihood estimator (Seber 1982; Kwak 1992) as an objective of this research. Our experiences and observations on several sampling occasions in the Deep and Rocky rivers suggested that estimation of Cape Fear shiner population sizes was not feasible using a removal method. We attempted sampling with and without the use block nets in a delineated sampling area, and in both cases, the number of Cape Fear shiners collected was based more on chance enclosure of a school of fish within the sampling area than actual population density in the area. Only small discrete areas can be sampled via the removal method, and the resulting population estimates would be unduly variable, and even with many replicate estimates, would not adequately represent actual population sizes. Thus, based on sampling experience and consultations with federal and state agency biologists, we concluded that due to fish behavior, habitat configuration, natural variation in density, clumped fish distributions, and limitations and assumptions of the removal method, that

meaningful Cape Fear shiner population estimates of reasonable accuracy and precision could not be obtained by the removal method, using electrofishing, seining, or any other netting technique.

Underwater observation is becoming a popular and useful method of estimating the distribution and abundance of a variety of fish species, including salmonids and marine reef species. However, success with benthic species, such as darters (Percidae), has been limited (Ensign et al. 1995). Most cyprinids, like the Cape Fear shiner, are considered water-column species, and are good candidates for use of this method. Comparisons of underwater observation and more traditional methods (i.e., depletion or mark-recapture) have shown that underwater observations estimate abundance as well or better than the traditional methods for some species (Hankin and Reeves 1988). I employed the strip transect method rather than distance sampling (Buckland et al. 2001), which can estimate the number of undetected individuals, because visibility was relatively low as compared to some pristine streams and thus, transects were narrow; therefore, it was likely that all Cape Fear shiners in a strip transect were detected.

Underwater observations require less time, expense, and effort than do the more traditional methods, and because of this, I was able to survey 10 strip transects at each site in a reasonable amount of time. Whereas, sampling 10 discrete areas at each site using depletion sampling would have been almost impossible under the logistic time constraints. Instead of using "representative reaches" which can give highly biased and misleading estimates of fish density (Hankin and Reeves 1988), I randomly chose the strip transects within a larger reach, that included riffle, pool, and run macrohabitats. Surveying strip

transects that are randomly selected, can yield estimates that are more representative of a larger area, rather than of the "representative reach".

Results of the strip transect sampling suggest some conclusions about the distribution of Cape Fear shiners. (1) The upper Deep River was thoroughly surveyed at two sites considered to have extant Cape Fear shiner populations, and Cape Fear shiners were either not detected (Coleridge Dam tailrace) or only two individuals were observed (SR 1456) after a large area of habitat was searched. This species is easily located at the three other extant sites used to estimate density, and therefore it appears that there is a large discrepancy between the density in the upstream reaches of the Deep River as compared to reaches downstream in the Deep River and in the lower Rocky River. This may indicate that reaches just upstream (SR 1456) or downstream (Coleridge Dam) of dams and reaches that are locally impacted by agricultural practices, as is the site at SR 1456, may not be able to sustain populations of Cape Fear shiners without substantial habitat restoration. (2) Cape Fear shiners are clumped in distribution and can be found in large groups (up to 82 individuals observed) within the small areas sampled. A clumped distribution that varies greatly depending on available habitat makes extrapolation of densities to larger reaches of river difficult. Cape Fear shiner habitat has been typically characterized by the presence of American water-willow, but because they were not exclusively observed in direct association with it, future efforts could be biased if only American water-willow is targeted. (3) A review of the literature revealed that Cape Fear shiner population density in the Rocky and Deep rivers is moderate to high when compared to the density of minnows in the genera *Notropis*, *Cyprinella*, *Luxilus*, and *Lythrurus* in other warmwater streams of the United States (Lotrich 1973; Vadas and Orth 1993; Vadas 1994; Rambo 1998; Radwell 2000). This

suggests that Cape Fear shiners may be locally abundant in remaining habitats, but are exceptionally rare with respect to the overall area occupied by the species.

Ecological and management implications

My findings related to habitat suitability and availability clearly relate to human uses and alterations of the upper Cape Fear River basin. Lower mean water depth at sites where the Cape Fear shiner is rare or extirpated and an extreme scarcity of suitable water velocity at all sites may be related to the presence and operation of dams, changes in hydrology, and the changes associated with riparian and watershed land use. The construction of small dams and impoundments on rivers and streams has been hypothesized as the reason for decline of other *Notropis* species, including the Topeka shiner, *Notropis topeka*. Schrank et al. (2001) found that the number of small impoundments within a watershed was an important factor in the extirpation of the Topeka shiner in much of its range. Other studies have found that damming has lead to the extirpation of obligate riverine cyprinids above dams (Winston et al. 1991) and reduced species richness and diversity below impoudments (Quinn and Kwak 2003). Dams alter the flow of rivers and fragment species home ranges, and impoundments can act as a source of predators, and both can prevent dispersal of individuals, causing local extirpations (Winston et al. 1991; Schrank et al. 2001). These aspects must be carefully considered to facilitate survival of the Cape Fear shiner and before further alteration of the river takes place or restoration or reintroduction is considered at extirpated locations.

Sedimentation is the greatest source of pollution in the Cape Fear drainage (NCDWQ 2000) and can greatly affect fish that utilize benthic resources. Burkhead and Jelks (2001) found that a benthic spawning *Cyprinella* species reduced its reproductive output with increasing concentrations of suspended sediment in laboratory experiments. Cape Fear

shiners deposit their eggs in clean gravel, and may be affected by sediment directly (i.e., reduced egg or juvenile survival) or may be behaviorally impacted by the presence of excessive sedimentation. Both effects are sublethal to adults, but can have grave consequences for overall population stability and longevity (Burkhead and Jelks 2001).

Between the two potential Cape Fear shiner reintroduction sites studied, the extirpated site on the Rocky River (site 3) contains the most suitable physical habitat. This conclusion is primarily based on substrate availability, as this river reach contains a relative abundance of gravel substrate similar to the two sites on that river where the fish is extant. However, suitable water depth at that site may be somewhat limited, and any reintroduction effort should include examining means to improve that condition.

The site on the Deep River where the species persists, but is rare (site 6) is a candidate reach for habitat restoration toward increasing mean water depth and substrate alteration to improve conditions for Cape Fear shiner population growth. Excessive sedimentation is a common detriment to habitat quality and ecological function of many river systems (Waters 1995), and such is the case for the Cape Fear River system (NCDWQ 2000), but dams also act as sediment traps, and may deprive downstream reaches of transported sediment and organic matter (Gordon et al. 1992). Site 6 is located proximately downstream of Coleridge Dam, which may explain the lack of fine sediments (5% by area) and gravel (5%) at that site. The lack of gravel substrate, as well as the lack of suitable depths, are likely related to the presence and operation of the dam and may be addressed to improve habitat suitability for the Cape Fear shiner.

In addition to influencing hydrology and material transport, dams are detrimental to the Cape Fear shiner by impounding suitable lotic stream reaches to create unsuitable lentic

habitat and by fragmenting the fish population. Cape Fear shiners are not found in impounded river reaches upstream of dams in the upper Cape Fear River system (personal observation). In addition to creating substantial reaches of river that are unusable by the Cape Fear shiner, dams block movement of individual fish between the fragmented subpopulations that exist today. Thus, if an acute or catastrophic event occurs, even if localized, that results in Cape Fear shiner mortality, individuals from other reaches would not be available to recolonize the otherwise recovered suitable habitat. Thus, for example, even if adequate water quality was attained and if the reduced mean depth in the extirpated reach on the Rocky River (site 3) was not critical to Cape Fear shiner occurrence, the species could not recolonize the area on its own due to dams.

It is critical that both adequate water quality and suitable instream habitat be considered in maintenance and recovery of the Cape Fear shiner throughout its range. My research uses an interdisciplinary approach to examine the habitat suitability of Cape Fear shiners using a holistic approach to assess the suitability of physical habitat and water quality. Such approaches that integrate knowledge among scientific disciplines (e.g., conservation and toxicology) are needed to facilitate long-term viability of ecosystems (Hansen and Johnson 1999). The results and interpretation above in this chapter on physical habitat suitability must be considered along with those from Chapter 2 on water quality and contaminants for recovery of the Cape Fear shiner to proceed. For example, except for reduced mean depth at Site 3, management agencies might proceed with an experimental reintroduction of the species, as this site contained all components of suitable instream physical habitat; however, survival of Cape Fear shiners in our *in situ* exposures was significantly reduced at that site (53%), and contaminants accumulated in surviving fish,

indicating a water quality problem there (see Chapter 2). Thus, until water quality is improved, and perhaps some flow augmentation is attained, this site remains unsuitable for potential reintroduction of the Cape Fear shiner. Conversely, another possible reintroduction site in the Deep River [at Parks Crossroads (site DR1), see Chapter 2] had 100% fish survival and significant fish growth during *in situ* exposures, indicating suitable water quality, but a survey of physical habitat in that reach would be necessary to determine if suitable physical habitat exists there in combinations known to support Cape Fear shiners.

Biotic interactions should not be ignored in the restoration of Cape Fear shiner populations. Exotic and introduced species have been cited as a major factor in the decline of native fishes, and it has been listed it as the second most common factor, following habitat alteration and preceding pollution (Lassuy 1999). Although the presence of introduced species was not originally a factor in the federal endangered listing of the Cape Fear shiner in 1987, the flathead catfish, *Pylodictis olivaris*, an obligate carnivorous apex predator, has been introduced into the upper Cape Fear River and has been collected from the Deep River in reaches with extant Cape Fear shiner populations. The impacts of this exotic predator may be severe and management should consider further study to investigate any possible habitat overlap and direct interactions between the species.

To conclude, the survival and recovery of the Cape Fear shiner depends greatly on the successful preservation and enhancement of remaining suitable physical habitat and water quality. This task will require a broad-scale approach that incorporates physical instream habitat, water quality and contaminants, biotic interactions with other organisms, as well as differences in landuse patterns and human activities that may contribute to habitat loss. This multidisciplinary research has provided insights into environmental interactions that have led

to Cape Fear shiner extirpations. The information herein should prove useful as a tool in the strategic planning and management necessary to ensure the Cape Fear shiner's long-term survival.

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Table 1. Categories used to describe river substrate composition based on a modified Wentworth particle size scale.

Particle Category	Size Class (mm)
Silt/Clay	<0.62
Sand	0.62–2.0
Gravel	3–64
Cobble	65–250
Small boulder	251–2000
Large boulder	2001–4000
Mammoth boulder (and bedrock)	>4000

Table 2. Retained component loadings from principal components analyses for the Rocky and Deep rivers during post-spawning (summer 2001) spawning (spring 2002) seasons.

	Post-spawning				Spawning			
	Rocky River (N = 516)		Deep River (N = 118)		Rocky River (N = 161)		Deep River (N = 101)	
Variable and statistic	PC 1	PC 2	PC 1	PC 2	PC 1	PC 2	PC 1	PC 2
Depth	0.71	0.01	0.55	-0.65	0.73	0.04	0.58	0.58
Substrate	0.66	0.33	0.50	0.76	0.38	0.80	0.62	0.13
Mean velocity	-0.25	0.94	0.67	-0.03	-0.56	0.59	-0.53	0.80
Threshold R-value[a]	0.124	0.124	0.255	0.255	0.20	0.20	0.274	0.274
Eigenvalue	1.32	1.00	1.23	0.96	1.12	1.02	1.30	0.90
Variance explained	44%	33%	41%	32%	37%	34%	43%	30%

[a]Threshold R-value is the critical absolute value of the correlation coefficient for statistical significance of the component loadings based on sample sizes.

Table 3. Statistical comparisons of Cape Fear shiner microhabitat use and availability for continuous (Kolmogorov-Smirnov two-sample tests) and categorical (chi-square test) variables in the Rocky and Deep rivers during the post-spawning (summer 2001) and spawning (spring 2002) seasons. Sample sizes appear in Table 2.

	Post-spawning		Spawning	
River and variable	Statistic	*P*	Statistic	*P*
Rocky River				
Component 1 scores	D = 0.230	0.003	D = 0.509	0.0001
Component 2 scores	D = 0.094	0.65	D = 0.339	0.0012
Depth	D = 0.296	0.0001	D = 0.298	0.0068
Mean velocity	D = 0.244	0.0013	D = 0.396	0.0001
Substrate	D = 0.217	0.0062	D = 0.383	0.0002
Cover	χ^2 = 48.127	0.0001	χ^2 = 24.374	0.0002
Deep River				
Component 1 scores	D = 0.297	0.033	D = 0.528	0.0001
Component 2 scores	D = 0.312	0.022	D = 0.248	0.16
Depth	D = 0.509	0.0001	D = 0.308	0.0395
Mean velocity	D = 0.211	0.2527	D= 0.349	0.0131
Substrate	D = 0.271	0.0652	D = 0.481	0.0001
Cover	χ^2 = 20.619	0.001	χ^2 = 12.492	0.0286

Table 4. Cape Fear shiner microhabitat use and availability statistics for reaches of the Rocky and Deep rivers during the post-spawning season (summer 2001).

River and variable	*N*	Mean	SE	Minimum–maximum
Rocky River microhabitat use[a]				
Depth (cm)	70	37.5	1.07	10–51
Mean velocity (m/s)	70	0.037	0.006	0–0.25
Focal depth (cm)	70	11.9	1.0	1–30
Focal velocity (m/s)	70	0.026	0.007	1–0.26
Rocky River microhabitat availability (extant sites)[a]				
Depth (cm)	516	43.0	1.51	1–225
Mean velocity (m/s)	516	0.031	0.003	0–0.70
Rocky River microhabitat availability (extirpated site)				
Depth (cm)	285	27.0	1.14	1–91
Mean velocity (m/s)	285	0.034	0.005	0–0.550
Deep River microhabitat use				
Depth (cm)	29	41.2	1.07	32–57
Mean velocity (m/s)	29	0.048	0.011	0–0.26
Focal depth (cm)	29	10.4	1.34	1–25
Focal velocity (m/s)	29	0.022	0.006	0–0.12
Deep River microhabitat availability (extant site)				
Depth (cm)	118	35.3	2.31	2–139
Mean velocity (m/s)	118	0.106	0.015	0–0.720
Deep River microhabitat availability (rare site)				
Depth (cm)	169	27.5	1.32	2–86
Mean velocity (m/s)	169	0.068	0.010	0–0.850

[a] Two sites.

Table 5. Comparison of suitable microhabitat availability for the Cape Fear shiner between reaches of the Rocky and Deep rivers during summer 2001 where the species is extant, rare, or extirpated.

		Rocky River		Deep River	
Variable	Suitable Category	Extant	Extirpated	Extant	Rare
Depth	40–49 cm	12.4%	11.2%	13.6%	17.8%
Mean velocity	0.16–0.19 m/s	0.8%	0.7%	1.7%	4.1%
Substrate	Gravel	11.6%	14.4%	26.3%	4.7%
Cover	American water-willow	13.2%	23.9%	9.3%	17.2%

Table 6. Cape Fear shiner microhabitat use and availability statistics for reaches of the Rocky and Deep rivers during the spawning season (spring 2002).

River and variable	*N*	Mean	SE	Minimum-maximum
Rocky River microhabiat use [a]				
Depth (cm)	40	32.5	1.47	12–60
Mean velocity (m/s)	40	0.026	0.006	0–0.19
Focal depth (cm)	40	13.0	0.90	3–25
Focal velocity (m/s)	40	0.016	0.003	0–0.07
Rocky River microhabitat availability (extant sites)[a]				
Depth (cm)	161	47.7	3.77	2–250
Mean velocity (m/s)	161	0.016	0.004	0–0.43
Deep River microhabitat use				
Depth (cm)	26	34.6	1.27	23–50
Mean velocity (m/s)	26	0.046	0.008	0–0.19
Focal depth (cm)	26	13.4	1.01	5–25
Focal velocity (m/s)	26	0.035	0.007	0–0.14
Deep River microhabitat availability (extant site)				
Depth (cm)	101	43.9	3.37	3–165
Mean velocity (m/s)	101	0.035	0.006	0–0.35

[a]Two sites

Table 7. Cape Fear shiner population density estimates and associated statistics from reaches of the Rocky and Deep rivers during summer 2002. Site descriptions appear in Methods section and 10 transects were surveyed at each site. SD and Min-max are standard deviation and minimum and maximum values, respectively.

Statistic	Site 1 (Rocky River)	Site 4 (Deep River)	Site 5 (Deep River)
No. observed			
Mean	19.2	6.4	10.1
SD	27.4	9.3	12.6
Min-max	0–82	0–26	0–32
Area surveyed (ha)			
Mean	0.012	0.0084	0.0079
SD	0.003	0.0025	0.0028
Min-max	0.0064–0.0162	0.0056–0.012	0.006–0.015
Mean stream width (m)	45	60	70
Density (no./ha)			
Mean	1,393	795	1,056
95% CI	97–2,690	0–1,773	179–1,933
Min-max	0–5,062	0–4,333	0–3,333
Density (no./km)			
Mean	6,270	4,768	7,392
95% CI	437–12,104	0–10,642	1,254–13,529
Min-max	0–22,778	0–26,000	0–23,333

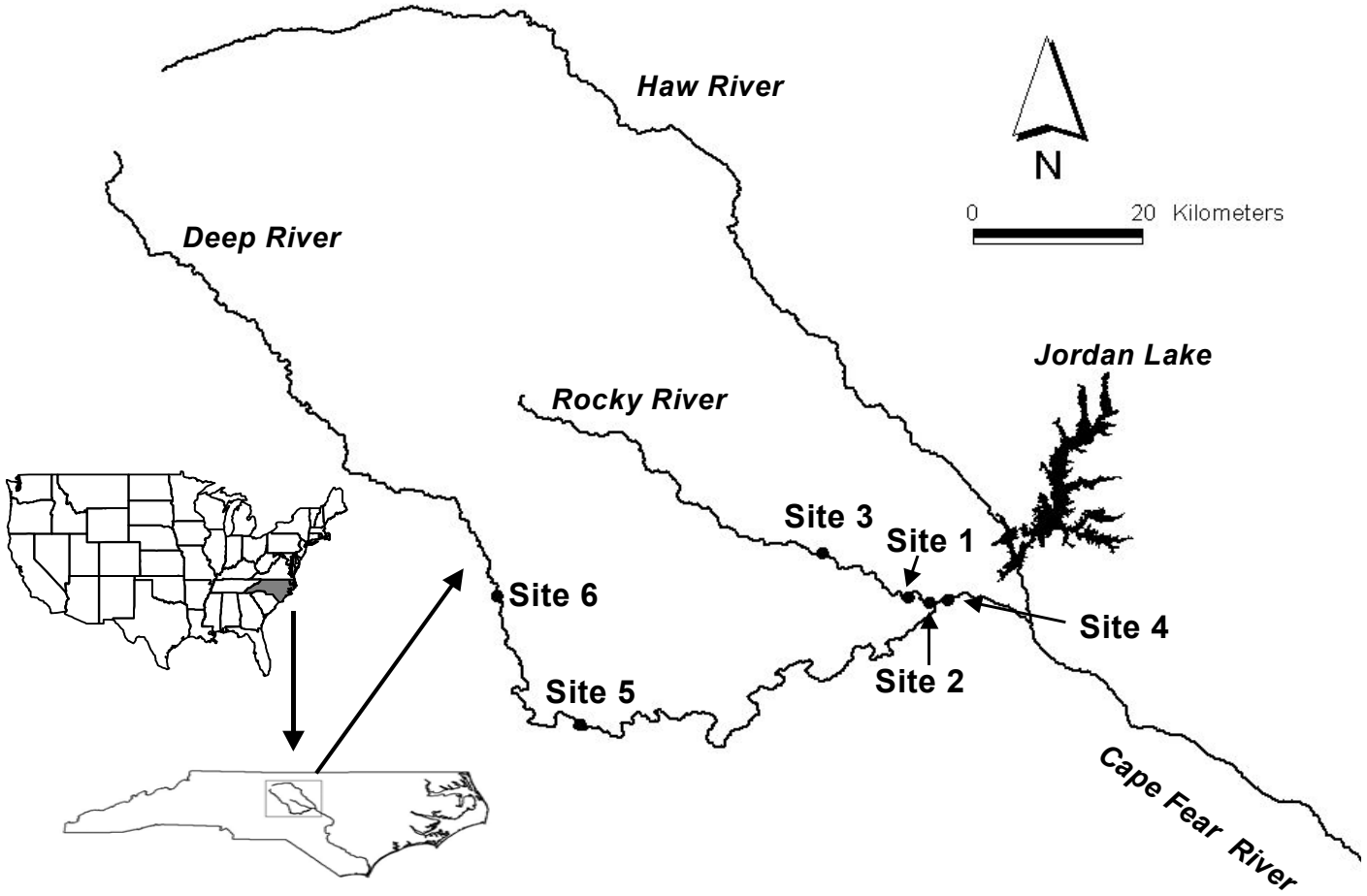


Figure 1. Map indicating six primary sites on the Deep and Rocky rivers selected for Cape Fear shiner instream physical habitat analyses and population density estimates.

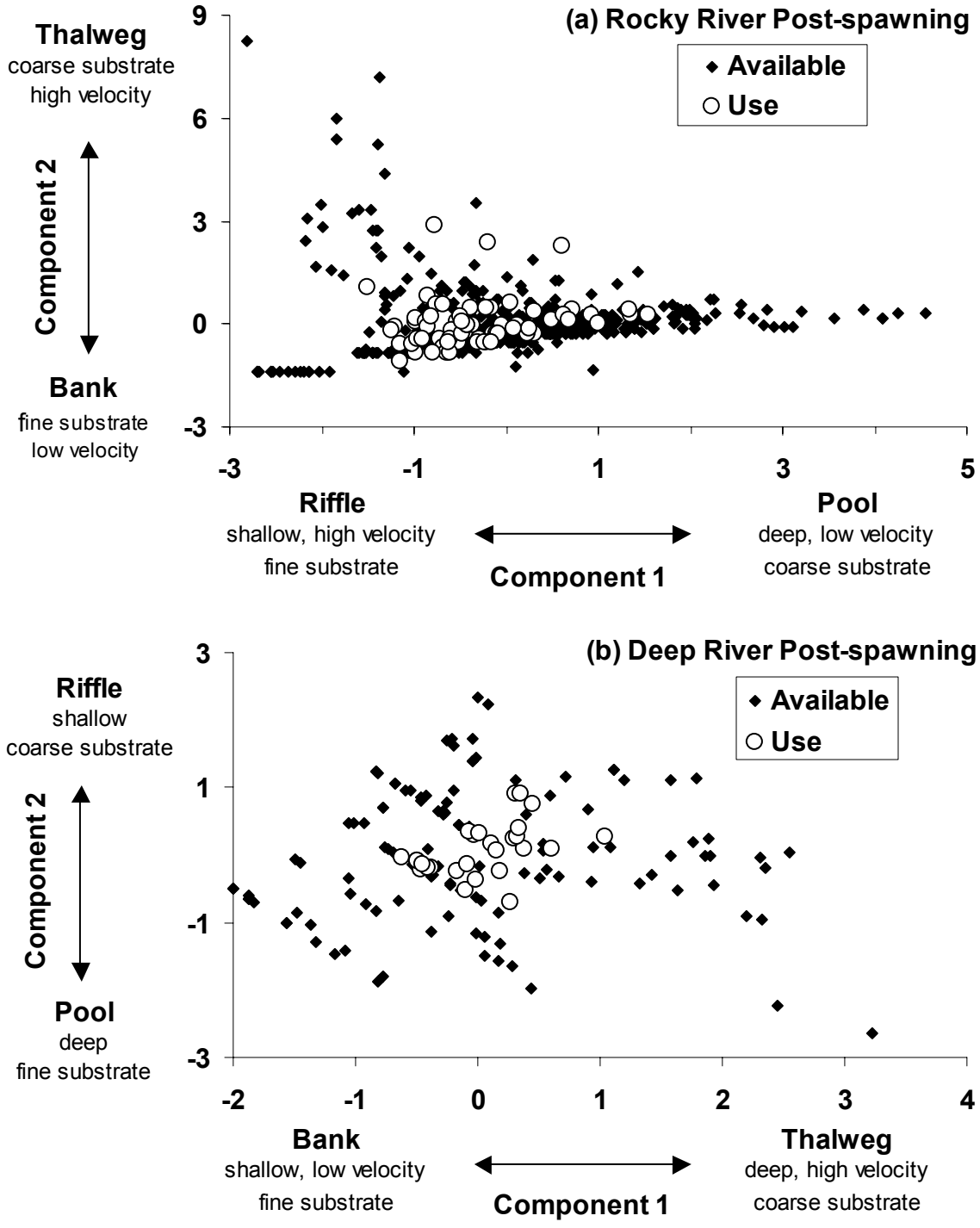
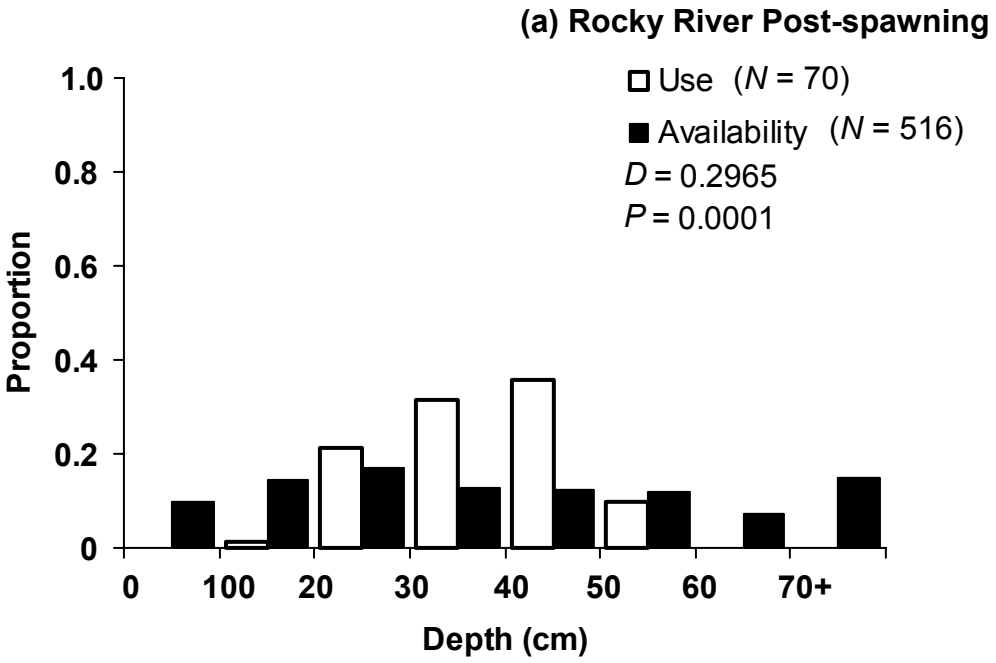


Figure 2. Plots of Cape Fear shiner microhabitat use and habitat available component scores in the (a) Rocky River and (b) Deep River during post-spawning (summer 2001). Principal component loadings and sample sizes appear in Table 2, and statistical comparisons appear in Table 3.



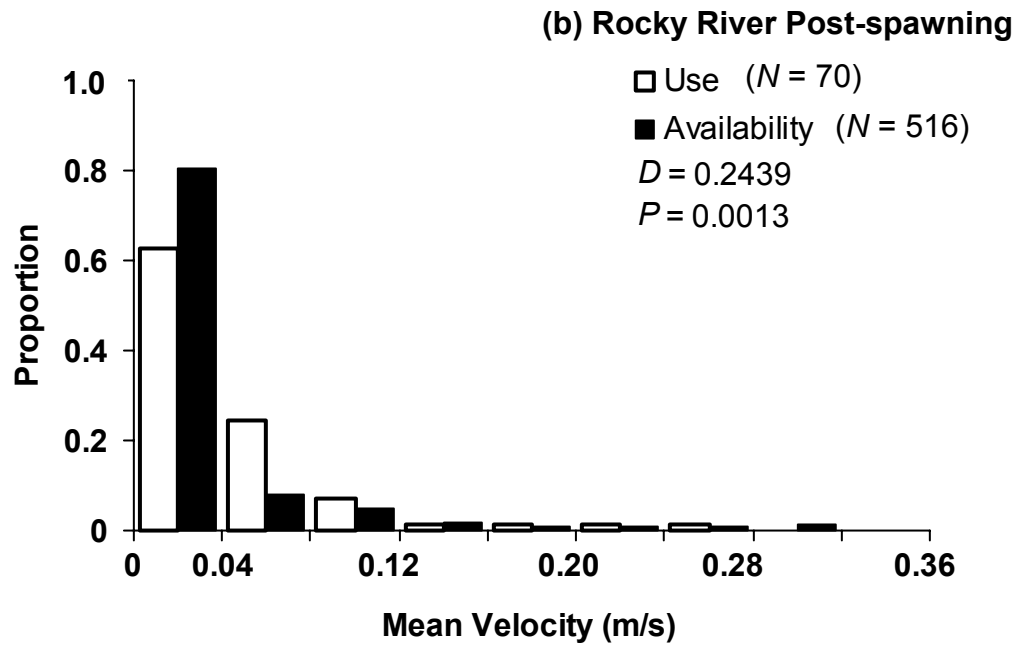
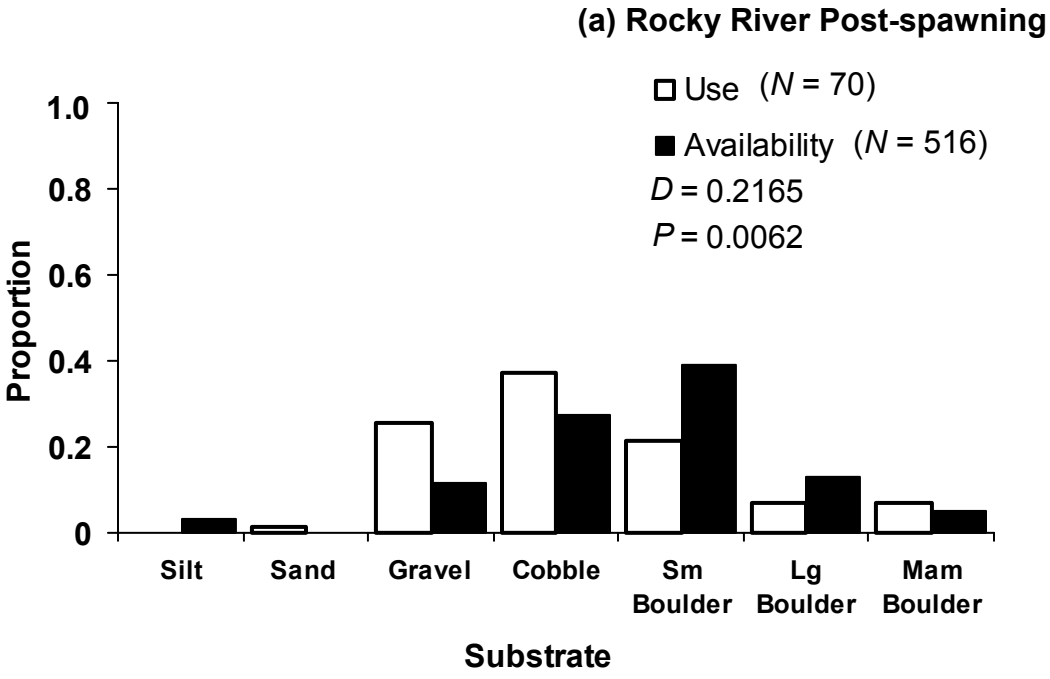


Figure 3. Frequency distributions of (a) depth and (b) mean column velocity for Cape Fear shiner microhabitat use and availability in the Rocky River during post-spawning (summer 2001). Use and availability distributions were compared using a Kolmogorov-Smirnov two-sample test.



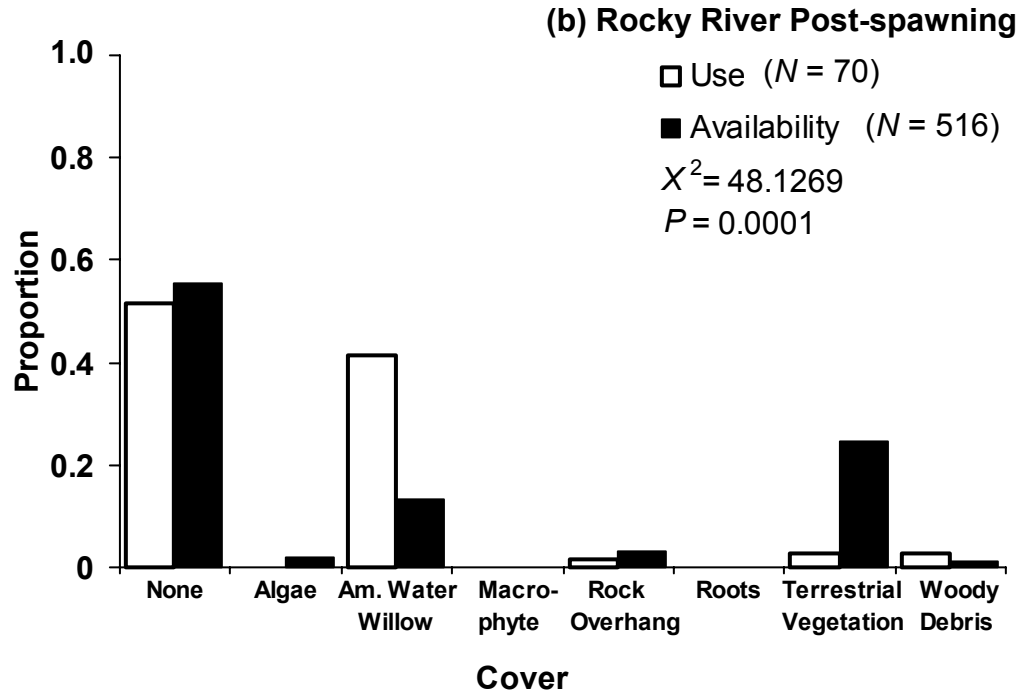


Figure 4. Frequency distributions of (a) substrate and (b) cover for Cape Fear shiner microhabitat use and availability in the Rocky River during post-spawning (summer 2001). Use and availability distributions were compared using a Kolmogorov-Smirnov two-sample test (substrate) or a Chi-square test (cover).

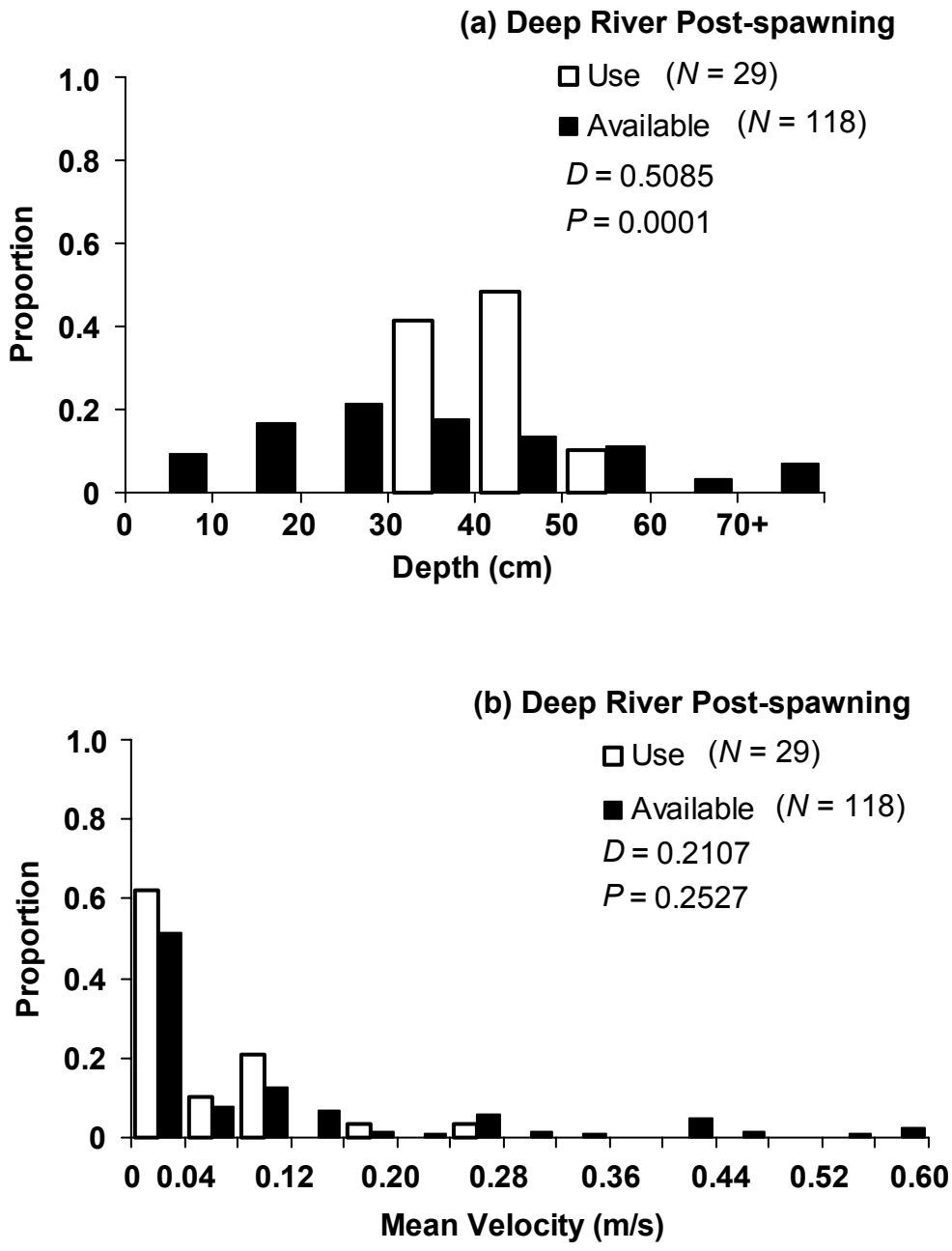


Figure 5. Frequency distributions of (a) depth and (b) mean column velocity for Cape Fear shiner microhabitat use and availability in the Deep River during post-spawning (summer 2001). Use and availability distributions were compared using a Kolmogorov-Smirnov two-sample test.

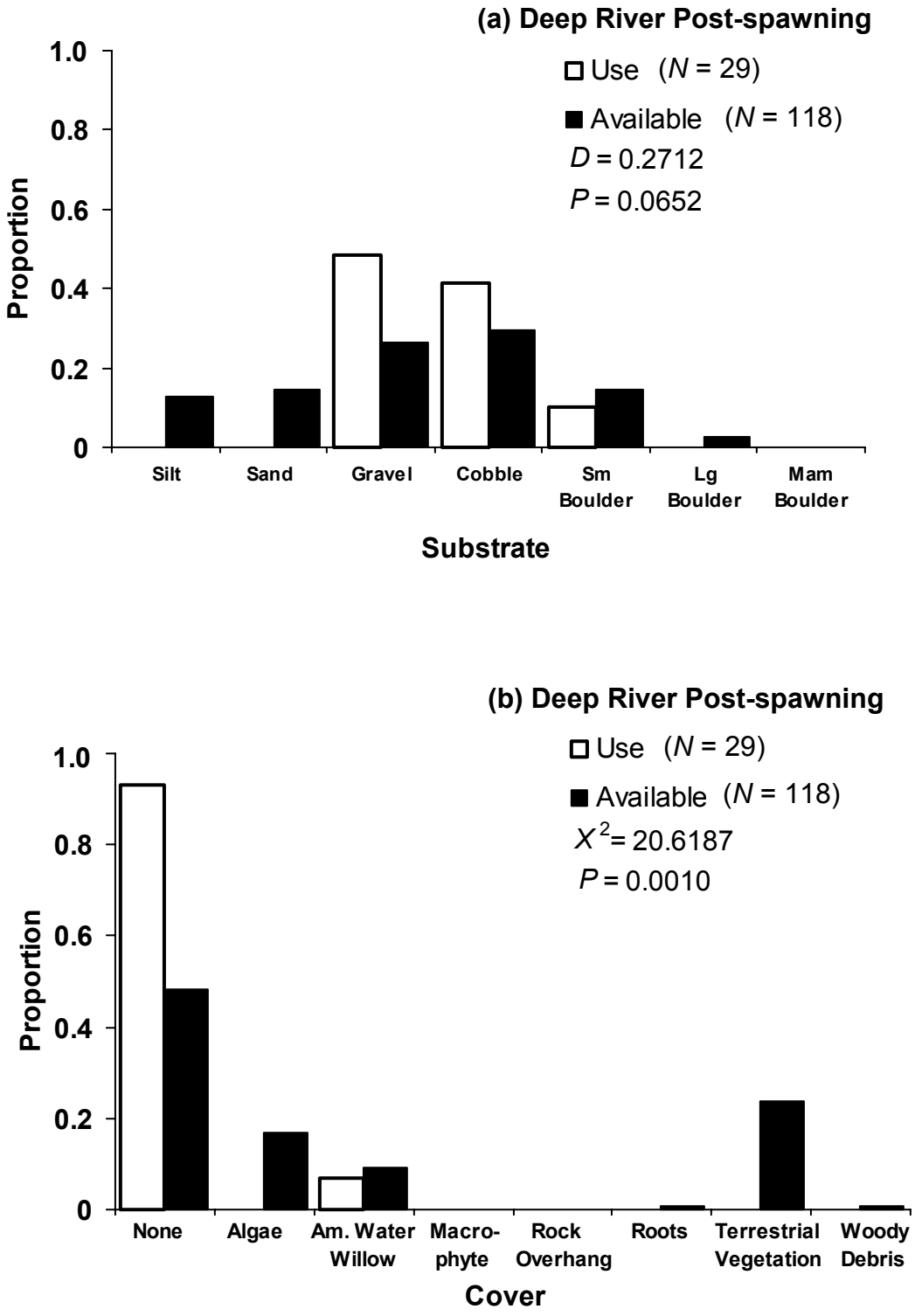
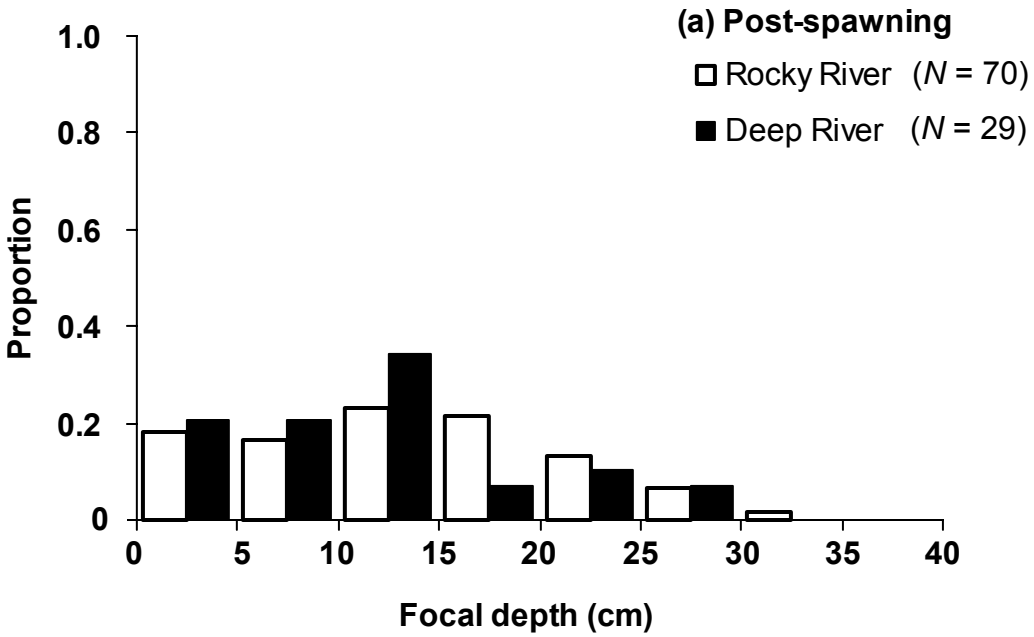


Figure 6. Frequency distributions of (a) substrate and (b) cover for Cape Fear shiner microhabitat use and availability in the Deep River during post-spawning (summer 2001). Use and availability distributions were compared using a Kolmogorov-Smirnov two-sample test (substrate) or a Chi-square test (cover).



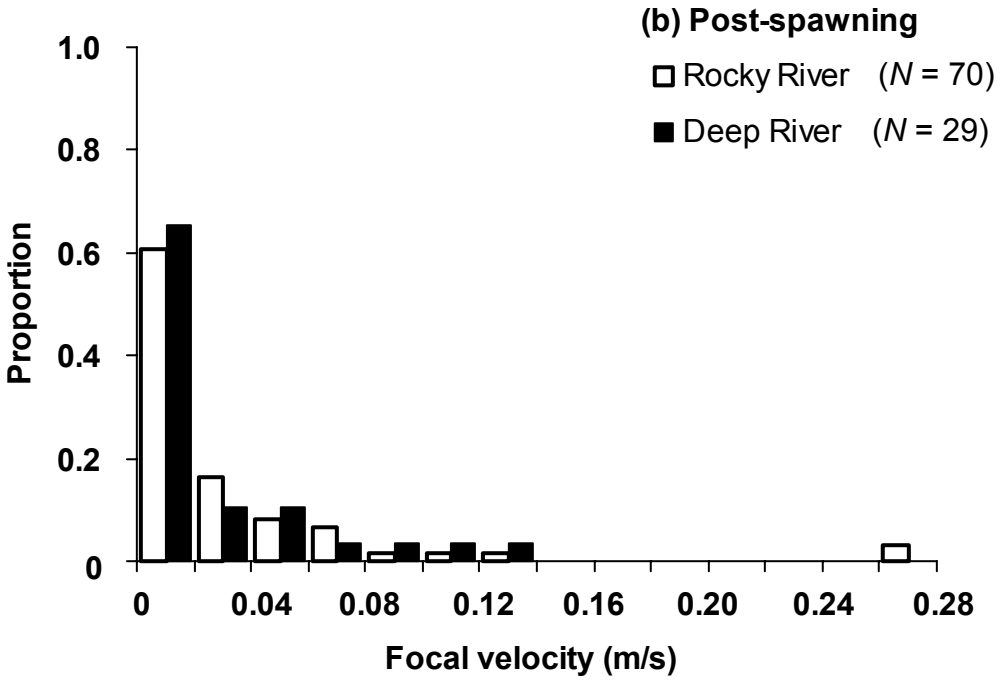


Figure 7. Frequency distributions of (a) focal depth and (b) focal velocity for Cape Fear shiner microhabitat use in the Rocky and Deep rivers during post-spawning (summer 2001).

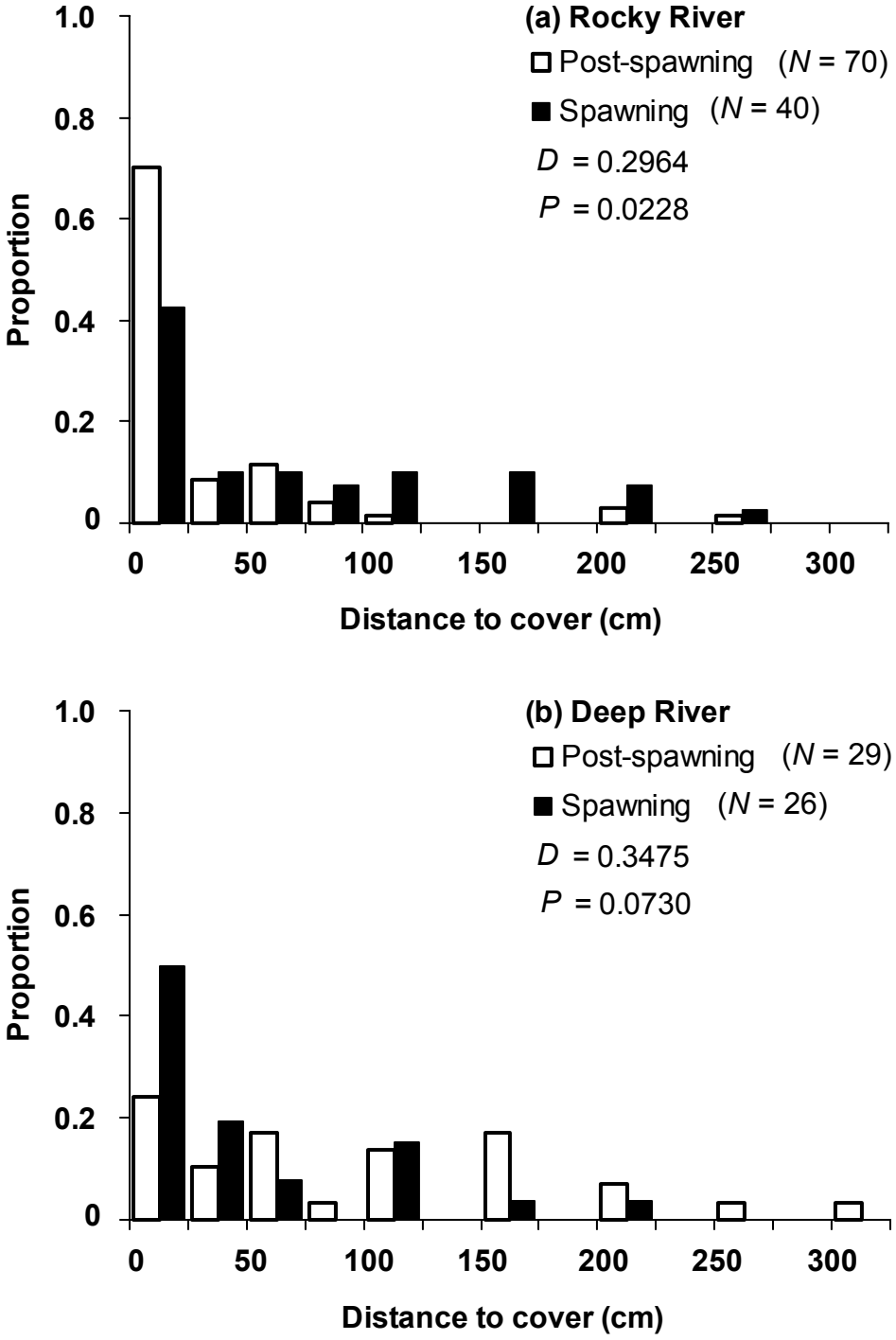


Figure 8. Frequency distributions of Cape Fear shiner distance to cover in the (a) Rocky River and (b) Deep River during the post-spawning (summer 2001) and spawning (spring 2002) seasons. Post-spawning and spawning distributions were tested using a Kolmogorov-Smirnov two-sample test.

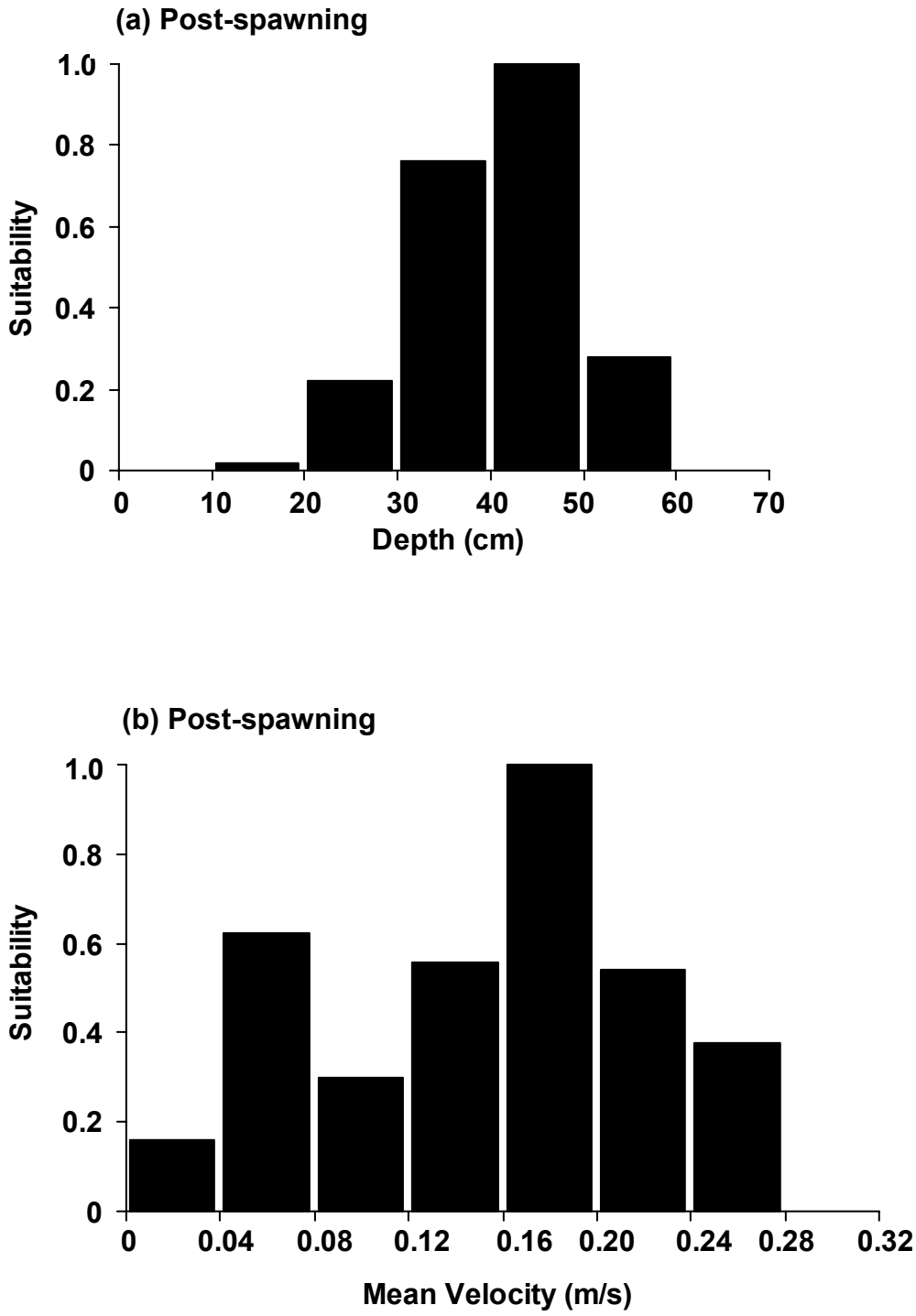


Figure 9. Cape Fear shiner microhabitat suitability for (a) depth and (b) mean column velocity, based on combined data collected from the Rocky and Deep rivers during post-spawning (summer 2001).

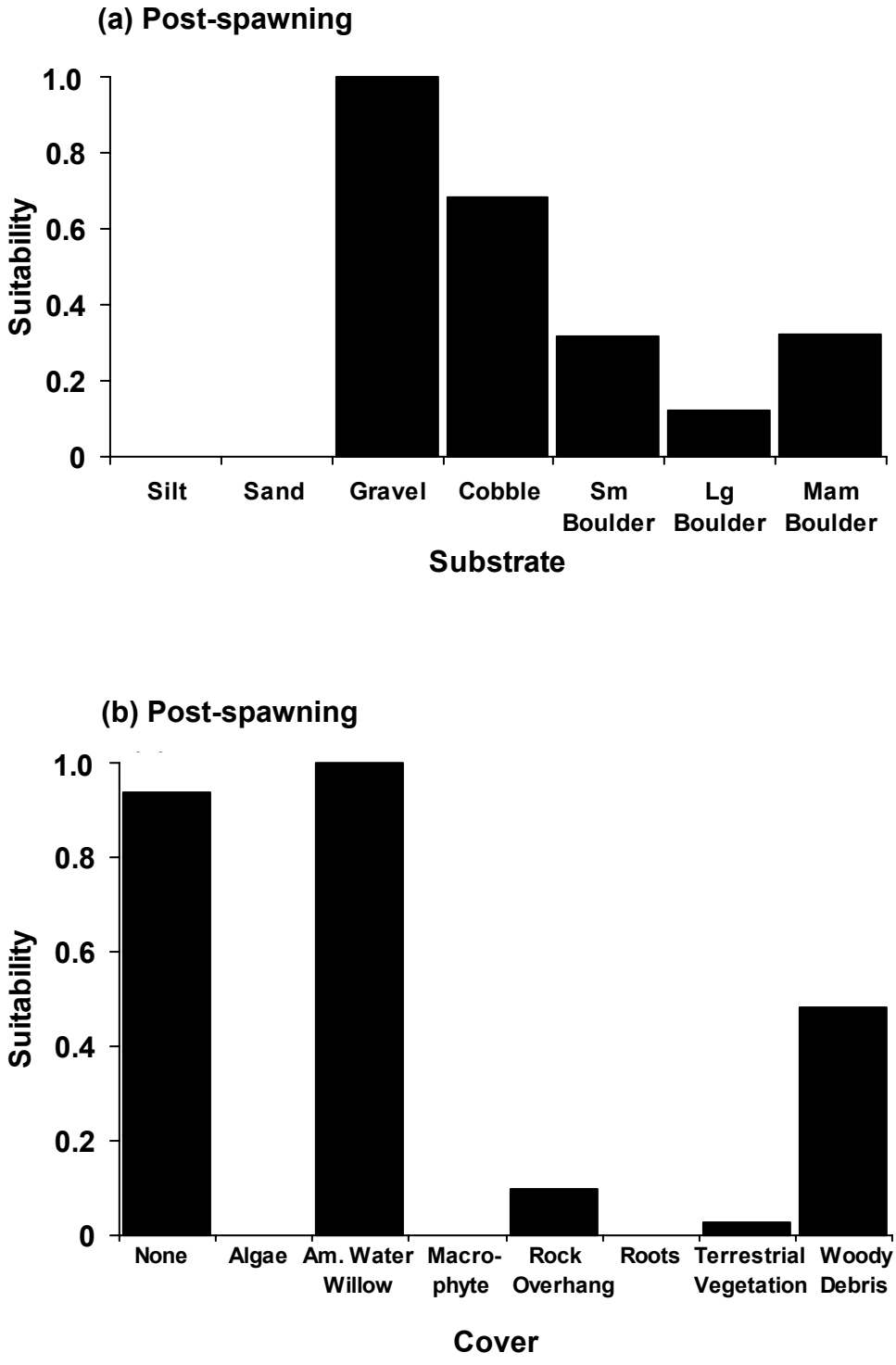


Figure 10. Cape Fear shiner microhabitat suitability for (a) substrate and (b) cover, based on combined data collected from the Rocky and Deep rivers during post-spawning (summer 2001).

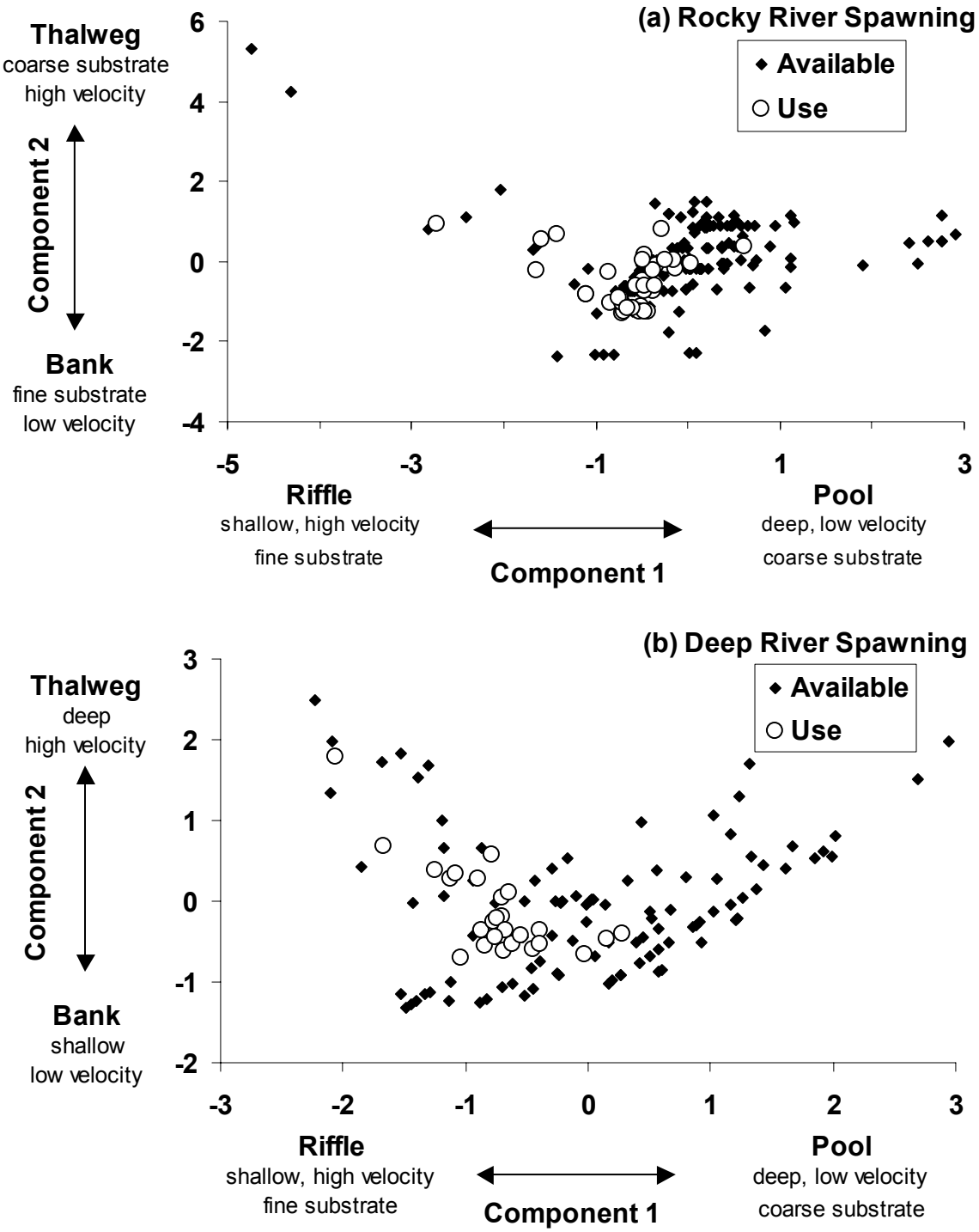


Figure 11. Cape Fear shiner microhabitat use and habitat available component scores in the (a) Rocky River and (b) Deep River during spawning (spring 2002). Principal component loadings and sample sizes appear in Table 2, and statistical comparisons appear in Table 3.

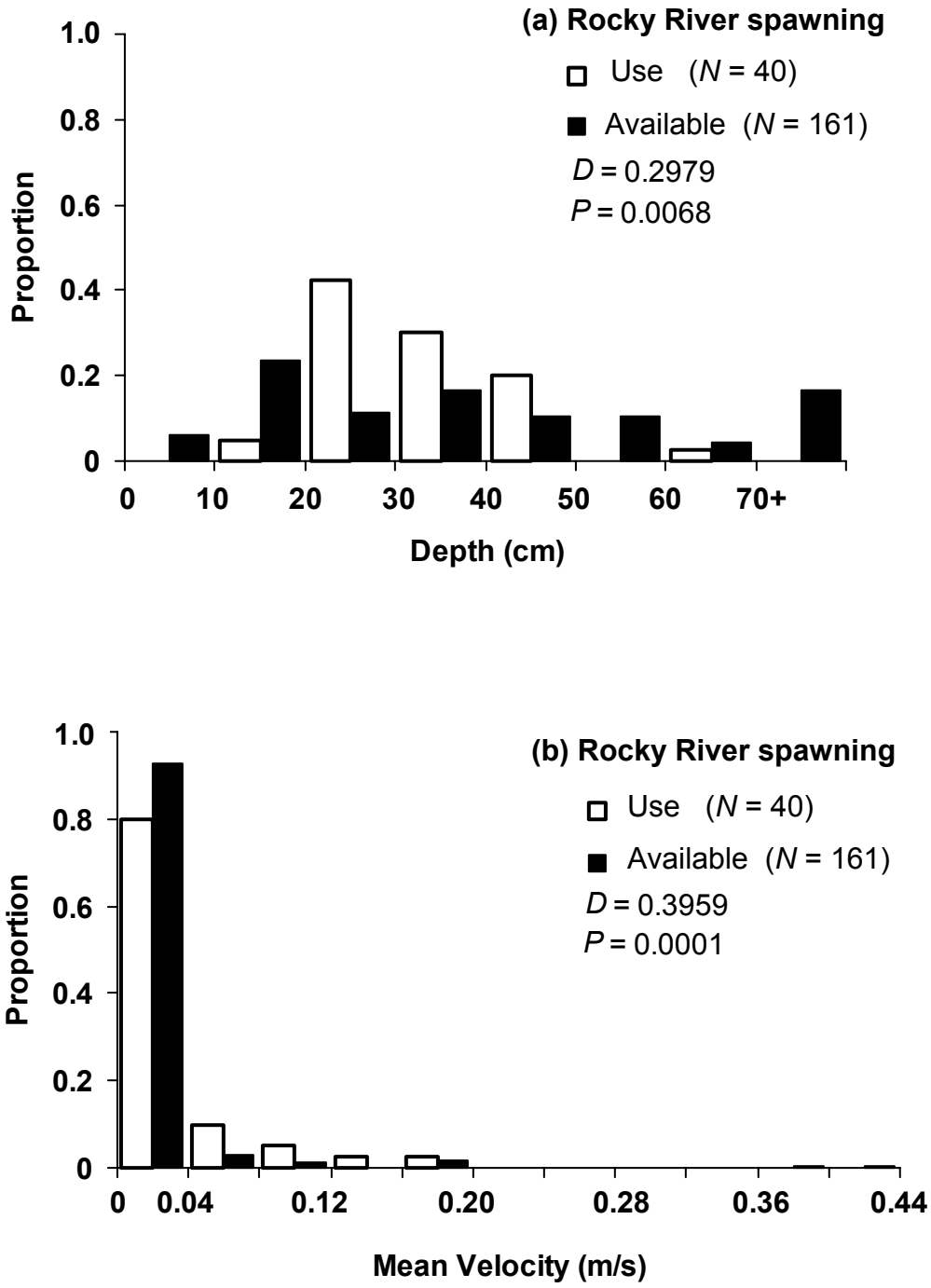


Figure 12. Frequency distributions of (a) depth and (b) mean column velocity for Cape Fear shiner microhabitat use and availability in the Rocky River during spawning (spring 2002). Use and availability distributions were compared using a Kolmogorov-Smirnov two-sample test.

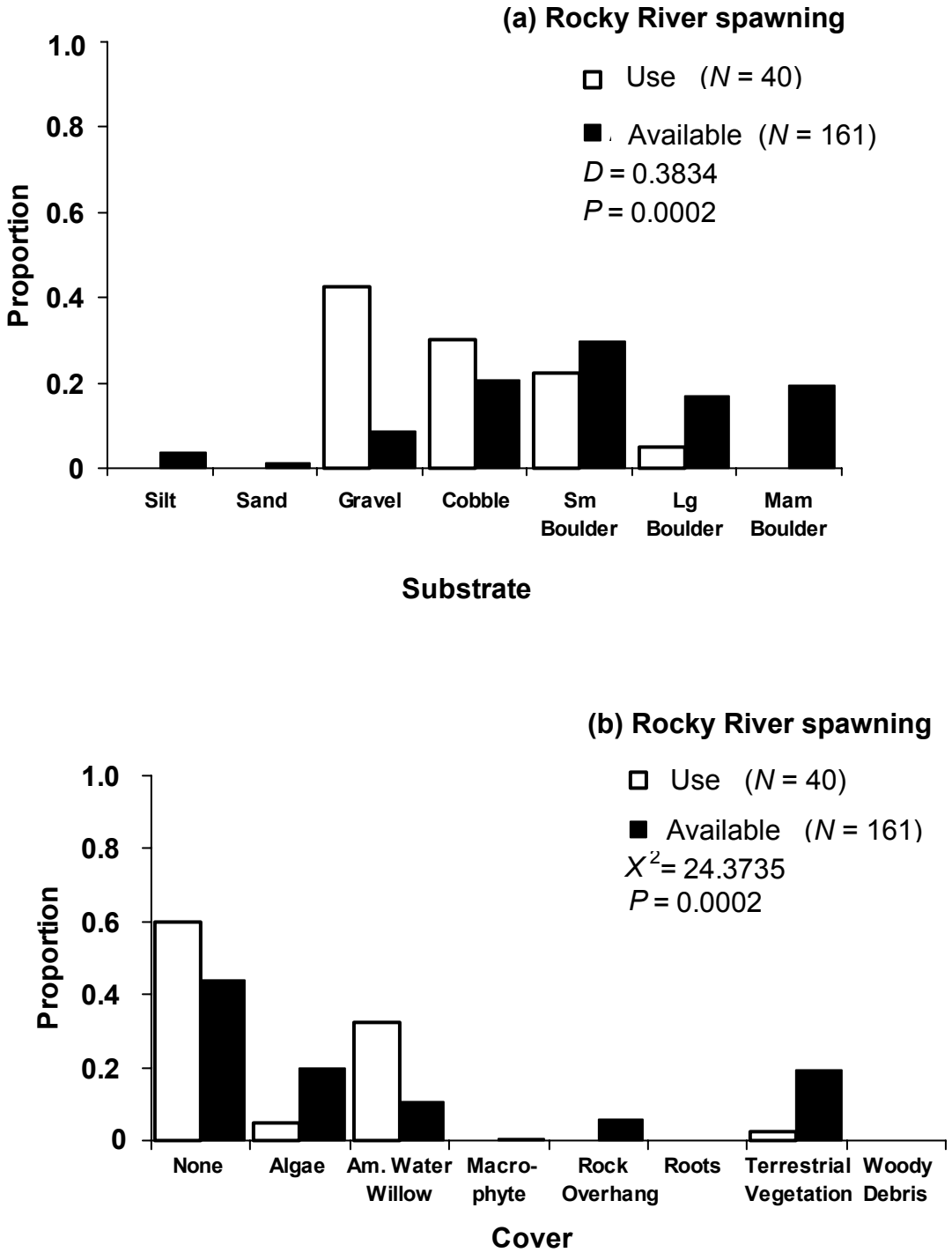


Figure 13. Frequency distributions of (a) substrate and (b) cover for Cape Fear shiner microhabitat use and availability in the Rocky River during spawning (spring 2002). Use and availability distributions were compared using a Kolmogorov-Smirnov two-sample test (substrate) or a Chi-square test (cover).

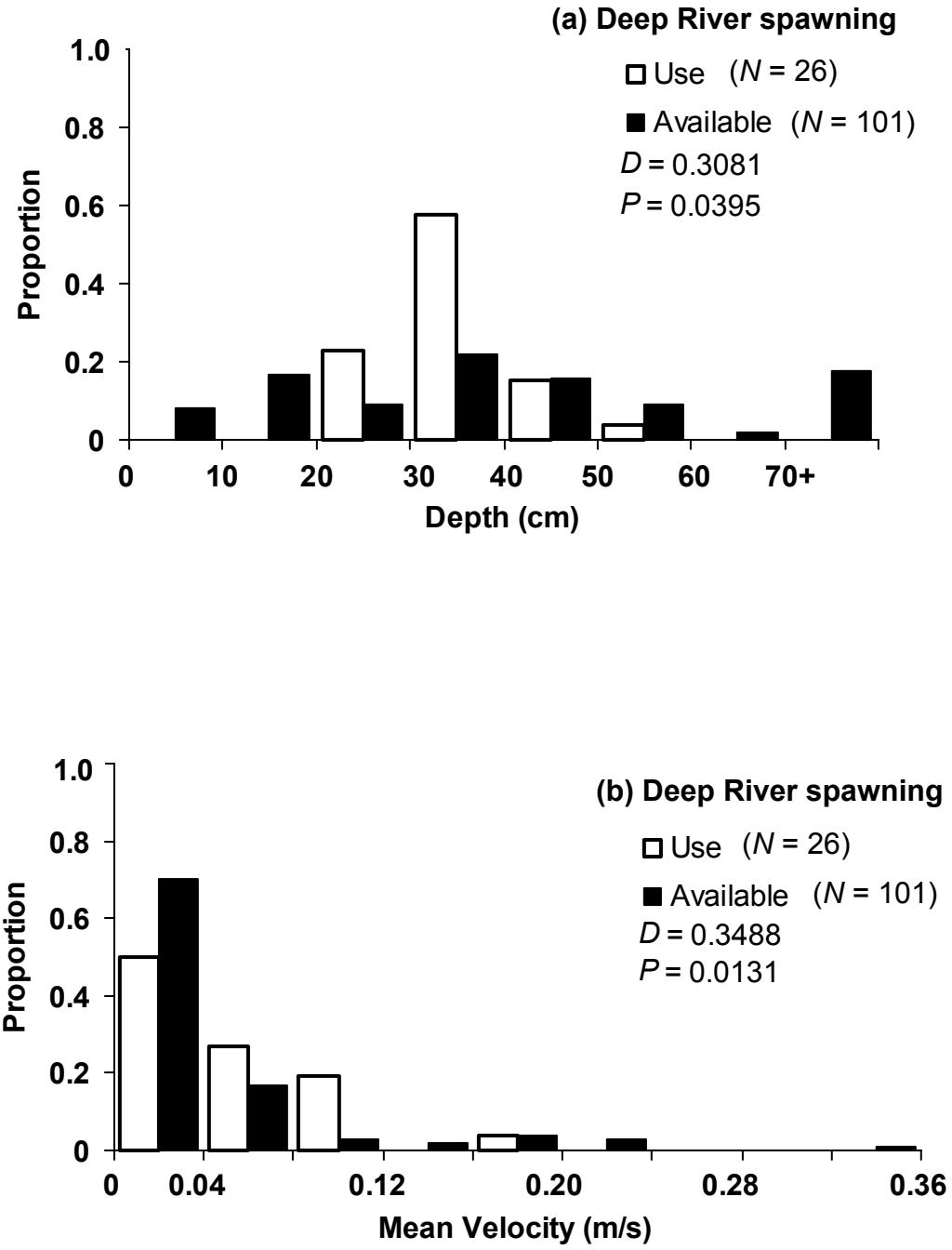


Figure 14. Frequency distributions of (a) depth and (b) mean column velocity of Cape Fear shiner microhabitat use and availability in the Deep River during spawning (spring 2002). Use and availability distributions were compared using a Kolmogorov-Smirnov two-sample test.

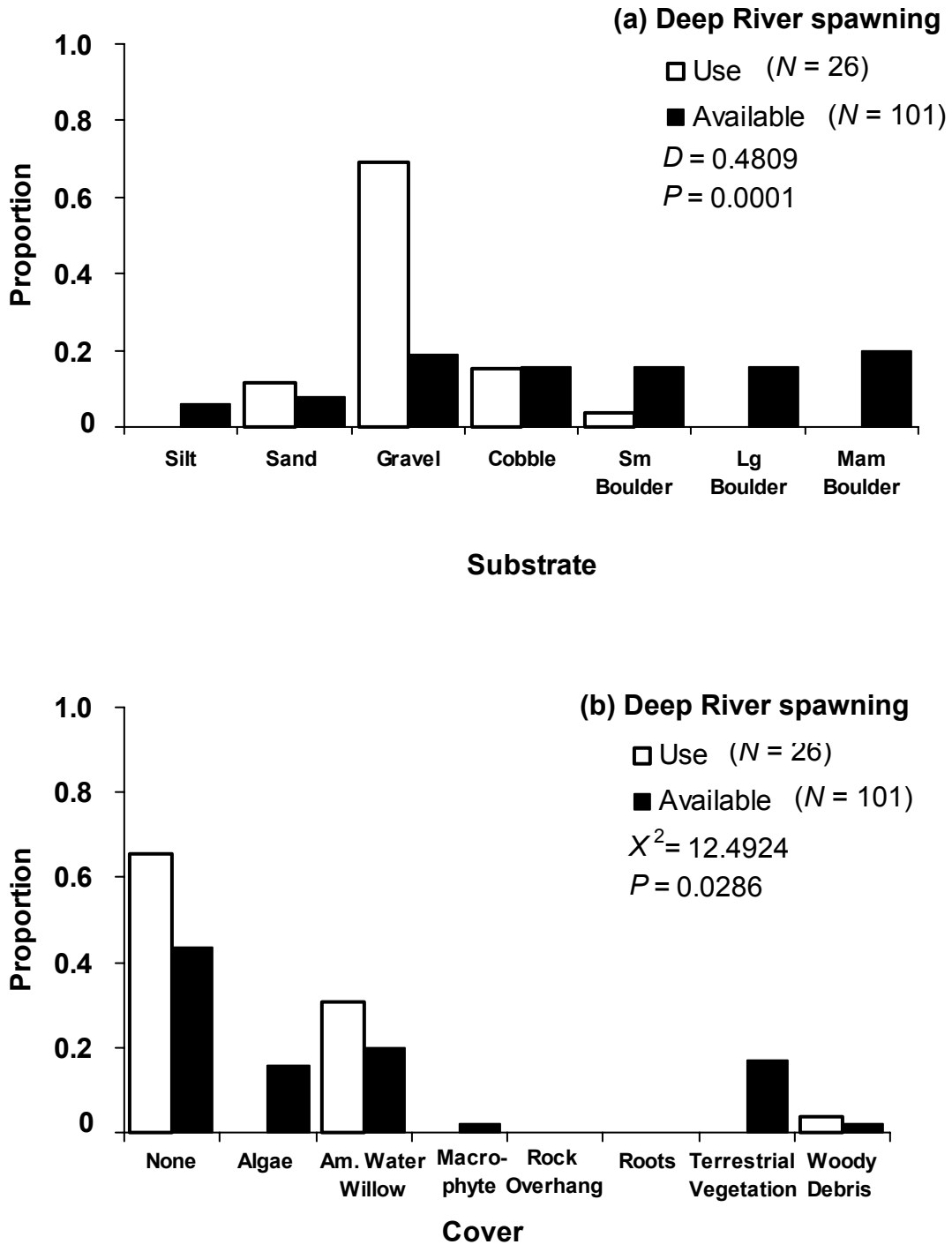


Figure 15. Frequency distributions of (a) substrate and (b) cover of Cape Fear shiner microhabitat use and availability in the Deep River during spawning (spring 2002). Use and availability distributions were compared using a Kolmogorov-Smirnov two-sample test (substrate) or a Chi-square test (cover)

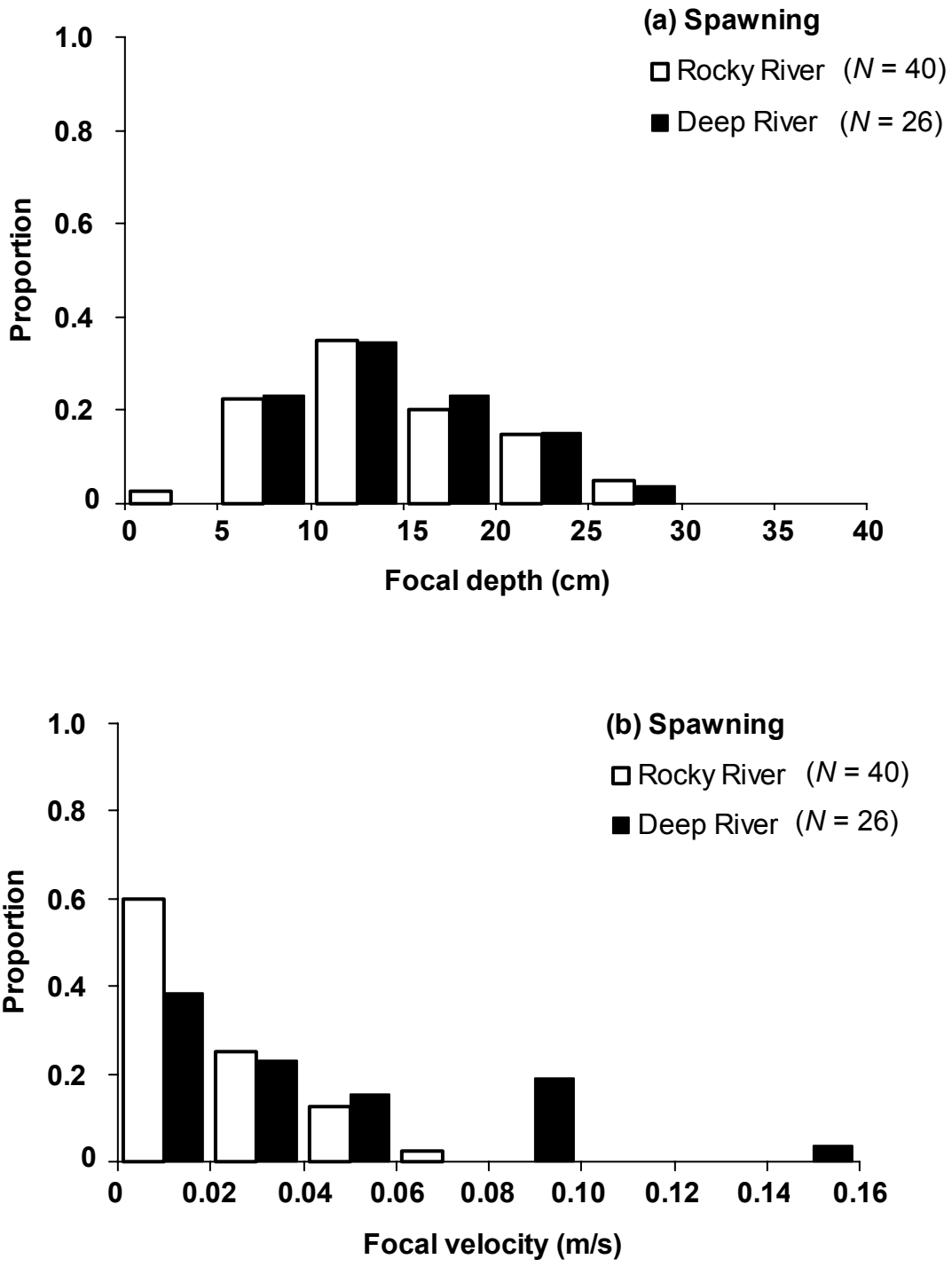


Figure 16. Frequency distribution of (a) focal depth and (b) focal velocity for Cape Fear shiner microhabitat use in the Rocky and Deep rivers during spawning (spring 2002).

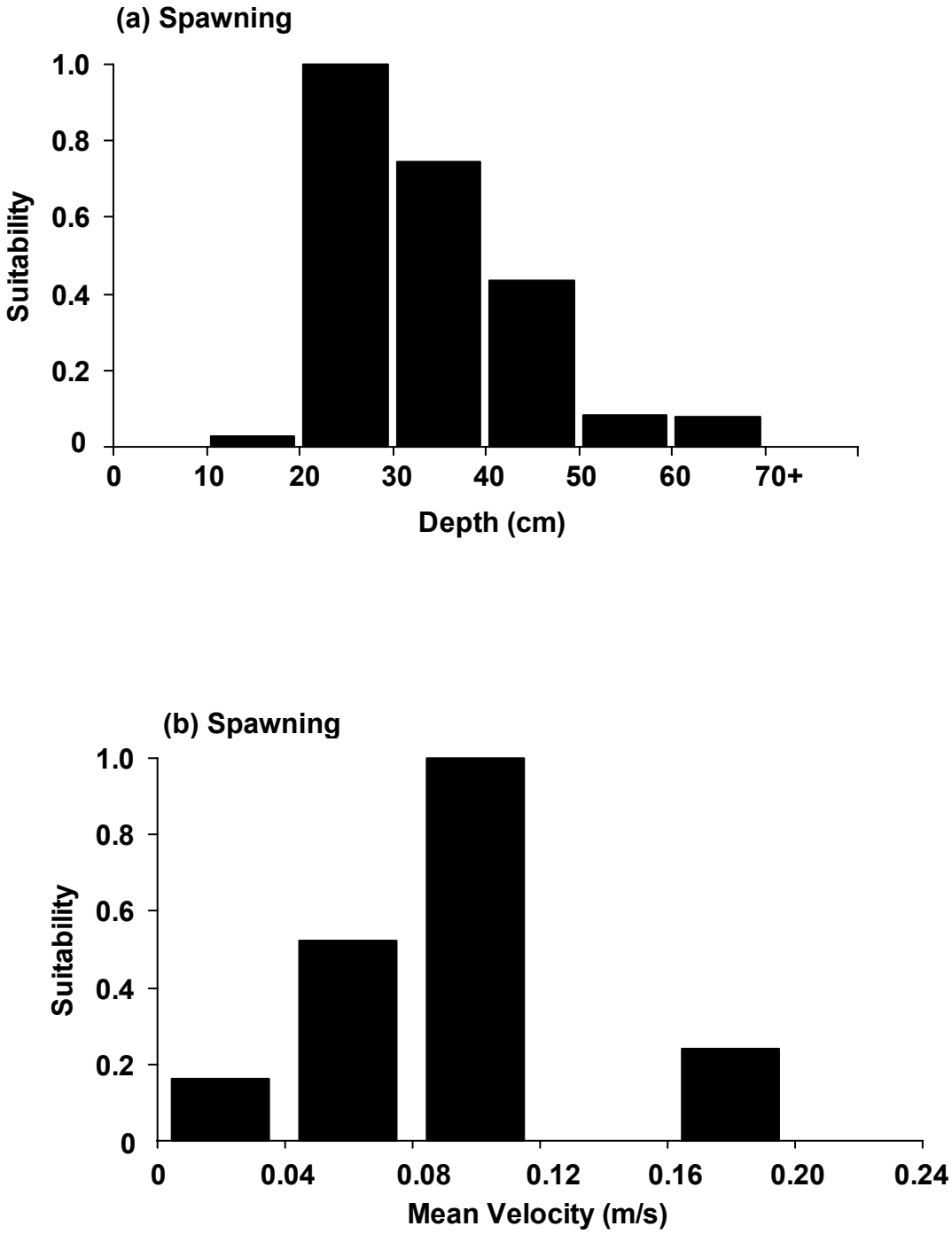


Figure 17. Cape Fear shiner microhabitat suitability for (a) depth and (b) mean column velocity, based on combined data collected from the Rocky and Deep rivers during spawning (spring 2002).

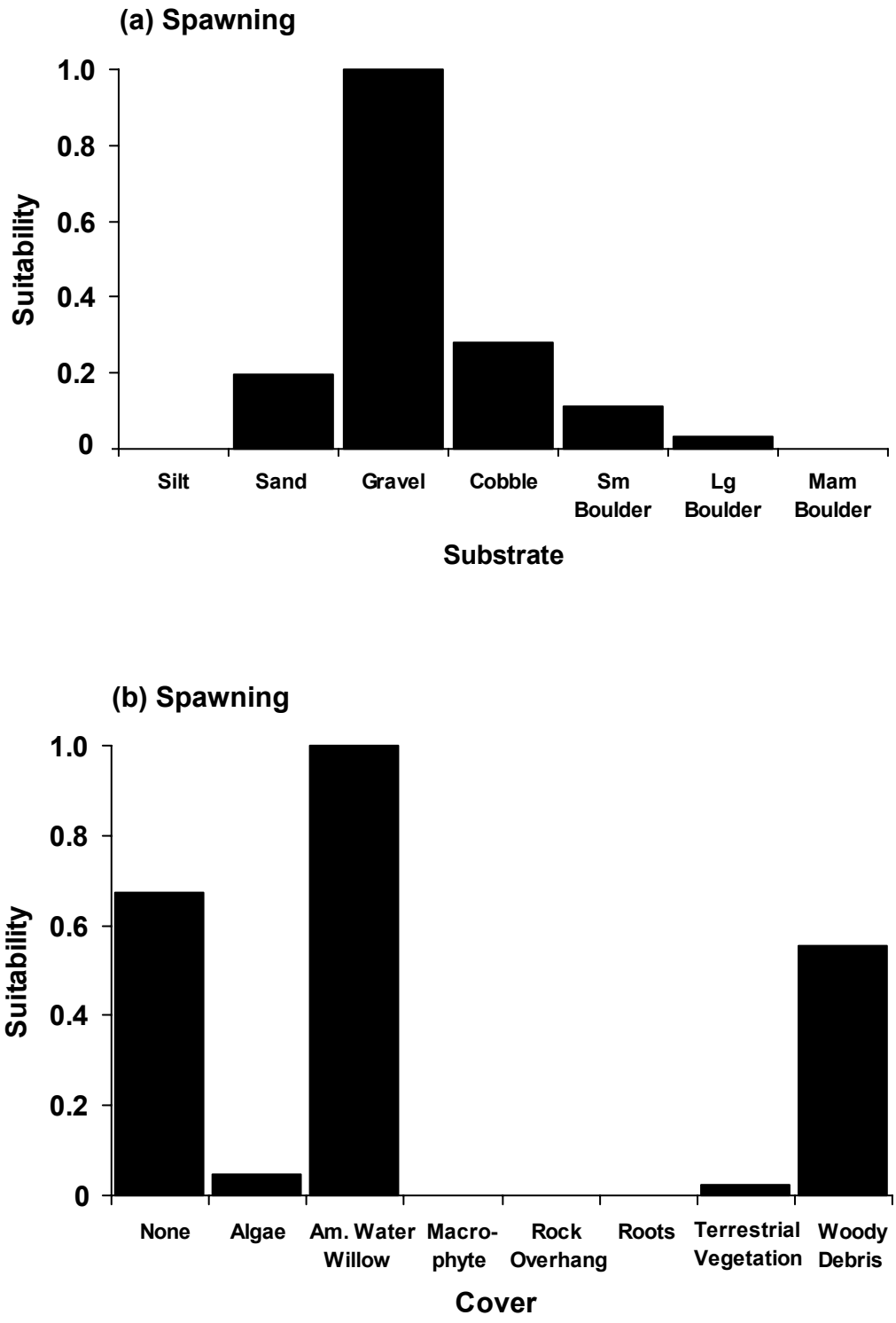


Figure 18. Cape Fear shiner microhabitat suitability for (a) substrate and (b) cover, based on combined data collected from the Rocky and Deep rivers during spawning (spring 2002).

Chapter 2
INFLUENCE OF WATER QUALITY AND ASSOCIATED CONTAMINANTS ON SURVIVAL AND GROWTH OF THE CAPE FEAR SHINER

Introduction

Populations of many native fishes in the southern United States have declined in recent years, and the threat of imperilment and extinction has increased dramatically within the last two decades (Burr and Mayden 1992; Warren et al. 1997, 2000). The Cape Fear shiner *Notropis mekistocholas* is, unfortunately, among this group of declining species, and the small cyprinid was added to the Federal Endangered Species list in 1987 (USFWS 1987). The Cape Fear shiner was first described by Snelson (1971) and is currently known from only five remaining populations in the Cape Fear River basin of North Carolina (Pottern and Huish 1985, 1986, 1987; NCWRC 1995, 1996). Like most of the other declining southern fish species, the Cape Fear shiner's decline has been attributed to human mediated changes in its endemic watershed from factors such as impoundments, water withdrawals, and altered land use patterns, which have led to degraded water quality and quantity, habitat loss and fragmentation, and increased influx of point and non-point source pollutants (NCDWQ 1996).

Most studies that aim to identify factors limiting the distribution and density of stream fishes, particularly threatened and endangered fishes, have focused on instream physical habitat as the primary target (Freeman and Freeman 1993; Kessler and Thorp 1993). A broader approach, which evaluates anthropogenic effects on water quality and contaminants, can lead to more effective management and possibly halt declines of populations that may not be limited by physical habitat alone (Wildhaber et al. 2000)

Pottern and Huish (1985) cited poor water quality in the upper reaches of the Cape Fear basin as a possible cause for the decline of the Cape Fear shiner. As this species and other endemic fish populations become increasingly isolated and rare, their vulnerability to

catastrophic events such as chemical spills and to cumulative, subtle degradation of physical habitat and water quality are greatly enhanced (USFWS 1988; Warren and Burr 1994; Burkhead et al. 1997). Although numeric water quality standards are designed to protect all aquatic organisms, they are developed from toxicity information derived from a small sample of the total freshwater fauna, such as the fathead minnow *Pimephales promelas* and the rainbow trout *Oncorhynchus mykiss*. These common test species often lack ecological relevance and may not serve as an adequate surrogate in toxicity testing. Sappington et al. (2001) found that the sensitivity of listed fish species in acute single chemical tests is similar to that of surrogate species with a few exceptions for some chemicals, and concluded that in order to effectively protect listed species, the most sensitive species should be used in development of water quality criteria. Therefore, it is important to understand if results from tests using the surrogate species, usually a member of the same family, can provide adequate protection to threatened or endangered species with unknown sensitivities to contaminants (Dwyer et al. 1999).

Fortunately, the Cape Fear shiner can be easily propagated in the laboratory and its relative sensitivity to five contaminants representing diverse chemical classes was recently assessed in acute tests (Dwyer et al. 1999). Those results indicated that the Cape Fear shiner was among the most sensitive (in the top 9) of the 16 fish species tested, and was more similar to Rainbow trout in sensitivity to chemicals than it was to the Fathead minnow. Therefore, it is a necessity to test the hypothesis that water quality may be a limiting factor to the species' ultimate restoration and sustainability. However, the tests conducted by Dwyer et al. (1999) were single-chemical laboratory exposures that lacked the realism of the natural

ecosystem in which fish are exposed to mixtures of chemical contaminants and other environmental stressors.

The *in situ* bioassay approach, which integrates conditions of the natural system with a degree of experimental control, has been successfully used to evaluate the effects of water quality on locally important fish species (Hall et al. 1985; Snyder-Conn 1993; Chappie and Burton 2000; Echols et al. 2000). This approach provides the environmental realism lacking in laboratory tests and combines the disciplines of toxicology and ecology (Hansen and Johnson 1999), which are both necessary for understanding and managing ecosystem health and diversity. Therefore, the purpose of this part of the project was to evaluate the influences of water quality on captively propagated Cape Fear shiners with a 28-d *in situ* bioassay in some of the best remaining and historical habitats for the species, focusing on sites that may be considered as potential reintroduction or population augmentation sites (USFWS 1988). The specific objectives were to (1) determine if water quality is a limiting factor to the occurrence, growth, and survival of the Cape Fear shiner, (2) document habitat suitability by assessing inorganic and organic contaminants through chemical analyses, and review of existing data, and (3) assess the protectiveness of water quality standards for primary pollutants based on comparisons of laboratory, field toxicity, and water chemistry data.

Methods

Study Area

The Cape Fear River rises in the north-central Piedmont region of North Carolina, near the cities of Greensboro and High Point and flows southeasterly to the Atlantic Ocean (Figure 1). It is one of only four basins located entirely within the state and is the largest among those, spanning a 15,000-km^2 watershed and 9,735 km of freshwater streams and

rivers. The basin supports approximately 22.1% of the state's human population, including 116 municipalities and all or portions of 26 counties (NCDWQ 2000). Land use in the Cape Fear Basin is 26% agriculture, 59% forest, 6% urban, and 9% other uses (NCDWQ 1996). From 1982 to 1992 there was a 43% increase in the amount of developed land in the basin. The basin contains 54% of the state's swine operations, and swine its population increased 90% from 1994 to 1998 (NCDWQ 2000).

The extant populations of the Cape Fear shiner are found in the Haw, Rocky, and Deep rivers in Randolph, Moore, Lee and Chatham counties, North Carolina (USFWS 1988; NCWRC 1996). I selected ten sites for this study, two on the Haw River, four on the Rocky River, and four on the Deep River (Figure 1). Of the 10 sites studied, six were in the extant range of the Cape Fear shiner and four were in the historic range or considered potential reintroduction sites for the species. One of the sites in the extant range (Rocky River at US 15-501) was deemed the best available habitat for the Cape Fear shiner by knowledgeable biologists and served as a reference control site for the test. The sites, listed from upstream to downstream for each river, were identified as follows.

Haw River

HR1: Chicken Bridge Rd. (SR 1545) crossing, Chatham Co., extant, 35.8331º N, 79.2193º W.

HR2: Downstream of Bynum Dam, Chatham Co., considered extant, but population is small and vulnerable, 35.7723º N, 79.1442º W.

Rocky River

RR1: US Hwy 64 crossing, Chatham Co., upstream of Siler City, NC wastewater treatment plant effluent, extirpated, 35.7351º N, 79.4229º W.

RR2: Rives Chapel Rd. (SR 2170) crossing, Chatham Co., downstream of Siler City wastewater treatment plant effluent, extirpated, 35.6988º N, 79.3760º W.

RR3: NC Route 902 crossing, Chatham Co., extirpated, potential reintroduction site, 35.6989º N, 79.3759º W.

RR4ref: US 15-501 crossing, Chatham Co., reference site, extant, 35.6225º N, 79.1882º W.

Deep River

DR1: Parks Crossroads Church Rd. (River Rd, SR 2628) crossing, Randolph Co., potential reintroduction site, extirpated, 35.6727º N, 79.6273º W.

DR2: Howard's Mill Rd. (SR 1456) crossing, Moore Co., extant, 35.5009º N, 79.5817º W.

DR3: Plank Rd. (SR 1007) crossing, Moore/Lee Co. line, NC, extant, 35.5551º N, 79.2874º W.

DR4: US 15-501 crossing, Moore/Lee Co. line, extant, 35.5788º N, 79.1939º W.

Bioassay design and fish deployment

About 900 captively reared Cape Fear shiners of a relatively uniform size (15-30 mm total length) and age (4-6 months) were obtained from Conservation Fisheries, Inc., Knoxville, Tennessee, on July 24, 2001. While at the hatchery, fish were cultured in reverse osmosis, filtered (passed through mechanical micron and carbon pre-filters) water, combined with de-chlorinated tap water that was buffered with Seachem Neutral Regulator with Reef Builder and/or Marine Buffer to maintain the system pH at about 7.5. Young fish were fed primarily live *Artemia* nauplii 2-3 times daily, augmented with Ocean Star International, Zeigler, or other dry larval fish foods as a supplement. When the fish were sufficiently large,

they were also fed frozen *Daphnia* spp. and chopped chironomids, with the latter being the staple food once they were able to feed on large items. Once fish were able to consume whole chironomids, they were generally fed *ad libitum* twice daily. The supply sources and potential contaminant burdens of these natural food items for Cape Fear shiners at the hatchery varied and are unknown. Once received, Cape Fear shiners were held for 3 days at the North Carolina Cooperative Fish and Wildlife Research Unit, Reedy Creek Laboratory for acclimation to test stream water quality conditions and temperatures. Before deployment, a subsample of 190 fish from the overall test population was taken, and individual fish were measured for length and weight to obtain a baseline for comparison of growth at the end of the test (none of these fish were used in the bioassay). In addition, five composite samples, of 10 fish each, from this group of 190 fish were promptly frozen after measurement and served as the baseline for comparison of contaminant concentrations after the test. On July 27, 2001 (day 0 of the test), fish were randomly allocated to 30 cages (three cages per site) with 20 fish per cage, at each of the 10 sites. Cages consisted of a clear plexiglass tube (25 cm long x 15 cm o.d.) covered on each end with tear-resistant nitex® mesh (2.0 mm) and held secure by stainless steel hose clamps. The size of mesh ensured that fish were retained, while allowing water and plankton to pass through the cage. Each cage was secured to a concrete block (39.5 cm long x 19.0 cm wide x 19.5 cm high) with two elastic binding straps and placed on the stream bottom in an area of typical Cape fear shiner habitat (determined from previous observations or historic reports) with suitable velocity and depth. Cage depths at all sites ranged from 1.0 to 1.5 m. As an additional measure to ensure that fish and cages would not be lost during potential high flow events, each block with cage was inconspicuously tied to a shoreline structure (e.g., tree or rock) with black nylon rope.

Sample collection and processing

Fish were monitored every 96-h throughout the 28-d exposure period for mortality, and any dead fish were removed. At each 96-h interval, temperature, dissolved oxygen (Yellow Springs Instrument model 58 meter), pH (Beckman model Φ110 ISFET meter), and conductivity (Hach model CO150 meter) were measured at each site. Water samples were also collected at each site at that time, held on ice, and analyzed for alkalinity, hardness, and turbidity (Hach model 2100 AN meter) at the laboratory within 24 h of collection with standard methods (APHA et al. 1995). Grab samples of water and surficial (top 5 cm) sediment were taken at the sites once during the 28-d test and stored for chemical contaminant (organic and inorganic) analysis. Water samples for inorganic constituents were promptly preserved to pH < 2 with concentrated HNO_3 and stored refrigerated (4EC) until analysis, and sediment samples were stored frozen at -20EC until analysis. A set of two passive sampling devices (PSDs), similar to semi-permeable membrane devices (Booij et al. 1998; Luellen 2000), was deployed along side the fish cages at each site for the 28-d period to obtain an estimate of cumulative waterborne organic contaminant exposure. The PSDs consisted of 10-mil (approximately 275 µm) 'virgin' low-density polyethylene (LDPE) tubing (Brentwood Plastics, Inc., St. Louis, MO), as described by Luellen (2000). The LDPE tubing was extracted with hexane for 48 h prior to use. After the 28-d deployment, the two PSDs, each 7.5 cm wide and 30 cm long, were combined to form a single composite sample from each site, placed in aluminum foil, sealed in a plastic bag, and stored frozen (-20EC) until analysis for chemical contaminants.

At the end of the bioassay (August 23, 2001), surviving fish were counted, measured, and weighed. Composite samples of 10 fish from each cage were then wrapped in aluminum

foil, sealed in plastic bags, and stored frozen (-80EC) for contaminant analysis. At the time of processing, fish samples were removed from the freezer, lyophilized (<-50EC, <145 millitorr) for 24 h, weighed, and ground to a fine powder with a mortar and pestle. Fish tissue samples were then split into two equal subsamples, one for inorganic analysis and one for organic analysis. Enough dry tissue mass was obtained to perform triplicate chemical analyses on fish samples from 20% of the sites. Samples of fish tissue and sediment were analyzed for 48 polycyclic aromatic hydrocarbons (PAHs) and alkylated homologues, 20 polychlorinated biphenyls (PCBs), 26 organochlorine (OC) pesticides and metabolites, chlorpyrifos, and a suite of 20 metals and metalloids (Appendices 1–6). Water samples were analyzed only for the suite of 20 metals and metalloids, and the PSD samples were analyzed only for PAHs, PCBs, OCs, and chlorpyrifos (Appendices 1–6).

Sample preparation and analysis

All inorganic chemical analyses were performed by the Midwest Research Institute in Kansas City, Missouri, or the Trace Element Research Laboratory at Texas A&M University, through contracts with the U.S. Fish and Wildlife Service, Patuxent Analytical Control Facility in Laurel, Maryland. All organic chemical analyses were performed by the Analytical Toxicology Laboratory at North Carolina State University.

Inorganics. Determinations of total mercury (Hg) in fish, sediment, and water were made on subsamples that had been digested with concentrated nitric acid, sulfuric acid, potassium permanganate, and potassium persulfate in polypropylene tubes in a water bath at 90-95EC. Hydroxylamine hydrochloride was added to reduce excess permanganate before analysis and aliquots of diluted digestate were analyzed by cold vapor atomic absorption spectrophotometry (CVAAS). Subsamples of homogenized fish tissue for total arsenic (As),

cadmium (Cd), and lead (Pb) determinations were digested in closed teflon reaction vessels with 3 mL of concentrated HNO_3 in a 130EC oven. Aliquots of diluted digestate were analyzed by graphite furnace atomic absorption spectroscopy (GFAAS). Subsamples of homogenized fish tissue for selenium (Se) were digested with the same procedure described for As, Cd, and Pb, but diluted digestates were analyzed by atomic fluorescence spectroscopy (AFS). All other metal analytes (Appendix 1) in fish tissue were analyzed by inductively coupled plasma (ICP) optical emission spectroscopy after digestion in closed teflon reaction vessels with 3 mL of concentrated HNO_3 in a 130EC oven.

All sediment samples (except for Hg) were digested in tall form beakers with 10 mL of Aqua Regia (1:4 v:v HNO_3:HCl) on a hot plate for 2 h and analyzed by ICP, except for As, Cd, and Pb, which were analyzed by GFAAS and Se, which was analyzed by AFS. All water samples (except for Hg) were digested for 2 h at 85EC in polyethylene containers with ultrapure 1% HCl and 0.5% HNO_3 and analyzed by ICP, except for As, Cd, and Pb, which were analyzed by GFAAS and Se, which was analyzed by AFS.

Organics. Field-deployed PSDs were cleaned with deionized water and a brush, followed by a rapid rinse in acetone to remove attached biological material from the surface of the LDPE. The PSDs were then cut into small strips and serially extracted three times in Teflon® bottles on a shaker table with a total of 75 mL of dichloromethane (DCM); total extraction time was 24 h. For sediment, a 25-g subsample of wet sediment was added to sodium sulfate and serially extracted three times in Teflon bottles on a shaker table with a total of 300 mL of DCM; total extraction time was 24 h. Samples of lyophilized fish tissue, ranging between 0.36 and 1.15 g dry weight, were serially extracted three times in Teflon bottles on a shaker table with a total of 45 mL of DCM; total extraction time was 24 h.

For all sample matrices, extracts were combined, filtered through a glass fiber 2-µm filter (baked at 400 ºC), and the combined extracts were reduced to approximately 2 mL by rotoevaporation and a gentle stream of nitrogen gas. Concentrated extracts were filtered through a 0.45-µm filter and fractionated with gel permeation chromatography (GPC). Lipid analysis was performed on the lipid GPC fraction. The fractionated extract was further purified with a 3-g silica column. The final extract volume was 200 µL.

The purified extracts were analyzed for PAHs with an Agilent 6890 gas chromatograph (GC) connected to an Agilent 5973N MSD (Avondale, PA) utilizing a Restek 30m x 0.25mm Rtx-5 (film thickness 0.25 µm) MS with an Integra-Guard column. The pressure was ramped to 40 psi before injection with a 1-min hold time. The flow was then decreased to a constant flow of 1 mL/min for the duration of the run. The temperature program for PAH analysis was as follows: initial temperature 40°C for 1 min with a ramp of 6°C /min to 290°C and a final hold time of 30 min; injector temperature 300°C, detector temperature 280°C. Selected ion monitoring (SIM) was used for analysis. Total PCBs and OC pesticides were analyzed by GC with electron capture detection (ECD) using a dual-column (30 m x 0.25-mm i.d., 0.25 um film, DB-1 and DB-17; J&W Scientific, Folsom, CA) dual ECD (Hewlett-Packard 5890 Series II, Avondale, PA) for confirmation. The GC temperature program was initially 60ºC (1 min hold) to 160ºC at 20ºC/min, held for 10 min, and ramped to 260ºC at 2ºC/min with a final hold of 20 min. Injector and detector temperatures were 260 and 280ºC, respectively. Samples were quantified from the DB-1 chromatograms and confirmed with the DB-17 chromatograms.

Analytical standards were obtained from AccuStandard, Inc. (New Haven, Connecticut); deuterated PAH standards were obtained from Cambridge Isotopes (Andover,

Massachusetts). Napthalene-d8, acenapthene-d10, chrysene-d12, and perylene-d12 were used as surrogate standards to estimate recoveries in PAH analyses and 4,4'-dibromooctafluorobiphenyl (DBOFB), PCB 112, and PCB 197 were used as surrogate standards to estimate recoveries in PCB and OC pesticide analyses.

Quality assurance

Inorganics. The accuracy of all determinations was assessed by analyzing one or more standard reference materials that approximated the matrix and concentration range of the samples, spiked samples, replicate samples, and procedural blanks with each batch of samples. With the water samples analyzed for alkalinity, hardness, and turbidity every 96 h from each site, we included 20% of samples analyzed in triplicate and certified reference materials from Spex CertiPrep, Inc. These analyses yielded concentrations of alkalinity, hardness, and turbidity within the certified concentration range in 22 of 24 determinations; two of the turbidity measurements were slightly (<5%) below the certified range. The relative standard deviation, estimated from analyses of 18 triplicate samples of river water, averaged 6% and ranged from 3 to 13%.

For analyses of Cape Fear shiners, the National Research Council of Canada (NRCC) standard reference material, DORM-2, (dogfish muscle) was used, and all analytes were within the certified range. The recovery of analytes from spiked fish samples averaged 95% (range 60–114%) and the mean percent difference from duplicate fish samples was 16% (range 0–70%). U.S. National Institute of Standards and Technology (NIST) standard reference materials (Buffalo River sediment, SRM 8704; Tennessee River sediment, SRM 8406) and NRCC MESS-3 (sediment) were analyzed with sediment samples and yielded concentrations within the certified range for all analytes, except for beryllium, chromium,

strontium, and vanadium, which had recoveries 39 to 42% below the certified range and manganese which had a recovery 72% above the certified range. The mean analyte recovery from spiked sediment samples was 93% and ranged from 68 to 118%. The percent difference from duplicate sediment samples averaged 12% (range 2–64%). Analysis of NIST, SRM 1640 with water samples yielded concentrations within the certified range of each analyte except for iron, which had a recovery 129% greater than the established limits.

Organics. Procedural blanks and polyethylene blanks (with the PSD samples) were analyzed with each batch of samples to determine background contamination in the materials and reagents or potential contamination introduced during extraction and cleanup. All of the blanks were extremely low; no PCBs or OC pesticides were detected, and only small amounts (<1 ng/g) of a several PAHs were detected. Recoveries of surrogate internal standards ranged from 40 to 120% for all analytes, except for several samples where naphthalene-d8 was between 30 and 40%. The lower recoveries for naphthalene were most likely due to evaporative losses during the solvent exchange step required for the silica column cleanup. Data were not corrected for surrogate recoveries. Matrix spike recoveries were also within the range of 40-120%, with several exceptions of higher recoveries, but only for analytes that were not detected in any samples. The percent difference between matrix spike and spike duplicates, and duplicate sample analysis, was usually less than 10% and always less than 30%.

Statistical analyses

Statistical analyses were performed with PC SAS v8.1 (SAS Institute Inc. 1999-2000). Variation among sites in mean survival, growth, and contaminant concentrations in fish, sediment, water, and PSDs was evaluated with the general linear models procedure in

SAS (PROC GLM). All variables were examined for normality and homogeneity of variance (PROC Univariate and Bartlett's test in SAS), and transformed, if necessary, to meet assumptions of statistical tests. The data for fish survival were arcsine transformed prior to analysis. A Ryan-Einot-Gabriel-Welsch multiple range test (PROC GLM, REGWQ option), which is a conservative test that controls the experimentwise error rate, was used to identify significant differences among site means for survival and growth of fish. A Type I error rate ($\forall$) of 0.05 was used to judge statistical significance.

RESULTS

The mean physicochemical characteristics of river water measured every 96 h during the 28-d bioassay at the 10 test sites ranged from 25.1 to 28.9EC for temperature, 5.81 to 12.46 mg/L for dissolved oxygen, 7.56 to 9.01 for pH, 121 to 617µS/cm for conductivity, 37 to 59 mg/L as $CaCO_3$ for alkalinity, 39 to 128 mg/L as $CaCO_3$ for hardness, and 2.1 to 40.8 NTU for turbidity (Table 1).

The length of Cape Fear shiners on day 0 of the test, as estimated from a subsample of 190 fish from the overall test population (about 900), averaged 21 mm and ranged from 14 to 33 mm. The mean wet weight of test fish before deployment was 0.080 g, and ranged from 0.022 to 0.283 g. After the 28-d exposure, the average length of surviving fish from all 10 sites was 24 mm (range 17-37 mm), and the corresponding average wet weight was 0.103 g (range 0.014-0.417 g). Relative to length at day 0, fish grew significantly at four of the 10 sites (Figure 2); one was in the Rocky River (RR4, reference site), and the remaining three were in the Deep River (DR1, DR2, and DR4).

Survival of fish over the 28-d exposure period at all sites averaged 76% and ranged from 53 to 100%. The sites with the greatest overall survival were on the Deep River (87%),

followed by those on the Rocky River (74%), and were lowest on the Haw River (66%). Five sites, two in the Haw River (HR1, HR2), two in the Rocky River (RR1, RR3) and one in the Deep River (DR2), had fish with significantly reduced survival (Figure 2). The surviving fish at the HR1, HR2, RR1, and RR3 sites, which had reduced survival rates, also had no detectable growth (as measured by an increase in length) over the duration of the test (Figure 2). However, mean survival and growth of fish were not significantly related ($r = 0.60$, $P = 0.06$) among all sites.

The lipid content of test fish, an indicator of relative health and condition, averaged 2.61% (range 2.59–2.63%) on day 0 of the test and decreased to an average of 0.83% (range 0.28–1.35%) at all sites by day 28. The sites with fish that had the lowest survival and growth rates consistently had the least lipid reserves (Figure 2). Among all sites, lipid concentrations in fish were significantly correlated with growth ($r = 0.76$, $P = 0.01$).

Captively propagated Cape Fear shiners accumulated quantities of certain inorganic and organic contaminants over the 28-d exposure. Unexpectedly, we also detected some of the more persistent, bioaccumulative contaminants (e.g., cadmium, mercury, PCBs, chlordanes, and DDTs) in our baseline control fish, which complicated assessing field exposure and accumulation for these chemicals. These persistent contaminants presumably originated in the test fish through dietary exposure at the hatchery.

Although there were no apparent relations between specific contaminant accumulation and reduced growth or decreased survival of fish among all of the sites, certain sites exhibited trends in cumulative contaminant accumulation with reduced survival and growth. Cadmium, copper, mercury, lead, and zinc (Figures 3–7) were detected in fish, water, and sediment samples from the 10 sites. The accumulation of cadmium, copper, and

lead in Cape Fear shiners was greatest at site DR2, which also had significantly reduced fish survival. In contrast, concentrations of copper, lead, and zinc in sediment were greatest in site RR3, another site with significantly reduced survival and no growth of fish. Zinc accumulation in fish tissue was greatest at RR2, a site with no significant growth. Mercury was greatest in both fish tissue and sediment at site DR4, but had no apparent effects on fish survival or growth.

Of the main organic contaminants of concern, PAHs were detected in sediment and water (PSDs), PCBs and chlordanes were detected in fish, water, and sediment, and DDTs were detected in fish and water at the sites (Figures 8–11). Again, several of the sites with reduced survival and growth of Cape Fear shiners (e.g., DR2, RR3) that had among the greatest concentration of metals measured, also had among the greatest concentrations of organic contaminants in the various compartments measured. A notable appearance among sites for the organic constituents was the occurrence of relative high concentrations of certain organics in fish, sediment, and water at the two Haw River sites (HR1, HR2), which also had reduced survival and growth of fish.

Because of the variation in measured contaminant concentrations among the sites for the various analytes and media (fish, water, and sediment), determining the overall trend for potential cumulative exposure and impacts of contaminants to Cape Fear shiners was difficult. Therefore, we devised a novel generalized hazard assessment tool that allowed us to evaluate relative cumulative exposure and contamination at a site. This general hazard assessment tool was based on ranking the highest three measured concentrations for a given analyte and media at a site and is presented in Table 2. Through this analysis, we found that certain sites and rivers could be identified as having pervasive contamination, which

generally corresponded to those sites and rivers that exhibited decreased survival and growth of Cape Fear shiners during the 28-d *in situ* bioassay. For example, the metals cadmium and zinc, and the organic contaminants PCBs, chlordanes, and DDTs, contribute to the overall degraded water quality in the Haw River (Table 2). The upper Haw River Basin is affected by point source and non-point source discharge, and NCDWQ has rated 6 streams from that basin as poor or poor/fair in the recent basinwide report (NCDWQ 2000). From sites on the Rocky River, inorganic contaminants were identified as most prevalent in the samples. Copper and zinc were detected in all three media at RR2, the site downstream of the Siler City wastewater treatment plant (WWTP) that discharges into Love's Creek, and were among the highest concentrations measured at any of the sites during the study. Organic contaminants such as PAHs and PCBs were also detected at sites in the Rocky River, but their concentrations were relatively low and not a concern for the protection of aquatic health. Overall, contamination of the Deep River was relatively low (Table 2) and clearly represents some of the best remaining water quality for Cape Fear shiners. However, some chlordane was surprisingly prevalent in Deep River sites and was among the highest three analytes measured in water and sediments at three of the four sites in the Deep River.

To assess whether any of the individual chemicals measured at the sites represented a potential hazard to aquatic life, we compared our results to existing water quality and sediment quality, and toxicity criteria (Tables 3 and 4). The majority of our results for contaminants in water were not above the US EPA's freshwater chronic continuous criterion (FW CCC; US EPA 2002), with a few exceptions. The RR2 site had a copper concentration of 0.007 mg/L; this value approaches the US EPA freshwater chronic criterion of 0.009 mg/L. RR2 is downstream of the Siler City WWTP, and as a result has had a problem with

elevated levels of copper. Lead concentration in water at DR1 was 0.003 mg/l, which is greater than the US EPA FW CCC of 0.0025 mg/L.

The PAHs were detected in all PSD samples, but estimated PAH concentrations in water (Figure 8) were relatively low with respect to thresholds for toxicity to aquatic species (US EPA 2002). Concentrations of PCBs in PSD samples were generally low or undetectable and estimated PCB concentrations in water were all below 1 ng/L at all sites (Figure 9), which is well below the US EPA numeric criteria of 14 ng/L (Table 3). Chlordanes were detected in all PSDs, except at the RR4 and DR3 sites. Estimated concentrations of chlordanes in water (Figure 10) ranged from 0.06 to 2.38 ng/L, with the greatest concentration occurring at the DR1 site. Concentrations above 1 ng/L can cause adverse affects in aquatic organisms, but concentrations known to affect fish are generally much greater (e.g., 200 ng/L; USGS 2000).

Again, the PAHs were detected in all sediment samples (Figure 8); however, all concentrations were extremely low compared to Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (SQGPAL; Environment Canada 2002). Concentrations of PCBs in sediment were extremely low (<1 ng/g dry weight) at the sites, and all were less than the probable effect level of 277 ng/g dry weight set by the Canadian SQGPAL. Chlordanes, which were detected in four sediment samples (RR3, DR1, DR2, and DR4), were all below the Canadian interim freshwater sediment quality (0.0045 µg/g dry weight) guidelines.

Cadmium was detected in sediment samples from all ten sites. However, all measured concentrations were <0.4 µg/g, which is below the protective level of 0.6 µg/g, established by the Canadian SQGPAL (Figure 3; Table 4). Copper and lead were also detected in sediments from all sites; their levels were less than 16.5 µg/g and 11.5 µg/g,

respectively—well below the Canadian SQGPAL protective level. Mercury was detected in sediment at six sites (RR2, RR3, RR4, DR1, DR3, and DR4; Figure 5; Table 4). The mercury concentration measured at the DR4 site was 0.178 µg/g dry weight, which was slightly greater than the Canadian SQGPAL; the other five sites were below the 0.17 µg/g dry weight criterion. Zinc was detected in all sediment samples (Figure 7), but concentrations were below the Canadian interim SQGPAL level of 123 µg/g. However, zinc concentrations at the RR2 and RR3 sites appeared elevated relative to the other sites.

Although only a few of the existing chemical-specific criteria for water and sediment were exceeded at sites during this study, the general hazard assessment (Table 2) showed that subtle, pervasive contamination existed at several of the sites. This contamination may lead to cumulative impairment of water and sediment quality for Cape Fear shiners. However, the overall potential for cumulative risk of chemicals below individual toxicity thresholds is unknown.

Discussion

In situ bioassays have become an increasingly important and useful tool to validate laboratory testing and to extrapolate results to field conditions (Pereira et al. 2000). Advantages of *in situ* bioassays include the incorporation of complex site-specific conditions, such as oxygen, pH, and temperature, which may alter the bioavailability and toxicity of contaminants, and the reduction in sampling artifacts (Chappie and Burton 2000). The *in situ* bioassay approach with caged fish has been successful in other studies in evaluating water quality and the effects of local contaminants (Nichols et al. 1999; Echols et al. 2000).

Comparison of contaminant availability among sites

The results of the *in situ* toxicity test indicate that water quality may be a limiting factor to the Cape Fear shiner in the Haw River. The two Haw River sites, HR1 (Chicken Bridge Rd.) and HR2 (downstream of Bynum Dam) are considered to be two of the five remaining populations of the Cape Fear shiner, but populations densities are low at these sites and could be prone to extirpation (NCWRC 1995). At HR1 and HR2, survival of caged fish was 65% and 67%, respectively. Fish at these two sites had statistically reduced survival compared to the Rocky River reference site (RR4), where survival was 98%. However, the surviving fish at both Haw River sites did not differ significantly from the pre-test control fish in total length; therefore, growth appears to have been limited at these sites. Zinc concentrations in water at these sites were among the highest of all sites sampled. Fish tissue contained relatively higher concentrations of zinc and lead relative to those of the background control fish. Lewis et al. (2002) sampled fish from near-coastal areas of the Gulf of Mexico receiving point source discharges, and the mean measured concentration of zinc in edible fish tissue (i.e., fillets) was approximately 5 μg/g wet weight. Measured concentrations of zinc in whole Cape Fear shiners from site HR1 were 20 times greater after only 28-days of exposure (Figure 7). Similarly, measured concentrations of zinc in liver and muscle from an endangered sucker (Catostomidae) species were 15.1 μg/g and 6.8 μg/g, respectively (much lower than in test Cape Fear shiners), and these concentrations were measured from adult fish taken from the wild (de Lafontaine et al. 2002). However, the concentrations measured in Cape Fear shiner tissue are similar to tissue residues in experiments with rainbow trout that did not produce significant effects on growth or survival (Jarvinen and Ankley 1999).

The organochlorine pesticide DDT and its metabolites are highly persistence and toxic compounds. There is much evidence of reproductive toxicity and adrenotoxicity in birds and mammals, and there is growing evidence of its adverse effects on the adrenal and reproductive systems in many fishes (Benguira and Hontela 2000; Benguira et al. 2002). The estimated concentrations of DDT in water were highest at HR1 and HR2. The levels of DDT in the water were about one-third of the US EPA freshwater chronic criterion for DDT and the metabolite 4,4'-DDE accounted for 64% of the total detected. Fish tissue, including the background sample contained concentrations of 4,4'-DDE. All fish were likely exposed to 4,4'-DDE prior to deployment through their diet, but after adjusting for lipid lost during the exposure, fish at HR1 had much greater concentrations of DDT than did the background sample. Fish deployed at HR2 did not show the same result. However, DDT metabolites are readily available in water at both Haw River sites and may limit the Cape Fear shiner in those reaches.

Interestingly, DDT metabolites were not detected in sediments. Sediment often serves as a reservoir for organochlorine pesticides and can act as a method of transport (Johnson et al. 1988; Gilliom and Clifton 1990; Eaton and Lydy 2000). Other studies have shown a positive relationship between DDT metabolites in soil and fish tissue (Eaton and Lydy 2000). However, the lack of DDT metabolites in sediment suggests that the source of DDT in the Haw River may be from non-point source pollution and not from a reservoir of the chemical. Possible non-points sources should be investigated in order to determine the source of the contamination.

PCBs and PAHs were found in low concentrations in fish tissue, water, and sediment and are not likely posing a threat to Cape Fear shiners at these sites or anywhere in the range

where our test took place. Chlordanes, however, were present in fish tissue, but the background control samples and test fish had different compositions of chlordane and chlordane metabolites, indicating that test fish most likely did accumulate chlordanes from the river. The concentration of chlordanes in water at HR1 was above 1 ng/L, which approaches the level known to have adverse affects on aquatic organisms (US EPA 2002) and HR2 had a concentration in water of 0.66 ng/L, which approaches the criterion level.

Extant populations in the Haw River are exposed to metals (zinc and lead) and organic pesticides (DDT and chlordanes) at levels that are questionable for health of aquatic organisms. Our results of non-significant growth and significantly reduced survival, compared with the reference site, support the conclusion that water quality may be a limiting factor in the Haw River.

Fish at site RR1 had statistically lower survival relative to the reference site (RR4), and fish total length was not significantly different than the pre-test control sample. Site RR1 (Rocky River at Hwy 64) is located in the upper Rocky River where the species has never been collected. It is possible that Cape Fear shiners were already extirpated from this reach when discovered in 1962. All organic contaminant concentrations in water and sediment at RR1 were low and not of concern. Fish tissue had detectable concentrations of chlordanes, but these values were comparable to those in the background control. Zinc concentrations were elevated in fish tissue with respect to the background control, so it is likely that zinc accumulation occurred in fish during the test. Although laboratory tests with other fish species at comparable concentrations had no or little effects (Jarvinen and Ankley 1999), it is possible that Cape Fear shiner sensitivities to zinc are greater than that of other species. This site also had low flow conditions at the time of the test, which could have contributed to fish

stress and reduced survival and growth. Water quality in this reach is comparable to the reference site, but lack of adequate flow, possibly due to the Siler City drinking water reservoir immediately upstream of this site, may have contributed to low survival.

Survival of fish at site RR2 (Rives Chapel Rd.) did not differ significantly from survival at the reference site, but total length was not significantly different from the pre-test control sample. Zinc concentration in fish tissue at this site was the highest, relative to other sites, and twice as high as background controls, suggesting that zinc uptake occurred. This site is just downstream of Love's Creek, a Rocky River tributary where point source discharge from Siler City's WWTP is released. Portions of Love's Creek are on the states list of impaired waters (NCDWQ 2000). North Carolina DWQ has monitored RR2 in recent years, and it received a Good-Fair rating in 1998 (NCDWQ 2000); however, this site is impacted by the discharge due to its high conductivity relative to the other sites in the study. Physiochemical characteristics, like conductivity, can influence the toxicity of contaminants, and therefore this site should continue to be monitored for effects of the upstream WWTP on the biological community. Chlordane concentration in fish tissue was higher than that of the control fish, and it is likely that the test fish accumulated chlordane during the test. Conditions at this site are degraded due to influences of the upstream urban areas; thus, combinations of site-specific interactions (including zinc and chlordane uptake) may be responsible for the lack of fish growth at this site.

The lack of fish growth, poor survival, and contaminant residues suggest that water quality is limiting at site RR3 (NC 902), where Cape Fear shiners have been extirpated. This site is considered a potential reintroduction site for the species. Survival of test fish was only 53% (significantly different from the reference site) at this site, and mean total length was not

significantly different from the pre-test controls. Zinc and chlordanes were both accumulated in fish tissue, and concentrations of zinc, copper, and lead in water and sediment were the greatest among all sites. This site is downstream of RR2 and is influenced by the upstream urban areas, but the immediate area is directly affected by agriculture practices.

Water quality is not limiting at site RR4 (US 15-501). It was considered to be the best reference site for this study by knowledgeable state and federal biologists because of its historic good water quality (NCDWQ 2000) and it is considered to have the most abundant population of Cape Fear shiners. Survival of test fish at this site was high (98%) and fish growth was highly significantly different from the pre-test controls. All concentrations of metals in fish tissue were similar to background controls, and concentrations in water and sediment were generally low and not of concern. Concentration of chlordanes in fish tissue was similar to background controls. Our results confirm that this lower reach of the Rocky River has good water quality and that it is not a limiting factor to the occurrence Cape Fear shiner in this reach.

Site DR1 (Parks Crossroads Rd./River Rd.) is in a reach of the Deep River where the Cape Fear shiner has been extirpated above Coleridge Dam, and test results suggest water quality may not be a limiting factor in this reach at the time of the test. It is also considered as a potential reintroduction site. Fish survival at this site was the highest (100%) measured during the study and fish growth was highly significant. Metal concentrations in fish tissue were similar to background controls. Lead concentration in water was near the US EPA chronic criterion for lead, although lead concentration in fish was similar to the background control, therefore site-specific conditions may have affected the availability of lead. Despite the significant growth and high survival, concentration of chlordanes in water (2.38 ng/L)

was over two times higher than the concentration known to cause adverse effects in some aquatic species. North Carolina Division of Water Quality has documented water quality problems in the upper Deep River over the last two decades, and over that time water quality has continuously improved, although some areas are impacted locally due to storm run-off and agricultural activities (NCDWQ 2000). Overall, water quality does not appear limited in this reach of the Deep River; however, consideration should be given to the to the source and concentration of chlordanes and lead before reintroduction efforts proceed.

Site DR2 (Howard's Mill Rd.) represents the uppermost population of the Cape Fear shiner in the Deep River, and this portion of the Deep River is classified as High Quality Waters (HQW) (NCDWQ 2000); however my results suggest water quality may be detrimental to the Cape Fear shiner in this reach. Fish growth was significant, but survival (63%) was significantly less than the reference site. Fish accumulated zinc, and zinc concentrations in water were near the chronic criterion set by the US EPA. The sum of chlordane concentrations in water was close to 1 ng/L, and chlordane concentrations in sediment were similar to the Canadian SQGPAL. Chlordane concentrations in test fish were similar to those in the background control; however, test fish had different compositions of chlordane metabolites and thus may have accumulated chlordane from the river water. This site is directly adjacent to an agricultural area with little or no riparian zone, and may be impacted by the use of pesticides in the area. Although fish growth was not affected significantly, survival was low relative to the reference site and may be due to a local combination of water quality factors.

My results show that water quality at site DR3 in the main stem river is not a limiting factor to the Cape Fear shiner. Site DR3 at Plank Rd is in the extant reach of Cape Fear

shiners on the Deep River downstream of Carbonton Dam, and represents the strongest remaining population; this area includes the Rocky River below the Rocky River Hydroelectric Dam near Bear Creek and the confluence of the Deep and Rocky rivers downstream to Indian Creek near US 1 on the Deep River. Survival of fish was high (90%) at this site, but fish growth was not significant. Concentrations of chlordane in fish, sediment, and water were all low with respect to critical threshold levels. Although zinc was detected in water and sediment and accumulated by test fish, concentrations were not of concern for aquatic health. Fish at this site also accumulated lead, but not at levels known to cause adverse effects (Jarvinen and Ankley 1999). Water quality problems are known from tributaries in this lower portion of the Deep River with two tributaries receiving a Fair or Poor classification in 1998 (NCDWQ 2000). In general, water quality increases in the downstream portion of this river. However, these tributaries have been impacted by local agricultural practices that have lead to stream bank erosion and degraded instream habitat.

Site DR4 (US 15-501), like site DR3, represents the range of the strongest population in the lower Rocky and Deep Rivers, and survival of fish at this site was high (95%), and growth was significant. The mercury concentration in sediment at this site was the highest measured at all 10 sites (0.178 mg/kg) and was above the standard for quality sediment set by the Canadian SQGPAL. However, the mercury concentration in fish did not indicate that test fish accumulated mercury. Fish accumulated lead and zinc, but concentrations were not high enough for concern to aquatic species. The surrounding watershed has high numbers of certified animal operations and two large permitted discharges (Sanford WWTP and Golden Poultry), and the classification at this site was reduced from Good to Good-Fair in 1998 (NCDWQ 2000). Despite declining water quality over the last five years at this site and from

river reaches upstream of this site (Good to Good-Fair), our results, including significant fish growth and high survival, indicate water quality is not likely a limiting factor in this reach of the Deep River.

Ecological and management implications

The quality of water in the historical and extant range of the Cape Fear shiner varied within and among rivers, and was likely due to differences in land use patterns and urbanization. The pesticides and organic contaminants detected in this study (i.e., chlordane, DDT, and PCBs) are substances that are now banned in the United States because of their persistence in the environment and potential to harm aquatic organisms; however these substances still pose a threat to the Cape Fear shiner due to their persistence. Sites varied in the composition of contaminants and therefore, potential effects on fish survival and growth were difficult to assess and predict. Water quality in the reaches of the extant populations of the Cape Fear shiner supported fish growth and high survival during the 28-d *in situ* bioassay. In contrast, presumed poor water quality in the extirpated reaches (inferred from contaminant profiles), may have contributed to the limited success of caged fish in these reaches. The Cape Fear shiner uses a narrow range of habitat conditions that are in relatively short supply in reaches of river where the fish is extant, extirpated, and rare. Past acute poor water quality events, combined with loss of riverine physical habitat and fragmentation of populations by dams, which prevent re-colonization, have produced the isolated and increasingly rare populations of the Cape Fear shiner that exist today.

Recommendations for restoration and management of Cape Fear shiners are to improve water quality in the lower Haw River where the species is vulnerable to extirpation and improve water quality and flow in the upper Rocky River where the fish has been

extirpated. The potential reintroduction site in the Rocky River (RR3) at NC 902 has similar physical habitat to the lower Rocky River (see Chapter 1), but water quality would most likely hinder any reintroduction efforts in the near future. Water quality at this site should be enhanced to that of the downstream reaches before reintroductions are planned. The other possible reintroduction site in the Deep River (DR1) at Parks Crossroads had 100% fish survival and significant fish growth. A survey of physical habitat in that reach of the Deep River is necessary to determine if percentages of suitable habitat similar to the extant reaches are present. Water quality in that reach appears suitable for reintroduction in the near future.

The sustainability of Cape Fear shiner populations depends on the protection and preservation of the extant populations and habitats. Pressure from urban development and the increasing demands of the human population for water resources will confound restoration efforts. This study identified areas requiring restoration prior to any reintroduction or population augmentation, and this information can be used to improve the management of aquatic resources that is necessary to ensure the long-term survival of the Cape Fear shiner.

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Table 1. Mean physiochemical characteristics of river water (standard error in parentheses) measured at each site every 96-hour during the 28-day bioassay with Cape Fear shiners.

River and Site	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Conductivity (µS/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Turbidity (NTU)
Haw River							
HR1	26.1 (0.81)	7.49 (0.26)	8.05 (0.10)	246 (38)	41 (4.4)	42 (3.8)	20.1 (6.6)
HR2	26.5 (0.82)	7.99 (0.24)	8.29 (0.12)	238 (37)	42 (3.1)	40 (2.4)	20.4 (6.5)
Rocky River							
RR1	27.2 (0.92)	6.10 (0.67)	7.56 (0.11)	121 (8)	43 (2.1)	43 (2.9)	6.6 (0.8)
RR2	25.1 (0.73)	7.08 (0.42)	7.79 (0.06)	617 (40)	59 (3.4)	128 (6.8)	5.8 (0.8)
RR3	25.3 (0.75)	8.53 (0.27)	8.04 (0.09)	445 (12)	56 (1.6)	94 (2.0)	2.1 (0.3)
RR4	28.9 (0.83)	12.46 (0.61)	9.01 (0.17)	194 (10)	37 (2.0)	46 (0.7)	2.6 (0.5)
Deep River							
DR1	26.4 (0.68)	7.96 (0.62)	7.86 (0.11)	214 (59)	45 (7.4)	48 (5.3)	37.9 (19.7)
DR2	28.1 (1.01)	8.74 (0.55)	8.19 (0.15)	316 (42)	49 (6.7)	51 (4.4)	40.8 (33.5)
DR3	27.9 (0.59)	5.88 (0.62)	7.59 (0.14)	230 (35)	41 (1.8)	40 (3.4)	3.6 (0.7)
DR4	27.7 (0.82)	5.81 (0.42)	7.56 (0.07)	223 (34)	40 (1.3)	41 (2.4)	5.8 (1.0)

Table 2. Summary of generalized hazard assessment for selected inorganic and organic contaminants among sites during the 28-d *in situ* bioassay with Cape Fear shiners. For a given triangle, a darkened compartment represents a measured concentration among the highest three for a given analyte at all sites; top = fish, middle = water, and bottom = sediment.

	Analyte								
River and Site	Cd	Cu	Hg	Pb	Zn	PCBs	PAHs[a]	Chlordanes	DDTs[b]
Haw River									
HR1									
HR2									
Rocky River									
RR1									
RR2									
RR3									
RR4									
Deep River									
DR1									
DR2									
DR3									
DR4									

[a]PAHs not measured in fish tissue, [b]DDTs not detected in sediment.

Table 3. Measured concentrations in river water (µg/L) of common contaminants at sites sampled in the Cape Fear shiner bioassay and the US EPA freshwater chronic continuous criterion (FW CCC) for each contaminant. (< preceding a value indicates that a sample was below the detection limit of the test.)

Analyte	FW CCC	HR1	HR2	RR1	RR2	RR3	RR4	DR1	DR2	DR3	DR4
Cd	2.2	0.25	0.11	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Cu	9.0	<5.0	<5.0	<5.0	7.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
Hg	0.77	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Pb	2.5	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	3.0	<2.0	<2.0	<2.0
Zn	120	27	16	<5	17	<5	<5	7	6	<5	<5
Chlordane	0.0043	0.00159	0.00066	0.00003	0.00013	0.00006	0	0.00240	0.00077	0	0.00016
PCBs	0.014	0.0006	0.00033	0	0.00019	0	0	0.00011	0.00007	0	0

Table 4. Measured concentrations in sediment of common contaminants at sites sampled in the Cape Fear shiner bioassay and the Canadian interim freshwater sediment quality guidelines (ISQG). All concentrations are μg/g dry weight.

Analyte	Canadian ISQG	HR1	HR2	RR1	RR2	RR3	RR4	DR1	DR2	DR3	DR4
Cd	0.6	0.158	0.276	0.224	0.330	0.0403	0.383	0.127	0.144	0.0929	0.207
Cu	35.7	4.11	6.38	4.88	8.7	16.5	6.29	3.96	3.55	1.96	9.3
Hg	0.17	0.009	0.0135	0.0109	0.0189	0.0238	0.0157	0.0186	0.0095	0.0158	0.178
Pb	35.0	4.27	7.7	6.71	9.37	11.5	9.66	3.94	4.28	2.81	6.47
Zn	123.0	24.3	37.6	21.9	43	52.7	33	13.3	16.4	10.3	24.6
Chlordane	0.0045	0	0	0	0	0.0004	0	0.0004	0.00299	0	0.00108
PCBs	0.0341	0.0001	0	0	0	0.00082	0	0.00017	0.00024	0.00005	0.0007

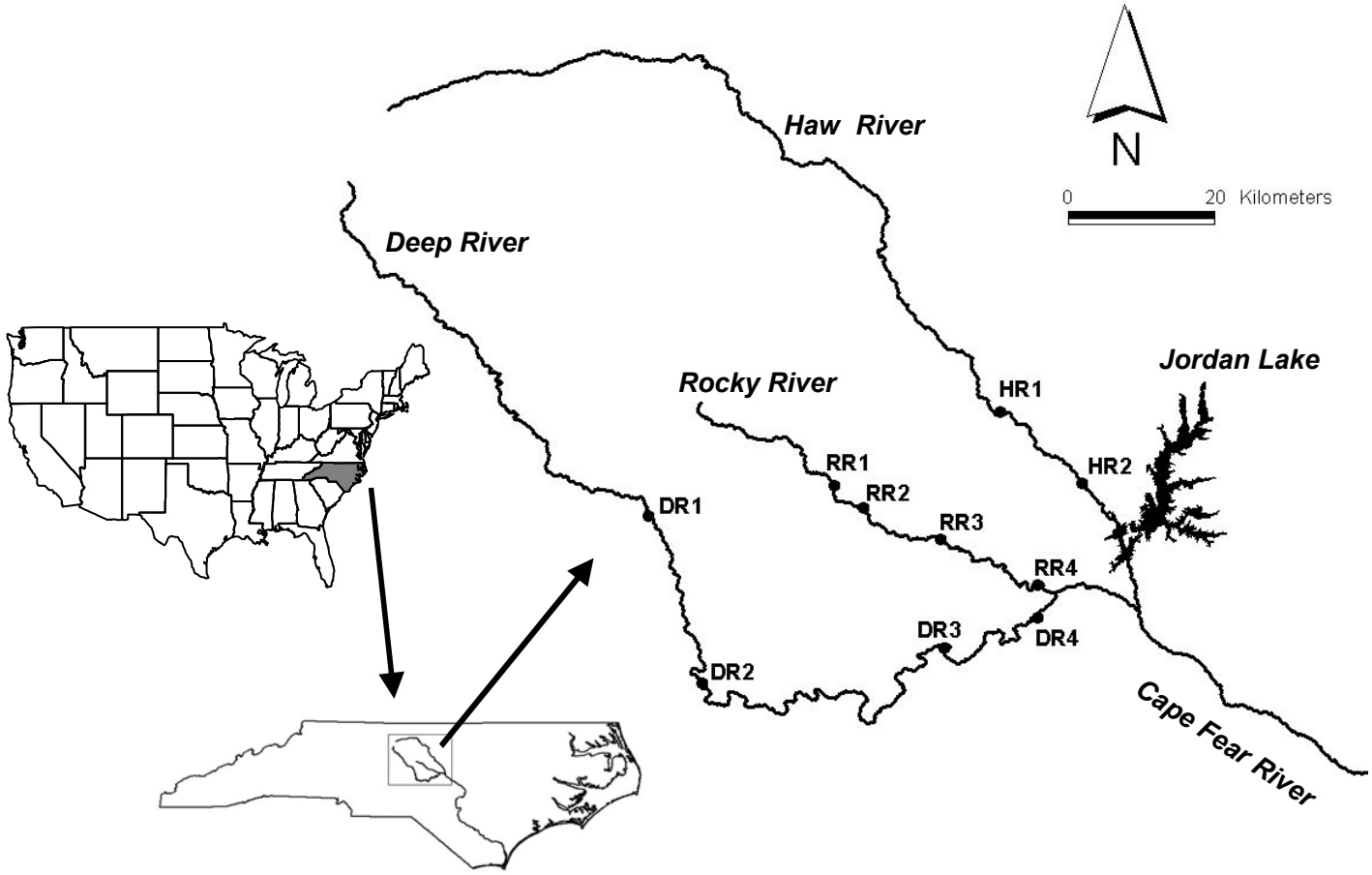

Figure 1. Study sites used in the Cape Fear shiner 28-d *in situ* bioassay.

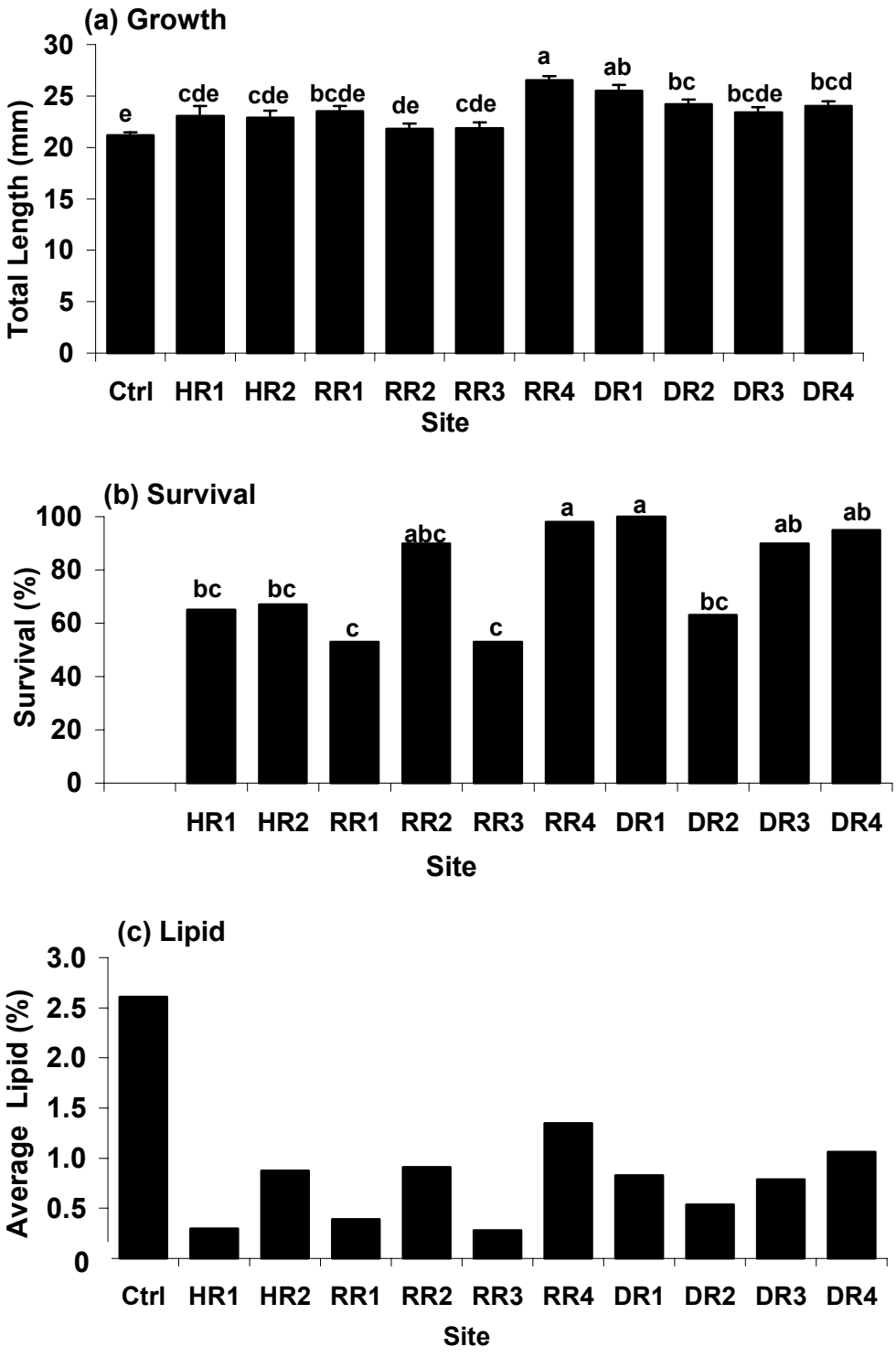

Figure 2. (a) Mean growth, (b) survival, and (c) lipid concentration of Cape Fear shiners after the 28-d bioassy (Ctrl = baseline control sample on day 0 of the test) at sites in the Haw, Rocky, and Deep rivers of North Carolina. Sites accompanied by the same letter were not significantly different ($P > 0.05$). Error bars represent the standard error.

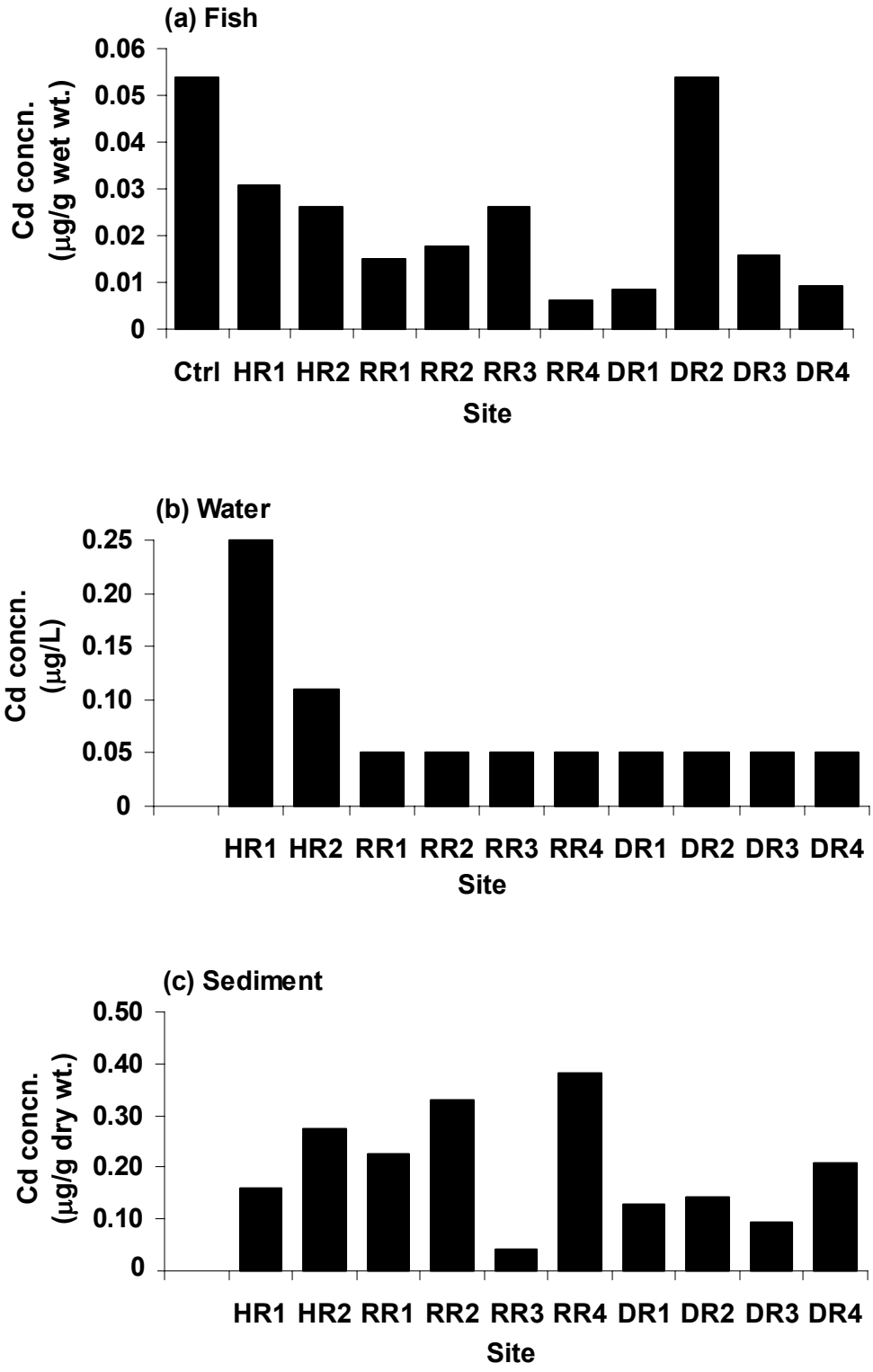

Figure 3. Mean concentration of cadmium (Cd) in (a) Cape Fear shiners, (b) water, and (c) sediment from sites in the Haw, Rocky, and Deep rivers of North Carolina. For fish samples, Ctrl = concentrations in fish on day 0 of the 28-d test.

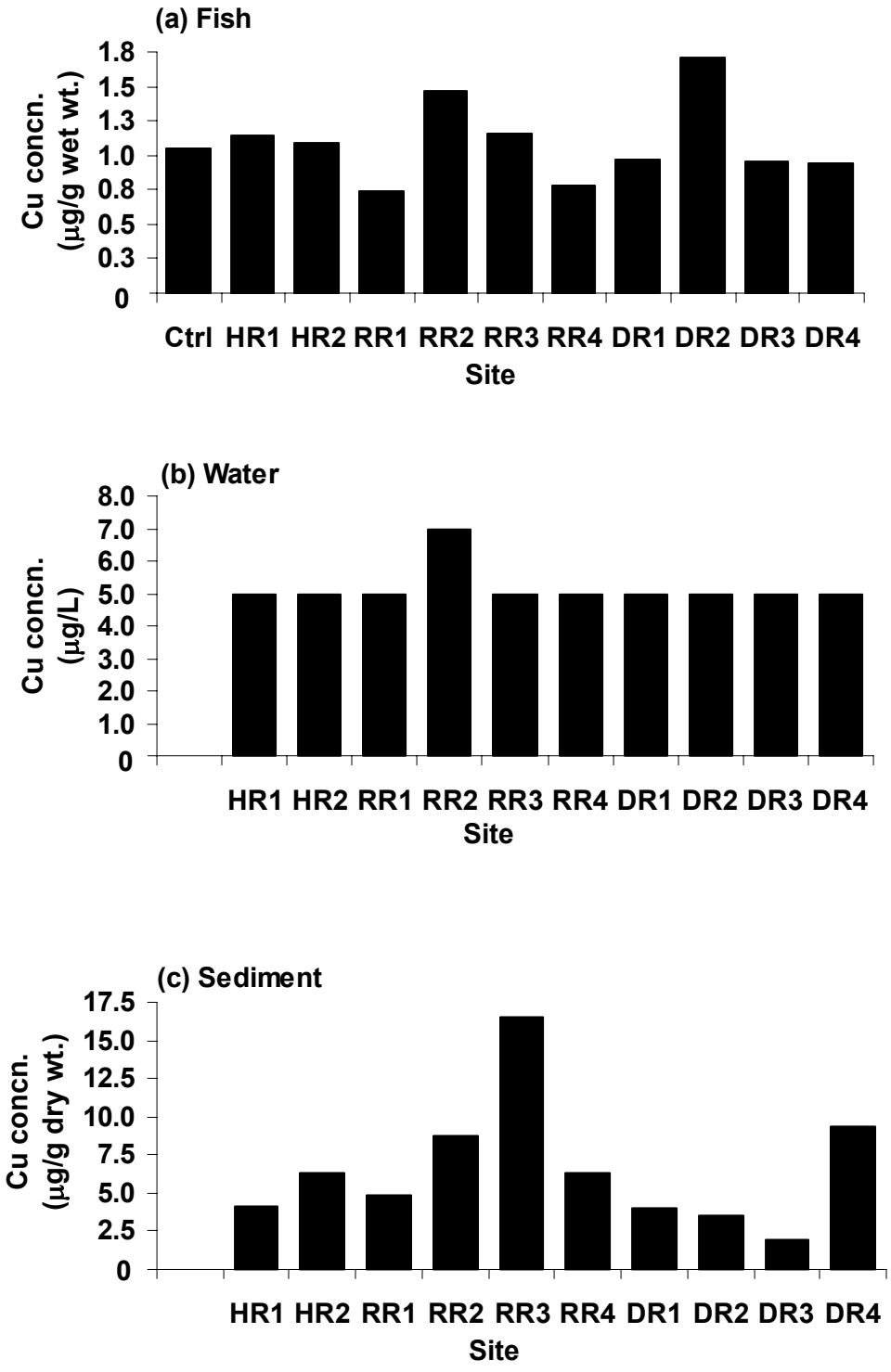

Figure 4. Mean concentration of copper (Cu) in (a) Cape Fear shiners, (b) water, and (c) sediment from sites in the Haw, Rocky, and Deep rivers of North Carolina. For fish samples, Ctrl = concentrations in fish on day 0 of the 28-d test.

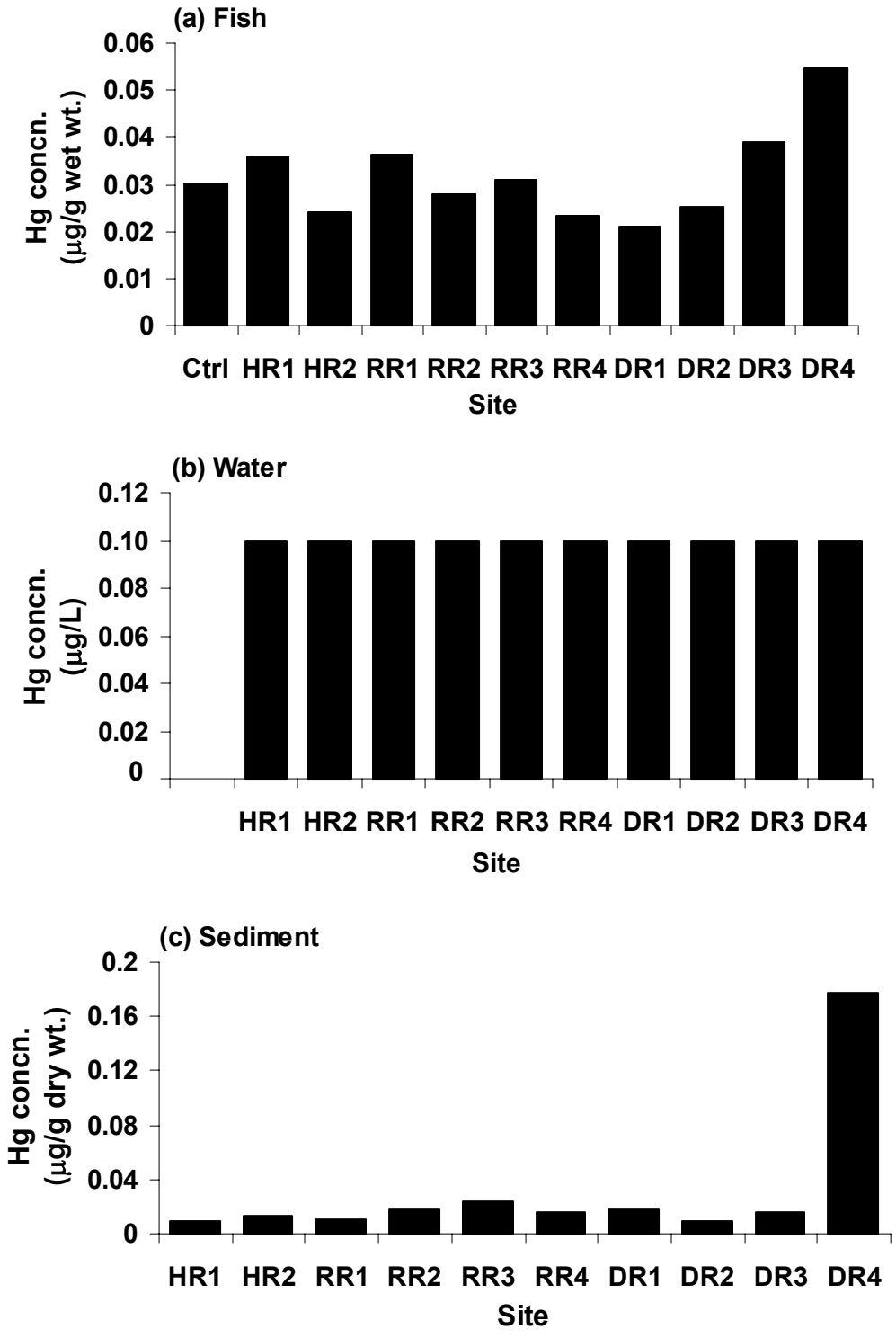

Figure 5. Mean concentration of mercury (Hg) in (a) Cape Fear shiners, (b) water, and (c) sediment from sites in the Haw, Rocky, and Deep rivers of North Carolina. For fish samples, Ctrl = concentrations in fish on day 0 of the 28-d test.

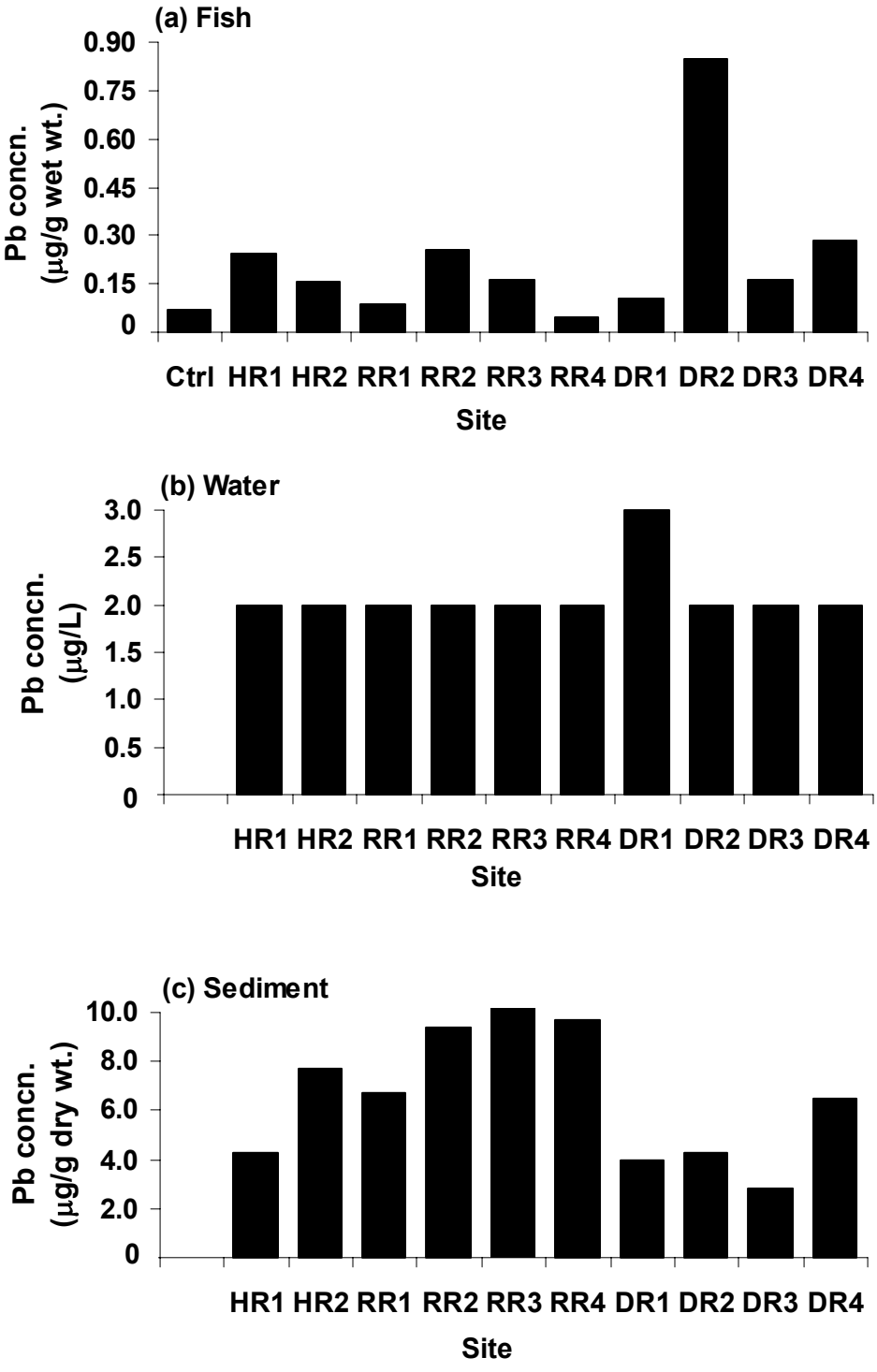

Figure 6. Mean concentration of lead (Pb) in (a) Cape Fear shiners, (b) water, and (c) sediment from sites in the Haw, Rocky, and Deep rivers of North Carolina. For fish samples, Ctrl = concentrations in fish on day 0 of the 28-d test.

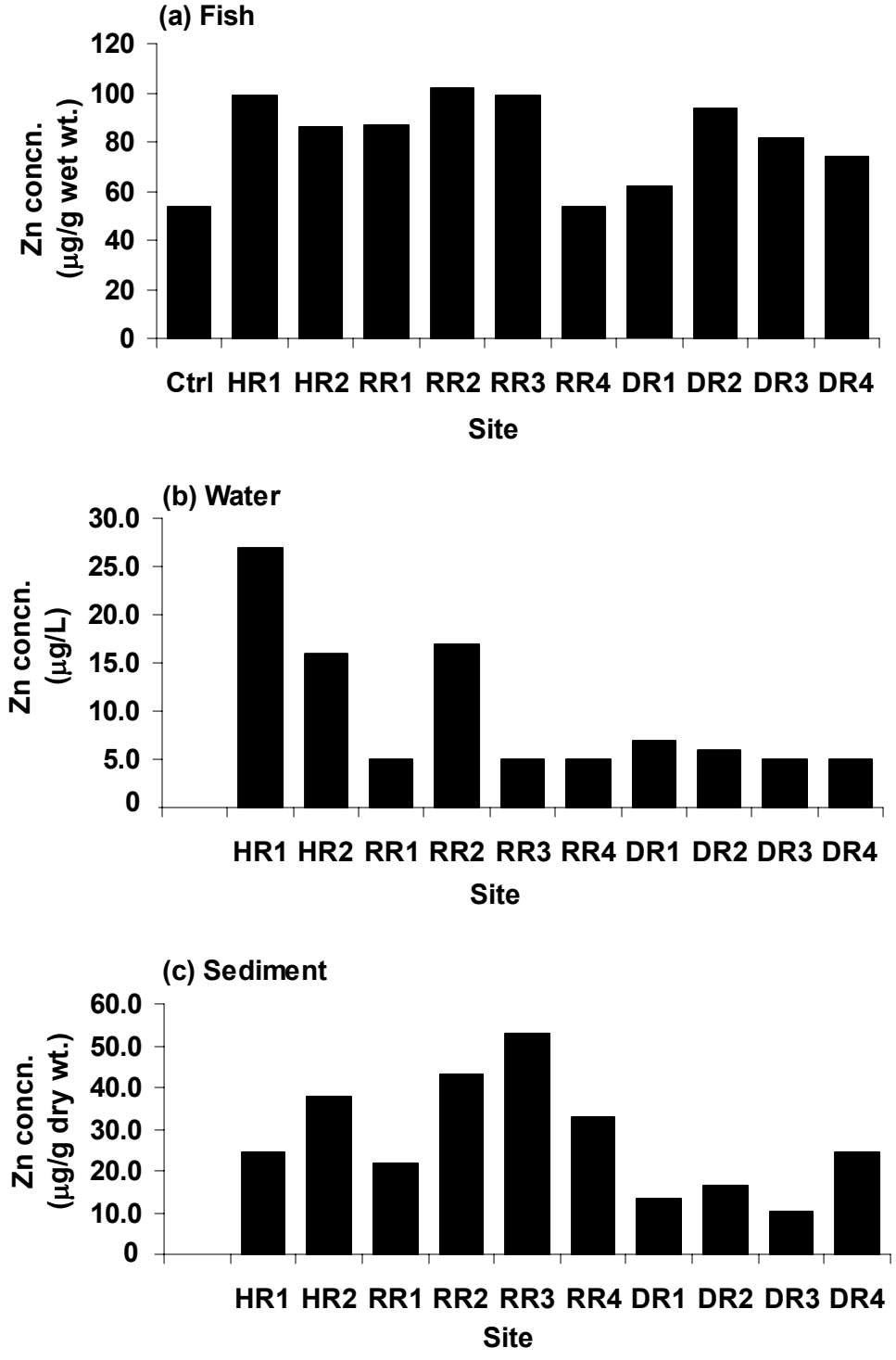

Figure 7. Mean concentration of zinc (Zn) in (a) Cape Fear shiners, (b) water, and (c) sediment from sites in the Haw, Rocky, and Deep rivers of North Carolina. For fish samples, Ctrl = concentrations in fish on day 0 of the 28-d test.

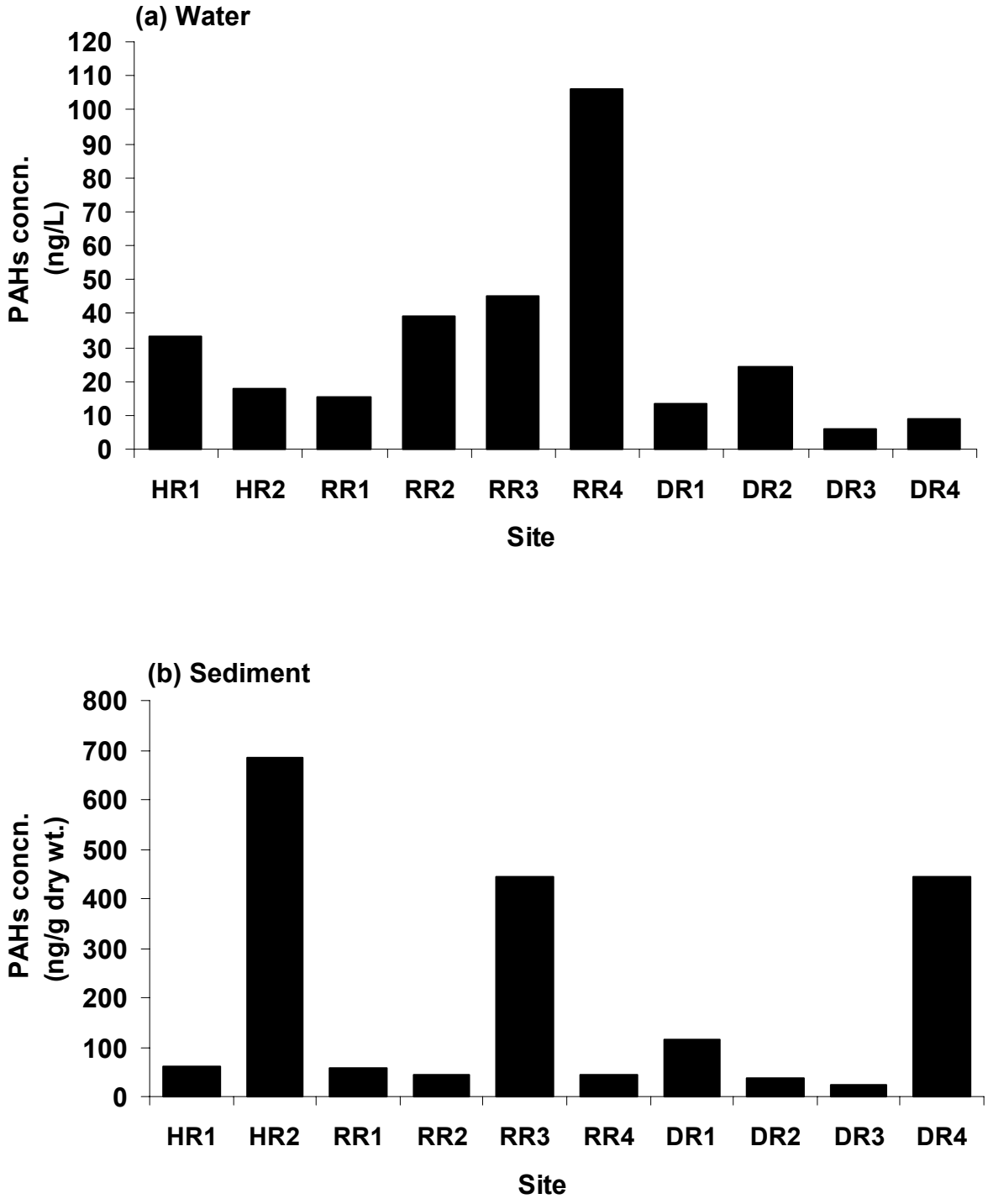

Figure 8. Mean concentration of polycyclic aromatic hydrocarbons (PAHs) in (a) water and (b) sediment from sites in the Haw, Rocky, and Deep rivers of North Carolina.

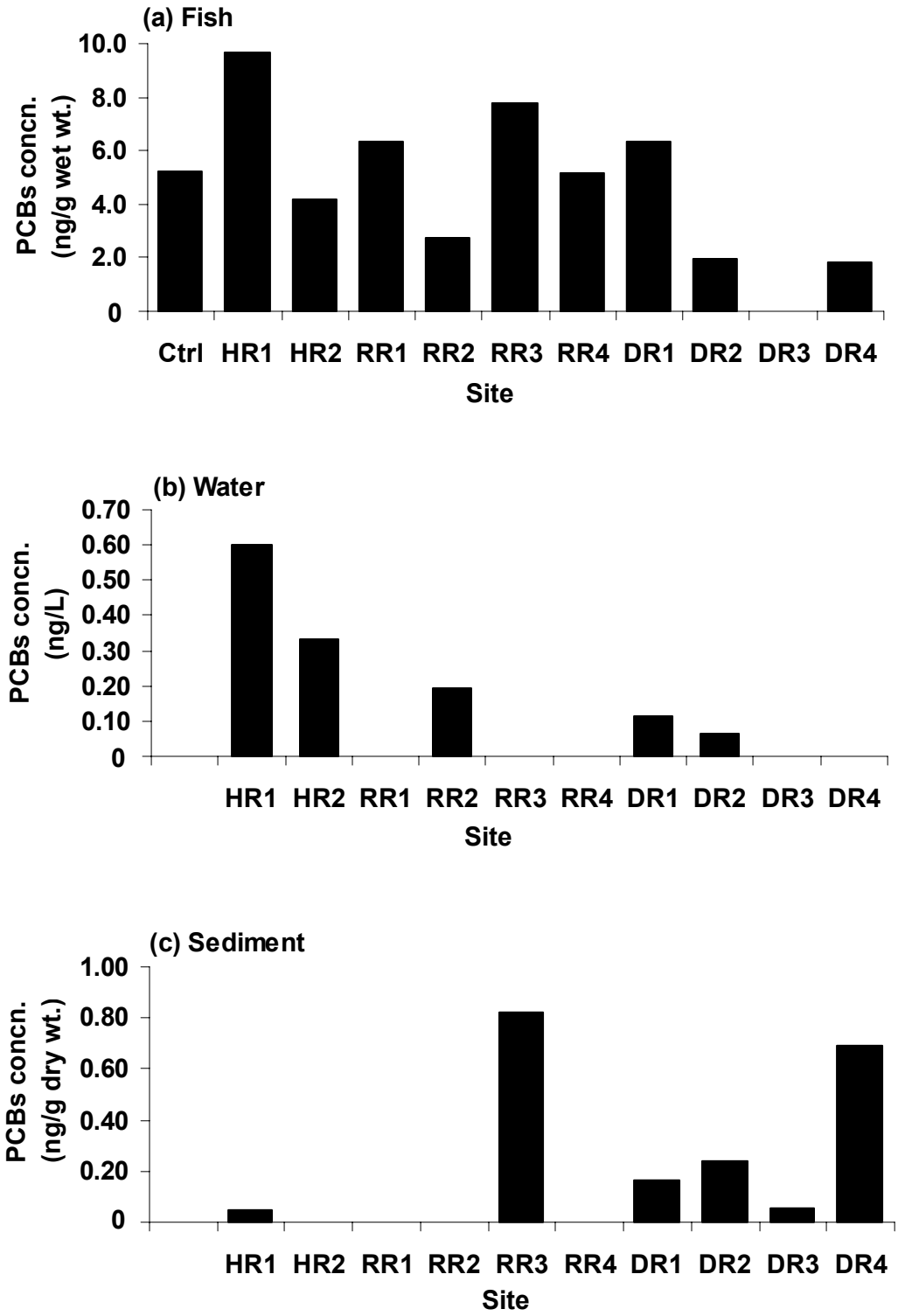

Figure 9. Mean concentration of polychlorinated biphenyls (PCBs) in (a) Cape Fear shiners, (b) water, and (c) sediment from sites in the Haw, Rocky, and Deep rivers of North Carolina. For fish samples, Ctrl = concentrations in fish on day 0 of the 28-d test.

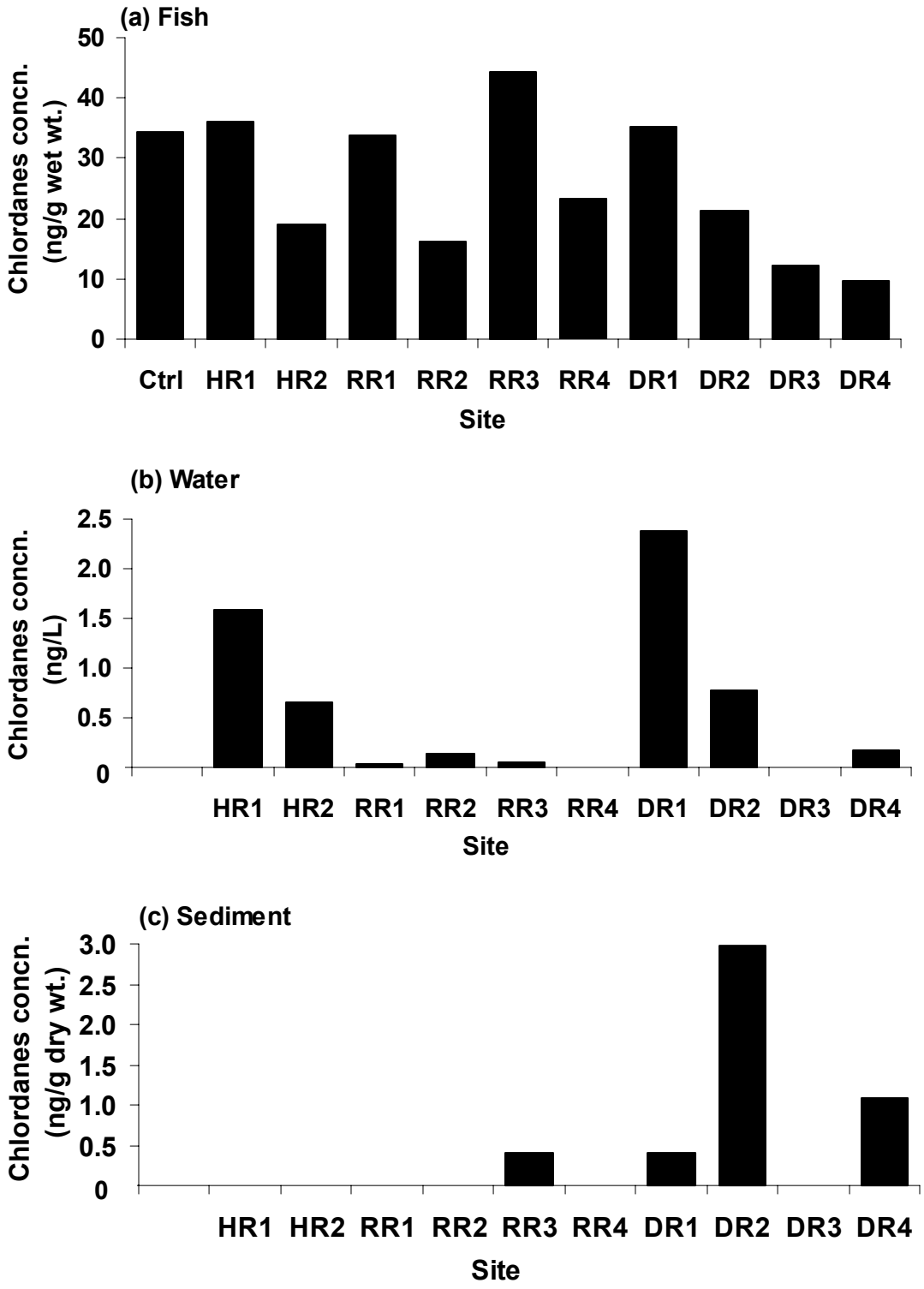

Figure 10. Mean concentration of chlordanes in (a) Cape Fear shiners, (b) water, and (c) sediment from sites in the Haw, Rocky, and Deep rivers of North Carolina. For fish samples, Ctrl = concentrations in fish on day 0 of the 28-d test.

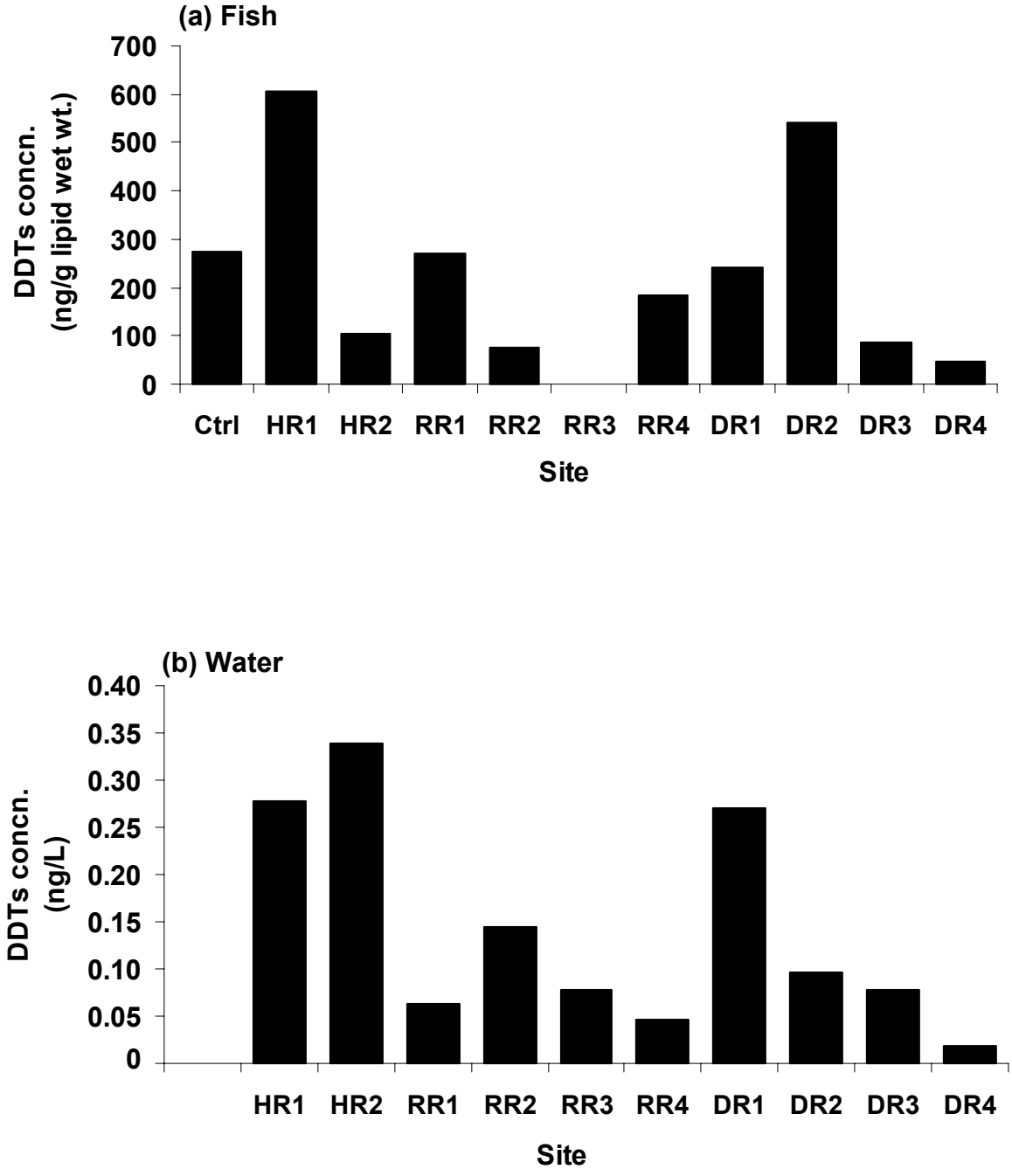

Figure 11. Mean concentration of DDTs in (a) Cape Fear shiners and (b) water (not detected in sediment) from sites in the Haw, Rocky, and Deep rivers of North Carolina. For fish samples, Ctrl = concentrations in fish on day 0 of the 28-d test.

APPENDICES

Appendix 1. Inorganic contaminant concentrations (:g/g wet wt.) in Cape Fear shiner tissue after the 28-d *in situ* bioassay; C = control samples, IRM = internal reference material, R = replicate samples, and DL = detection limit.

	Sample																	
Analyte	C1	C2	C3	IRM	HR1	HR2	RR1	RR2	RR3	RR4-R1	RR4-R2	RR4-R3	DR1-R1	DR1-R2	DR1-R3	DR2	DR3	DR4
Al	16.93	6.58	5.92	6.94	560.84	465.55	124.14	666.84	564.21	109.75	96.681	83.19	345.44	332.07	302.36	1319.08	413.54	685.75
Al (DL)	1.11	0.92	1.00	0.86	1.02	0.78	0.88	0.98	0.84	0.96	0.997	1.44	1.27	0.78	1.21	1.26	0.99	0.81
As	0.189	0.178	0.147	0.095	0.186	0.086	0.128	0.178	<DL	0.175	0.1815	<DL	<DL	0.089	0.192	0.307	0.206	0.178
As (DL)	0.111	0.092	0.100	0.086	0.102	0.078	0.088	0.098	0.084	0.096	0.0997	0.1441	0.127	0.078	0.121	0.126	0.099	0.081
B	<DL	<DL	<DL	<DL	0.2185	<DL	<DL	<DL	0.2296	<DL	<DL	<DL	0.2685	0.2605	<DL	0.3606	<DL	0.1672
B (DL)	0.2213	0.1836	0.2015	0.1720	0.2039	0.1567	0.1751	0.1967	0.1673	0.1921	0.2002	0.2874	0.2554	0.1567	0.2423	0.2520	0.1972	0.1625
Ba	3.2091	1.723	1.7229	1.7705	8.4309	7.2839	9.1773	6.4623	6.9050	5.7145	4.9962	5.0343	6.2540	4.6966	5.4650	30.5395	10.5391	9.2723
Ba (DL)	0.0221	0.018	0.0202	0.0172	0.0204	0.0156	0.0175	0.0197	0.0167	0.0192	0.0200	0.0287	0.0255	0.0156	0.0242	0.0252	0.0197	0.0163
Be	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.0105
Be (DL)	0.0111	0.0092	0.0100	0.0086	0.0102	0.0078	0.0088	0.0098	0.0084	0.0096	0.00997	0.0144	0.0127	0.0078	0.01211	0.0126	0.0099	0.0081
Ca	6042	4365	6082	6127	11872	9053	9803	10433	11022	8500	7857	8212	8595	8201	9071	13835	10818	8003
Ca (DL)	0.44	0.37	0.40	0.34	0.41	0.31	0.35	0.39	0.33	0.38	0.40	0.58	0.51	0.31	0.48	0.50	0.40	0.33
Cd	0.1036	0.0270	0.0305	0.0366	0.0306	0.0261	0.0149	0.0176	0.0261	0.0073	0.0060	0.0055	0.0110	0.0075	0.0070	0.0537	0.0158	0.0091
Cd (DL)	0.0044	0.0018	0.0020	0.0017	0.0020	0.0016	0.0018	0.0020	0.0017	0.0019	0.0020	0.0029	0.0026	0.0016	0.0024	0.0025	0.0020	0.0016
Cr	1.60455	2.30266	0.82969	0.48774	0.52625	0.3934	0.21419	0.36339	0.36083	<DL	0.1394	0.17316	0.21611	0.17154	0.27044	0.77453	0.25475	0.47987
Cr (DL)	0.1111	0.09182	0.1004	0.08609	0.10215	0.07834	0.08771	0.09797	0.08381	0.09614	0.09973	0.14407	0.12737	0.07834	0.12113	0.12565	0.09893	0.08111
Cu	1.359	0.904	0.881	1.427	1.149	1.087	0.746	1.469	1.155	0.825	0.723	0.784	0.945	0.96501	0.999	1.707	0.954	0.947
Cu (DL)	0.111	0.092	0.100	0.086	0.102	0.078	0.088	0.098	0.084	0.096	0.100	0.144	0.127	0.078	0.121	0.126	0.099	0.081
Fe	28.329	11.005	11.7646	11.8031	298.631	271.427	137.425	406.472	387.076	82.520	73.7982	69.98	183.363	165.666	156.627	855.473	233.814	383.895
Fe (DL)	0.221	0.184	0.202	0.17201	0.204	0.157	0.175	0.197	0.167	0.192	0.200	0.287	0.255	0.157	0.242	0.25204	0.197	0.163
Hg	0.035	0.023	0.032	0.030	0.036	0.024	0.036	0.028	0.031	0.026	0.023	0.020	0.019	0.023	0.020	0.025	0.039	0.055
Hg (DL)	0.002	0.002	0.00202	0.002	0.00204	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.003	0.002	0.002	0.003	0.002	0.002
Mg	314.270	211.901	283.221	279.455	429.738	376.219	337.700	404.598	362.474	375.466	348.968	348.113	353.629	332.066	368.093	456.252	399.578	356.032
Mg (DL)	0.221	0.184	0.202	0.17201	0.204	0.157	0.175	0.197	0.167	0.192	0.200	0.287	0.255	0.157	0.242	0.252	0.197	0.163
Mn	1.191	0.581	0.595	0.802	57.177	56.3469	182.921	62.1883	51.3368	39.610	33.7527	36.5965	73.8365	53.204	46.7628	610.789	86.8951	110.370
Mn (DL)	0.044	0.037	0.040	0.034	0.041	0.031	0.03502	0.039	0.033	0.038	0.040	0.058	0.051	0.031	0.048	0.050	0.040	0.033
Mo	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.180	<DL	0.258	<DL	0.183
Mo (DL)	0.221	0.184	0.202	0.172	0.204	0.157	0.175	0.204	0.167	0.192	0.200	0.287	0.255	0.157	0.242	0.252	0.197	0.163
Ni	0.189	0.134	0.115	0.132	0.260	0.239	0.107	0.324	0.225	0.112	<DL	0.187	0.174	0.140	0.122	0.557	0.258	0.299
Ni (DL)	0.111	0.092	0.100	0.086	0.102	0.078	0.088	0.09797	0.084	0.096	0.0997	0.144	0.127	0.078	0.121	0.126	0.099	0.081

Appendix 1. (continued) Inorganic contaminant concentrations (:g/g wet wt.) in Cape Fear shiner tissue after the 28-d *in situ* bioassay; C = control samples, IRM = internal reference material, R = replicate samples, and DL = detection limit.

	Sample																	
Analyte	C1	C2	C3	IRM	HR1	HR2	RR1	RR2	RR3	RR4-R1	RR4-R2	RR4-R3	DR1-R1	DR1-R2	DR1-R3	DR2	DR3	DR4
Pb	0.113	0.051	0.046	0.045	0.242	0.155	0.089	0.257	0.163	0.050	<DL	<DL	0.117	0.097	0.097	0.850	0.162	0.282
Pb (DL)	0.044	0.037	0.040	0.034	0.041	0.031	0.03502	0.039	0.033	0.038	0.0399	0.058	0.051	0.031	0.048	0.050	0.040	0.033
Se	0.635	0.44076	0.537	0.566	0.514	0.440	0.410	0.513	0.471	0.318	0.294	0.26599	0.380	0.358	0.336	0.410	0.422	0.361
Se (DL)	0.011	0.00918	0.010	0.009	0.010	0.008	0.009	0.010	0.008	0.010	0.010	0.014	0.013	0.008	0.012	0.013	0.010	0.008
Sr	31.648	23.0266	30.5008	31.2434	52.4426	38.4808	43.4633	39.5233	44.1201	29.0883	26.5063	27.6705	31.5974	28.987	31.363	62.7347	45.3669	32.8168
Sr (DL)	0.011	0.009	0.010	0.009	0.010	0.008	0.009	0.010	0.008	0.010	0.010	0.014	0.013	0.008	0.012	0.013	0.010	0.008
V	<DL	<DL	<DL	<DL	0.92503	0.728	0.23295	1.043	1.048	0.235	0.212	<DL	0.545	0.492	0.485	1.877	0.605	0.799
V (DL)	0.221	0.184	0.202	0.17201	0.204	0.157	0.175	0.197	0.167	0.192	0.200	0.287	0.255	0.157	0.242	0.25204	0.197	0.163
Zn	62.633	41.9565	56.2812	59.1889	98.694	86.2383	86.7702	101.524	99.0653	57.7641	53.0126	50.8781	65.9781	58.3409	61.4114	93.4581	81.137	73.9927
Zn (DL)	0.111	0.092	0.100	0.086	0.102	0.078	0.088	0.09797	0.084	0.096	0.100	0.144	0.127	0.078	0.121	0.126	0.099	0.081

Appendix 2. Concentrations of chlorinated pesticides and PCBs in Cape Fear shiner tissue (ng/g wet wt.) after the 28-d *in situ* bioassay.

	Sample															
Analyte	C-1	C-2	HR1	HR2	RR1	RR2	RR3	RR4-R1	RR4-R2	RR4-R3	DR1-R1	DR1-R2	DR1-R3	DR2	DR3	DR4
Cl2 (08)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Alpha-BHC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hexachlorobenzene	0.00	0.00	0.00	7.07	0.00	3.98	0.00	15.55	13.18	12.01	0.00	0.00	0.00	5.08	2.63	2.09
Beta-BHC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lindane (gamma-BHC)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl3 (18)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Delta-BHC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl3 (28)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Heptachlor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl4 (52)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aldrin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl4 (44)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chlorpyrifos	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Heptachlor Epoxide	25.75	23.56	36.15	19.13	33.70	14.32	44.45	22.23	18.15	29.68	53.72	19.96	27.16	18.30	10.93	8.64
Cl4 (66)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Trans-Chlordane	5.24	4.01	0.00	0.00	0.00	1.88	0.00	0.00	0.00	0.00	0.00	1.67	1.79	3.00	1.22	1.02
2,4'-DDE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl5 (101)	0.00	0.00	4.72	1.66	4.08	1.01	3.88	2.43	0.00	0.00	5.53	1.97	2.12	1.39	0.00	0.00
Endosulfan I	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cis-Chlordane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Trans-Nonachlor	4.65	4.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dieldrin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,4'-DDE	6.61	7.26	1.79	0.93	1.06	0.68	0.00	3.32	1.96	2.20	2.26	2.08	1.65	2.90	0.68	0.50
Cl4 (77)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2,4'-DDD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Endrin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Endosulfan II	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Appendix 2. (continued) Concentrations of chlorinated pesticides and PCBs in Cape Fear shiner tissue (ng/g wet wt.) after the 28-d *in situ* bioassay.

Analyte	C-1	C-2	HR1	HR2	RR1	RR2	RR3	RR4-R1	RR4-R2	RR4-R3	DR1-R1	DR1-R2	DR1-R3	DR2	DR3	DR4
Cl5 (118)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Endrin Aldehyde	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,4'-DDD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2,4'-DDT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl6 (153)	1.93	1.98	1.40	1.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.21	1.53	0.00	0.00	0.45
Cl5 (105)	3.90	2.56	3.54	1.48	2.25	1.72	3.88	5.12	3.03	4.30	0.00	2.65	3.43	0.59	0.00	0.72
Endosulfan Sulfate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,4'-DDT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl6 (138)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.66	0.00	0.00	0.00	0.00
Cl5 (126)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl7 (187)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl6 (128)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Endrin Ketone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Methoxychlor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl7 (180)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mirex	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl7 (170)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.75	0.00	0.00	0.00	0.00	0.00	0.00	0.68
Cl8 (195)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl9 (206)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl10 (209)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sum of PCBs	5.82	4.55	9.66	4.20	6.33	2.73	7.76	7.55	3.78	4.30	5.53	6.49	7.08	1.97	0.00	1.85
Sum of DDTs	6.61	7.26	1.79	0.93	1.06	0.68	0.00	3.32	1.96	2.20	2.26	2.08	1.65	2.90	0.68	0.50
Sum of Chlordanes	35.64	32.24	36.15	19.13	33.70	16.20	44.45	22.23	18.15	29.68	53.72	21.64	28.94	21.30	12.15	9.66

Appendix 3. Inorganic contaminant concentrations (mg/L) in water from all sites in the Cape Fear shiner 28-d *in situ* bioassay; D: = detection limit.

	Sample										
Analyte	DL	HR1	HR2	RR1	RR2	RR3	RR4	DR1	DR2	DR3	DR4
Al	0.05	0.224	0.288	<DL	0.194	<DL	0.09	1.17	0.488	0.07	0.144
As	0.005	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
B	0.01	0.129	0.1	<DL	0.06	0.03	0.01	0.07	0.106	0.129	0.109
Ba	0.001	0.031	0.028	0.026	0.031	0.028	0.019	0.023	0.025	0.023	0.024
Be	0.0005	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Ca	0.02	14.7	12.2	10.1	44.3	27.9	13.1	9.67	12.6	12.1	9.9
Cd	0.00005	0.00025	0.00011	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Cr	0.005	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Cu	0.005	<DL	<DL	<DL	0.007	<DL	<DL	<DL	<DL	<DL	<DL
Fe	0.01	0.379	0.477	2.27	0.226	0.07	0.17	1.34	0.695	0.188	0.325
Hg	0.0001	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Mg	0.1	5.01	4.43	3.65	5.43	5.07	3.49	2.74	3.83	4.46	4.1
Mn	0.002	0.062	0.071	0.522	0.104	0.013	0.035	0.095	0.057	0.0135	0.101
Mo	0.01	0.01	0.01	<DL	0.01	<DL	<DL	<DL	<DL	<DL	<DL
Ni	0.005	<DL	0.006	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Pb	0.002	<DL	<DL	<DL	<DL	<DL	<DL	0.003	<DL	<DL	<DL
Se	0.0001	0.00037	0.00025	<DL	0.00025	<DL	<DL	<DL	<DL	0.00017	0.0001
Sr	0.0005	0.114	0.0966	0.0799	0.104	0.0959	0.0665	0.0585	0.0818	0.0883	0.0753
V	0.01	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Zn	0.005	0.027	0.016	<DL	0.017	<DL	<DL	0.007	0.006	<DL	<DL

Appendix 4. Concentrations of PCBs, chlorinated pesticides, and PAHs in PSDs (ng/PSD) and estimates for water (ng/L) from PSDs deployed at all sites during the Cape Fear shiner 28-d *in situ* bioassay.

	Sample									
Analyte	HR1	HR2	RR1	RR2	RR3	RR4	DR1	DR2	DR3	DR4
PCBs (ng/PSD)										
Cl2 (08)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl3 (18)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl3 (28)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl4 (52)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl4 (44)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl4 (66)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl5 (101)	35.45	0.00	0.00	10.68	0.00	0.00	0.00	0.00	0.00	0.00
Cl4 (77)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl5 (118)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl6 (153)	0.00	10.93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl5 (105)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl6 (138)	8.75	11.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl5 (126)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl7 (187)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl6 (128)	0.00	0.00	0.00	5.65	0.00	0.00	0.00	0.00	0.00	0.00
Cl7 (180)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl7 (170)	0.00	0.00	0.00	0.00	0.00	0.00	8.70	5.53	0.00	0.00
Cl8 (195)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl9 (206)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl10 (209)	6.28	5.03	0.00	0.00	0.00	0.00	0.85	0.00	0.00	0.00
Chlorinated Pesticides (ng/PSD)										
Alpha-BHC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hexachlorobenzene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Beta-BHC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lindane (gamma-BHC)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	66.73	50.75	39.80

Appendix 4. (continued) Concentrations of PCBs, chlorinated pesticides, and PAHs in PSDs (ng/PSD) and estimates for water (ng/L) from PSDs deployed at all sites during the Cape Fear shiner 28-d *in situ* bioassay.

	Sample									
Analyte	HR1	HR2	RR1	RR2	RR3	RR4	DR1	DR2	DR3	DR4
Delta-BHC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Heptachlor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aldrin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chlorpyrifos	41.25	32.78	0.00	0.00	0.00	0.00	22.93	14.35	6.05	38.93
Heptachlor Epoxide	0.00	0.00	0.00	0.00	0.00	0.00	34.08	0.00	0.00	0.00
Trans-Chlordane	29.65	0.00	0.00	0.00	0.00	0.00	12.78	10.40	0.00	13.85
2,4'-DDE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Endosulfan I	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cis-Chlordane	29.78	55.13	0.00	11.23	4.88	0.00	13.23	9.98	0.00	0.00
Trans-Nonachlor	73.88	0.00	2.23	0.00	0.00	0.00	139.88	44.40	0.00	0.00
Dieldrin	98.90	80.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,4'-DDE	11.20	14.10	3.80	8.98	6.48	2.50	16.63	8.06	3.13	1.60
2,4'-DDD	6.60	8.68	0.00	0.00	0.00	0.00	3.35	0.00	0.00	0.00
Endrin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Endosulfan II	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.78	0.00	0.00
Endrin Aldehyde	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,4'-DDD	5.58	5.68	0.00	0.00	0.00	0.00	2.68	0.00	0.00	0.00
2,4'-DDT	0.00	0.00	1.48	3.23	0.00	1.35	0.00	0.00	3.40	0.00
Endosulfan Sulfate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,4'-DDT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Endrin Ketone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Methoxychlor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl7 (180)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mirex	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Estimate for Water (ng/L)										
Sum of PCBs	0.60	0.33	0.00	0.19	0.00	0.00	0.11	0.07	0.00	0.00
Sum of DDTs	0.28	0.34	0.06	0.15	0.08	0.05	0.27	0.10	0.08	0.02
Sum of Chlordanes	1.59	0.66	0.03	0.13	0.06	0.00	2.38	0.77	0.00	0.16
Dieldrin	1.18	0.95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Appendix 4. (continued) Concentrations of PCBs, chlorinated pesticides, and PAHs in PSDs (ng/PSD) and estimates for water (ng/L) from PSDs deployed at all sites during the Cape Fear shiner 28-d *in situ* bioassay.

	Sample									
Analyte	HR1	HR2	RR1	RR2	RR3	RR4	DR1	DR2	DR3	DR4
Lindane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.79	0.60	0.47
Chlorpyrifos	0.49	0.39	0.00	0.00	0.00	0.00	0.27	0.17	0.07	0.46
PAHs										
Napthalene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-Methylnapthalene	0.00	9.75	3.55	5.85	3.75	4.40	0.00	4.38	0.00	10.75
1-Methylnapthalene	0.00	8.20	4.85	6.30	3.55	5.53	0.00	0.00	0.00	13.83
Biphenyl	5.38	4.93	2.78	4.63	3.35	4.38	4.13	5.78	4.83	6.48
2,6-Dimethylnapthylene	0.00	40.58	2.35	3.83	2.18	2.40	0.00	0.00	0.00	0.00
Acenapthylene	0.00	5.95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.30
Acenapthene	14.05	0.00	16.63	14.65	2.28	8.53	17.45	7.03	10.33	35.20
Dibenzofuran	6.18	5.63	3.08	6.30	0.00	3.20	8.98	5.35	2.93	13.30
2,3,5-Trimethylnapthalene	0.00	78.48	11.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1 - Napthalenes	12.03	22.80	10.78	17.38	11.15	15.13	13.88	14.45	16.70	27.43
C2 - Napthalenes	35.90	164.50	31.43	38.68	22.65	31.15	44.70	32.70	47.88	55.73
C3 - Napthalenes	75.43	436.40	80.80	57.43	49.80	61.38	78.30	47.70	98.28	99.78
C4 - Napthalenes	129.15	765.95	62.20	77.83	49.13	64.40	110.25	54.40	130.98	139.38
Fluorene	15.40	17.63	7.78	13.25	0.00	4.20	20.28	12.60	11.68	34.13
1-Methylfluorene	13.03	37.70	6.88	4.83	0.00	3.55	14.18	6.10	9.13	9.90
C1 - Fluorenes	34.18	128.25	27.43	24.10	0.00	21.83	49.35	33.20	26.00	37.65
C2 - Fluorenes	173.68	666.25	52.33	82.93	0.00	53.15	121.68	75.53	98.05	167.28
C3 - Fluorenes	230.55	788.33	0.00	0.00	0.00	0.00	184.25	41.88	30.38	144.80
Dibenzothiophene	13.88	17.33	16.20	8.95	0.00	0.00	14.48	9.08	0.00	10.18
C1 - Dibenzothiophenes	45.75	84.48	20.70	26.18	1.33	0.00	33.28	15.33	0.00	0.00
C2 - Dibenzothiophene	78.13	240.88	26.08	38.53	0.00	0.00	41.00	32.83	19.18	36.03
C3 - Dibenzothiophene	62.75	192.30	17.13	32.58	0.00	0.00	33.85	26.33	0.00	28.88
Phenanthrene	104.90	117.98	131.08	83.50	32.80	66.28	147.68	109.98	40.00	142.83
Anthracene	18.38	20.58	0.00	12.68	3.75	3.28	22.00	16.08	5.88	34.28
1-Methylphenanthrene	31.33	90.38	12.30	16.38	5.15	7.98	32.23	22.18	16.10	28.53
C1 - Phenanthrenes/Anthracenes	211.30	762.88	82.85	116.18	40.60	58.83	195.55	116.15	98.60	210.15

Appendix 4. (continued) Concentrations of PCBs, chlorinated pesticides, and PAHs in PSDs (ng/PSD) and estimates for water (ng/L) from PSDs deployed at all sites during the Cape Fear shiner 28-d *in situ* bioassay.

	Sample									
Analyte	HR1	HR2	RR1	RR2	RR3	RR4	DR1	DR2	DR3	DR4
C2 - Phenanthrenes/Anthracenes	164.35	733.73	47.95	96.15	43.23	35.53	110.90	62.38	59.03	101.55
C3 - Phenanthrenes/Anthracenes	96.00	405.68	22.45	87.95	0.00	17.45	58.45	33.98	30.75	56.73
C4 - Phenanthrenes/Anthracenes	0.00	290.20	0.00	0.00	0.00	0.00	76.45	0.00	0.00	0.00
Fluoranthrene	664.15	646.85	190.38	390.95	84.05	109.83	510.93	270.13	166.55	741.68
Pyrene	742.43	792.58	97.45	286.58	46.28	53.40	394.78	164.80	123.78	518.78
C1 - Fluoranthenes/Pyrenes	205.85	361.43	21.73	87.35	14.00	16.03	104.75	51.58	37.90	156.30
Retene	51.95	106.95	34.93	40.60	32.88	37.18	83.00	68.88	107.33	91.23
Benz[a]anthracene	42.03	52.78	6.98	23.48	2.58	5.15	21.55	11.13	8.13	44.50
Chrysene	211.65	319.53	28.38	116.28	19.30	15.73	100.70	61.38	25.15	113.83
C1 - Chrysenes	30.83	69.45	7.15	28.25	6.90	5.28	27.45	16.13	6.15	25.23
C2 - Chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3 - Chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C4 - Chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Benzo[b]fluoranthene	64.63	111.90	11.38	54.35	8.23	0.00	32.08	23.03	9.65	38.33
Benzo[k]fluoranthene	31.58	42.83	5.20	23.25	1.60	0.00	11.03	7.10	2.88	16.40
Benzo[e]pyrene	81.58	124.28	11.15	46.13	6.33	3.78	34.10	19.40	7.55	34.33
Benzo[a]pyrene	15.50	19.38	0.00	8.63	0.00	0.00	0.00	0.00	0.00	6.43
Perylene	40.10	62.33	16.60	42.40	14.30	19.98	45.53	38.83	35.60	36.50
Indeno[1,2,3-c,d]perylene	14.20	20.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dibenz[a,h]anthracene	4.03	5.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[g,h,i]perylene	22.05	33.18	0.00	9.55	0.00	0.00	7.80	0.00	0.00	0.00
Coronene	4.08	5.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Estimate for Water (ng/L)										
Sum of PAHs	45.22	106.10	13.49	24.22	6.08	8.80	33.42	18.07	15.33	38.97

Appendix 5. Inorganic contaminant concentrations (:g/g dry wt.) in sediment from all sites in the Cape Fear shiner 28-d *in situ* bioassay; DL = detection limit.

	Sample																			
Analyte	HR1	DL	HR2	DL	RR1	DL	RR2	DL	RR3	DL	RR4	DL	DR1	DL	DR2	DL	DR3	DL	DR4	DL
Al	2085	0.921	3685	0.931	3071	1	4939	0.971	8620	1.09	5105	0.987	1985	0.988	1778	1.01	1397	0.95	3839	0.989
As	0.533	0.474	1.32	0.479	1.54	0.515	1.22	0.5	4.4	0.544	2.71	0.508	1.1	0.509	1.37	0.519	0.992	0.49	1.33	0.51
Ba	27.7	0.0186	50.5	0.0188	40.1	0.0202	43.1	0.0196	60.4	0.11	39.4	0.0199	21.2	0.02	21.6	0.0204	15.5	0.0192	49.9	0.02
Be	0.153	0.0186	0.338	0.0188	0.312	0.0202	0.501	0.0196	0.517	0.0544	0.447	0.0199	0.171	0.02	0.145	0.0204	0.0911	0.0192	0.235	0.02
Cd	0.158	0.0372	0.276	0.0376	0.224	0.0404	0.33	0.0392	0.0403	0.00544	0.383	0.0399	0.127	0.0399	0.144	0.0407	0.0929	0.0384	0.207	0.04
Cr	22.6	0.093	41.2	0.094	19.1	0.101	28.7	0.0981	49.1	0.218	60.1	0.0997	8.22	0.0998	7.92	0.102	3.47	0.096	8.46	0.0999
Cu	4.11	0.0186	6.38	0.0188	4.88	0.0202	8.7	0.0196	16.5	0.218	6.29	0.0199	3.96	0.02	3.55	0.0204	1.96	0.0192	9.3	0.02
Fe	7207	0.372	16864	0.376	14478	0.404	24954	0.392	40200	0.54	29028	0.399	7274	0.399	8043	0.407	4391	0.384	8718	0.4
Hg	<DL	0.0091	<DL	0.0135	<DL	0.0109	0.0189	0.013	0.0238	0.0109	0.0157	0.0118	0.0186	0.012	<DL	0.0095	0.0158	0.0119	0.178	0.0107
Mg	606	2.06	1121	2.08	645	2.23	824	2.17	1510	1.1	952	2.2	358	2.2	338	2.25	316	2.12	690	2.21
Mn	384	0.0186	776	0.0188	902	0.0202	883	0.0196	738	0.109	730	0.0199	344	0.02	388	0.0204	160	0.0192	545	0.02
Mo	0.178	0.0744	0.195	0.0752	<DL	0.0808	0.354	0.0785	<DL	1.09	<DL	0.0798	0.274	0.0798	0.333	0.0814	0.227	0.0768	0.52	0.0799
Ni	2.35	0.0465	2.4	0.047	1.41	0.0505	2.62	0.0491	4.63	0.544	2.98	0.0499	1.29	0.0499	2.04	0.0509	1.84	0.048	3.37	0.05
Pb	4.27	0.158	7.7	0.16	6.71	0.172	9.37	0.167	11.5	0.871	9.66	0.17	3.94	0.17	4.28	0.173	2.81	0.163	6.47	0.17
Se	<DL	0.484	<DL	0.489	<DL	0.525	0.783	0.51	0.306	0.0109	0.872	0.518	<DL	0.519	<DL	0.529	<DL	0.499	<DL	0.52
Sr	4.33	0.0186	6.72	0.0188	4.06	0.0202	4.49	0.0196	14.2	0.02	6.84	0.0199	5.29	0.02	4.47	0.0204	2.36	0.0192	7.81	0.02
V	18.6	0.0372	46.9	0.0376	35	0.0404	61	0.0392	95.1	0.544	68.7	0.0399	19	0.0399	14.5	0.0407	7.15	0.0384	17.3	0.04
Zn	24.3	0.0837	37.6	0.0846	21.9	0.0909	43	0.0883	52.7	0.218	33	0.0897	13.3	0.0898	16.4	0.0916	10.3	0.0864	24.6	0.0899

Appendix 6. Concentrations of PCBs, chlorinated pesticides, and PAHs in sediment (ng/g dry wt.) collected from all sites in the Cape Fear shiner 28-d *in situ* bioassay; bdl = below detection limit.

	Sample									
Analyte	HR1	HR2	RR1	RR2	RR3	RR4	DR1	DR2	DR3	DR4
PCBs										
Cl2 (08)	0.00	0.00	0.00	0.00	0.82	0.00	0.00	0.00	0.00	0.00
Cl3 (18)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl3 (28)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl4 (52)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl4 (44)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl4 (66)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl5 (101)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00
Cl4 (77)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl5 (118)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl6 (153)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl5 (105)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl6 (138)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl5 (126)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl7 (187)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl6 (128)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl7 (180)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl7 (170)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.70
Cl8 (195)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl9 (206)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cl10 (209)	0.05	0.00	0.00	0.00	0.00	0.00	0.17	0.08	0.00	0.00

Appendix 6. (continued) Concentrations of PCBs, chlorinated pesticides, and PAHs in sediment (ng/g dry wt.) collected from all sites in the Cape Fear shiner 28-d *in situ* bioassay; bdl = below detection limit.

	Sample									
Analyte	HR1	HR2	RR1	RR2	RR3	RR4	DR1	DR2	DR3	DR4
Chlorinated Pesticides										
Alpha-BHC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hexachlorobenzene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Beta-BHC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lindane (gamma-BHC)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Delta-BHC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Heptachlor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aldrin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chlorpyrifos	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Heptachlor Epoxide	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**2.85**	0.00	0.00
Trans-Chlordane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2,4'-DDE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Endosulfan I	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.20**	0.00
Cis-Chlordane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Trans-Nonachlor	0.00	0.00	0.00	0.00	**0.40**	0.00	**0.40**	**0.14**	0.00	**1.08**
Dieldrin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,4'-DDE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2,4'-DDD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Endrin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Endosulfan II	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Endrin Aldehyde	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,4'-DDD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2,4'-DDT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Endosulfan Sulfate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,4'-DDT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Endrin Ketone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Methoxychlor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mirex	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Appendix 6. (continued) Concentrations of PCBs, chlorinated pesticides, and PAHs in sediment (ng/g dry wt.) collected from all sites in the Cape Fear shiner 28-d *in situ* bioassay; bdl = below detection limit.

	Sample									
Analyte	HR1	HR2	RR1	RR2	RR3	RR4	DR1	DR2	DR3	DR4
PAHs										
naphthalene	0.97	1.66	1.66	0.98	1.74	1.12	0.93	0.94	1.27	5.78
2-methylnaphthalene	0.28	0.48	0.48	0.20	0.34	0.38	0.22	0.26	0.30	1.59
1-methylnaphthalene	0.17	0.33	0.33	0.14	0.21	0.30	0.15	0.16	0.20	0.96
biphenyl	bdl	bdl	bdl	0.33	0.72	2.35	bdl	0.27	0.94	8.06
2,6-dimethylnaphthalene	bdl	0.28	0.28	bdl	0.13	0.29	0.13	bdl	bdl	bdl
acenaphthylene	0.43	1.38	1.38	1.24	0.90	2.82	bdl	0.82	bdl	2.89
acenaphthene	0.06	1.25	1.25	bdl	0.08	0.64	bdl	0.03	bdl	0.72
dibenzofuran	0.08	0.31	0.31	bdl	0.11	0.46	bdl	0.05	0.11	1.09
2,3,5-trimethylnaphthalene	0.13	0.36	0.36	0.15	0.18	0.30	0.19	0.15	0.22	0.52
C1-naphthalenes	0.51	0.86	0.86	0.39	0.60	0.70	0.39	0.47	0.59	2.68
C2-naphthalenes	0.54	1.17	1.17	0.41	1.03	1.85	0.78	0.65	0.61	4.21
C3-naphthalenes	bdl	1.47	1.47	bdl	bdl	bdl	bdl	bdl	bdl	6.57
C4-naphthalenes	bdl	0.93	0.93	bdl	bdl	bdl	bdl	bdl	bdl	3.69
fluorene	bdl	1.58	1.58	bdl	0.15	1.12	0.10	bdl	bdl	1.87
1-methylfluorene	0.12	0.38	0.38	bdl	0.29	0.34	bdl	0.16	0.38	2.95
C1-fluorenes	0.27	1.77	1.77	bdl	0.58	bdl	bdl	0.26	0.68	4.90
C2-fluorenes	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
C3-fluorenes	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
dibenzothiophene	0.09	1.71	1.71	bdl	0.12	1.90	bdl	bdl	bdl	bdl
C1-dibenzothiophenes	bdl	0.20	0.20	bdl	bdl	0.90	bdl	bdl	bdl	bdl
C2-dibenzothiophenes	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
C3-dibenzothiophenes	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
phenanthrene	0.96	34.40	34.40	1.92	1.29	35.45	1.12	0.90	1.88	16.11
anthracene	0.53	12.61	12.61	0.86	0.75	3.18	0.26	0.91	0.28	7.58
1-methylphenanthrene	0.42	2.97	2.97	0.41	0.14	3.56	bdl	0.25	bdl	1.42
C1-phenanthrenes/anthracenes	1.65	21.57	21.57	2.22	0.98	bdl	bdl	1.60	bdl	14.49
C2-phenanthrenes/anthracenes	1.67	6.24	6.24	0.92	bdl	bdl	bdl	1.26	bdl	10.60
C3-phenanthrenes/anthracenes	2.10	1.92	1.92	bdl	bdl	bdl	bdl	0.84	bdl	bdl
C4-phenanthrenes/anthracenes	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl

Appendix 6. (continued) Concentrations of PCBs, chlorinated pesticides, and PAHs in sediment (ng/g dry wt.) collected from all sites in the Cape Fear shiner 28-d *in situ* bioassay; bdl = below detection limit.

	Sample									
Analyte	HR1	HR2	RR1	RR2	RR3	RR4	DR1	DR2	DR3	DR4
fluoranthene	3.78	115.72	115.72	6.11	2.60	75.30	1.14	2.24	1.20	41.20
pyrene	3.68	89.29	89.29	5.03	2.17	53.89	0.92	2.61	1.06	34.08
C1-fluoranthenes/pyrenes	2.36	40.50	40.50	3.56	1.26	22.75	bdl	2.61	0.84	16.22
retene	17.80	0.67	0.67	0.31	0.68	7.21	15.53	0.54	2.93	41.58
benz[a]anthracene	1.95	53.69	53.69	3.66	1.29	18.31	0.80	1.75	0.38	13.82
chrysene	2.24	44.16	44.16	3.70	1.68	30.13	0.68	2.17	1.17	20.47
C1-chrysenes	1.16	12.13	12.13	1.76	bdl	13.49	0.35	1.89	0.91	10.82
C2-chrysenes	1.48	bdl	bdl	bdl	bdl	bdl	bdl	1.59	bdl	6.64
C3-chrysenes	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
C4-chrysenes	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
benzo[b]fluoranthene	2.30	42.94	42.94	3.84	2.47	33.32	0.92	1.95	0.93	27.91
benzo[k]fluoranthene	2.44	38.50	38.50	3.71	1.41	23.25	0.42	1.69	0.45	17.67
benzo[e]pyrene	1.95	27.73	27.73	3.17	2.07	19.90	0.52	1.76	0.88	17.95
benzo[a]pyrene	1.95	41.41	41.41	3.46	1.60	21.56	0.41	1.86	0.34	15.95
perylene	3.16	14.55	14.55	2.05	10.55	19.88	19.06	1.80	2.49	31.91
indeno[1,2,3-c,d]pyrene	2.03	29.38	29.38	3.07	2.57	19.81	bdl	1.81	0.40	18.02
dibenz[a,h]anthracene	0.35	6.74	6.74	0.63	0.49	6.15	bdl	0.31	0.09	2.79
benzo[g,h,i]perylene	1.71	26.12	26.12	2.93	2.67	18.61	bdl	1.80	0.64	19.06
coronene	0.37	3.93	3.93	0.62	0.71	2.71	bdl	0.42	0.18	8.09