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INFLUENCE OF WATER QUALITY AND ASSOCIATED CONTAMINANTS ON SURVIVAL AND GROWTH OF THE ENDANGERED CAPE FEAR SHINER (*NOTROPIS MEKISTOCHOLAS*)

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Abstract—The Cape Fear shiner (*Notropis mekistocholas*) is a recently described cyprinid species endemic to the Cape Fear River Basin of North Carolina, USA. Only five populations of the fish remain; thus, it is listed as endangered by the U.S. Government. Determining habitat requirements of the Cape Fear shiner, including water quality and physical habitat, is critical to the survival and future restoration of the species. To assess water quality in the best remaining and in the historical habitats, we conducted a 28-d in situ bioassay with captively propagated Cape Fear shiners. Fish were deployed at 10 sites in three rivers, with three cages per site and 20 fish per cage. Water and sediment samples were collected and analyzed for selected metals and organic contaminants. Passive sampling devices also were deployed at each site and analyzed for organic contaminants at test termination. Fish survival, growth (as measured by an increase in total length), and contaminant accumulation were measured on completion of the bioassay. Survival of caged fish averaged 76% (range, 53–100%) and varied significantly among sites and rivers. Caged fish accumulated quantities of cadmium, mercury, polychlorinated biphenyls, and other persistent contaminants over the test duration and grew significantly at only four sites. No apparent relations were observed between exposure to or accumulation of a specific contaminant and reduced growth or survival of fish among all the sites. However, a generalized hazard assessment showed that certain sites exhibited trends in cumulative contaminant presence with reduced fish survival and growth, thereby enabling the identification of the existing riverine habitat most suitable for reintroduction or population augmentation of this endangered fish.

Keywords—Biomonitoring In situ toxicity Endangered species Water quality Cape Fear shiner

INTRODUCTION

Populations of many native fish in the southern United States have declined during recent years, and the threat of imperilment and extinction has increased substantially within the last two decades [1–3]. The Cape Fear shiner (*Notropis mekistocholas*) is among this group of declining species, and the small cyprinid was added to the U.S. List of Endangered and Threatened Wildlife and Plants in 1987 [4]. The Cape Fear shiner, which lives for two to three years in the wild, was first described by Snelson [5] and is currently known from only five remaining populations in the Cape Fear River Basin of North Carolina, USA [6–10]. Like most other declining southern fish species, the decline of the Cape Fear shiner 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 nonpoint-source pollutants [11].

Most studies that aim to identify factors limiting the distribution and density of stream fish, particularly threatened and endangered fish, have focused on instream physical habitat as the primary target [12,13]. Segmentation and alteration of the Cape Fear shiner's lotic habitat by several dams is a priority cause of their current status, but impacts of degraded water quality have not been assessed. 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 [14].

Pottern and Huish [6] cited poor water quality in the upper reaches of the Cape Fear River 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 ([15–17]; http://nc-es.fws.gov/fish/RecPlan_Cape_Fear_Shiner.pdf). Although numeric water-quality standards are designed to protect the majority of aquatic organisms, they are developed from toxicity information derived from a subset of the freshwater fauna, such as the fathead minnow (*Pimephales promelas*) and the rainbow trout (*Oncorhynchus mykiss*). These common test species may not serve as adequate surrogates for evaluation of specific taxa.

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 [18]. Those results indicated that the Cape Fear shiner was among the more sensitive (in the top 9) of the 16 threatened and endangered fish species tested, and it was more

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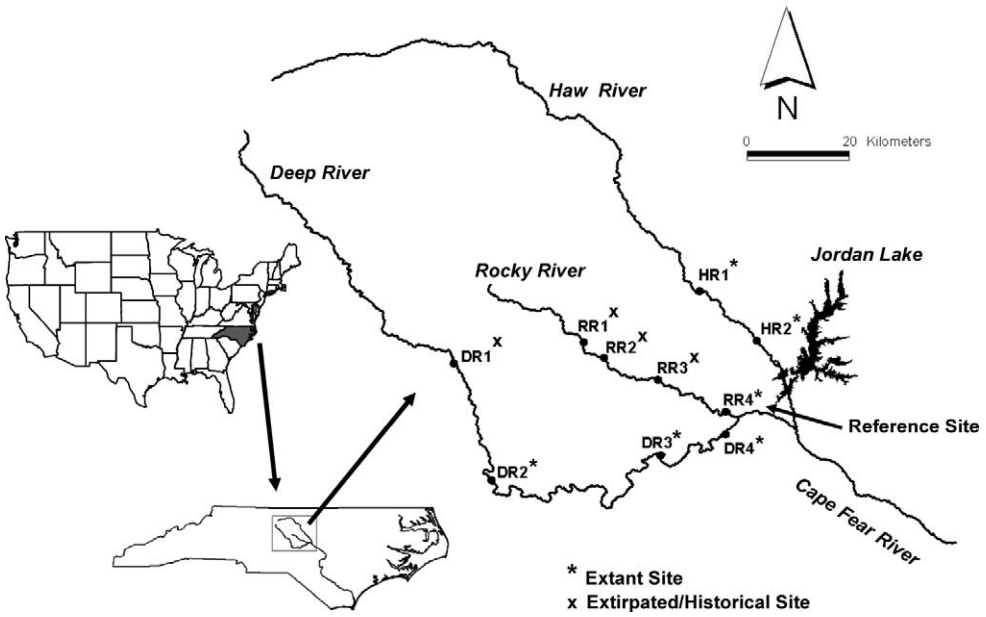

Fig. 1. Study sites tested in the Cape Fear shiner 28-d in situ bioassay in the Haw River (HR), Rocky River (RR), and Deep River (DR) of North Carolina, USA.

similar to rainbow trout than to the fathead minnow, another cyprinid species, in terms of its sensitivity to chemicals. Whereas the single chemical laboratory exposures have lacked the realism of the natural ecosystem in which fish are exposed to mixtures of chemical contaminants and other environmental stressors, they point out the need to test the hypothesis that water quality may be a limiting factor in the ultimate restoration and sustainability of the species.

The in situ bioassay approach, which integrates conditions of the natural system with a degree of experimental control, has been used successfully to evaluate the effects of water quality on locally important fish species [19–23]. This approach provides the environmental realism currently lacking in laboratory tests, and it combines the disciplines of toxicology and ecology [24], both of which are necessary for understanding and managing ecosystem health and diversity. Therefore, the purpose of our research was to evaluate the influences of water quality and associated contaminants 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 for potential reintroduction or population augmentation [15]. Specific objectives were to determine if water quality is a limiting factor in the occurrence, growth, and survival of the Cape Fear shiner; to document habitat suitability by assessing inorganic and organic contaminants through chemical analyses; and to review existing data and assess the protectiveness of water-quality standards for primary pollutants based on comparisons of laboratory, field toxicity, and water chemistry data. The availability and suitability of instream physical habitat and the existing population density of Cape Fear shiners at the same sites studied here have been reported in a companion study [25], which found that suitable microhabitat combinations of water depth, velocity, and substrate composition (i.e., streambottom materials) were critical physical habitat characteristics for the Cape Fear shiner.

MATERIALS AND METHODS

Study area

The Cape Fear River Basin begins in the northcentral Piedmont region of North Carolina, USA, near the cities of Greensboro and High Point, and it flows southeasterly to the Atlantic Ocean (Fig. 1). It is one of only four river basins located entirely within the state and is the largest basin in the state, covering 14,756 km^2 and having 10,006 km of freshwater streams and rivers. The basin encompasses approximately 25% of the state's population, including 114 municipalities and all or portions of 27 counties. Land use in the Cape Fear River Basin is composed of 26% agriculture, 59% forest, 6% urban, and 9% other [11]. The extant populations of the Cape Fear shiner are found in three tributaries to the Cape Fear River: The Haw, Rocky, and Deep rivers in Randolph, Moore, and Lee and Chatham counties of North Carolina, respectively [10,15]. Ten sites were selected for the present study: Two on the Haw River, four on the Rocky River, and four on the Deep River (Fig. 1). Of the 10 sites studied, six were in the extant range of the Cape Fear shiner, and four were in the historical range or were considered to be potential sites for reintroduction of the species. One of the sites in the extant range (RR4, Rocky River at US Highway 15–501) was deemed to be the best available habitat for, and the best remaining population of, the Cape Fear shiner based on existing information at the time the present study was initiated [6–10], and it served as a reference site for the test (Table 1). Additional information regarding the study area and sites has been provided by Kwak et al. [25].

Test design and fish deployment

Approximately 900 captively reared Cape Fear shiners of a relatively uniform size (total length, 15–30 mm) and age (four to six months) were obtained from Conservation Fisheries (Knoxville, TN, USA) on July 24, 2001. Fish were cultured in reverse osmosis–filtered (passed through mechanical micron and carbon prefilters) water combined with dechlorinated tap water that was buffered with Seachem Neutral Reg-

Table 1. List of rivers, sites, site identification (ID) codes, fish population status, and latitude/longitude of the 10 sites studied in the 28-d bioassay with Cape Fear shiners in the Haw, Rocky, and Deep rivers of North Carolina, USA. SR = State Route

River	Site	Site ID	Population status	Latitude/Longitude
Haw River	SR 1545 crossing, Chatham County	HR1	Extant	35.8331°N 79.2193°W
Haw River	Downstream of Bynum Dam, Chatham County	HR2	Extant	35.7723°N 79.1442°W
Rocky River	U.S. Highway 64 crossing, Chatham County	RR1	Extirpated	35.7351°N 79.4229°W
Rocky River	SR 2170 crossing, Chatham County	RR2	Extirpated	35.6988°N 79.3760°W
Rocky River	NC Route 902 crossing, Chatham County	RR3	Extirpated	35.6989°N 79.3759°W
Rocky River	U.S. Highway 15–501 crossing, Chatham County	RR4	Extant (reference site)	35.6225°N 79.1882°W
Deep River	SR 2628 crossing, Randolph County	DR1	Extirpated	35.6727°N 79.6273°W
Deep River	SR 1456 crossing, Moore County	DR2	Extant	35.5009°N 79.5817°W
Deep River	SR 1007 crossing, Moore/Lee County line	DR3	Extant	35.5551°N 79.2874°W
Deep River	U.S. Highway 15–501 crossing, Moore/Lee County line	DR4	Extant	35.5788°N 79.1939°W

ulator with Reef Builder and/or Marine Buffer (Seachem Laboratories, Madison, GA, USA) to maintain the system pH at approximately 7.5. Young fish were primarily fed live *Artemia* nauplii, augmented with Ocean Star International (Snowville, UT, USA), Zeigler (Gardners, PA, USA), or other dry larval fish foods as a supplement. When the fish were sufficiently large, they also were fed frozen *Daphnia* spp. and chopped chironomids, with the latter being the staple food once the fish were able to feed on large items. The supply sources and potential contaminant burdens of these natural food items at the hatchery varied and are unknown. However, we analyzed a subsample of fish for baseline contaminants before deployment at the riverine sites to assess any predeployment burdens. Test fish were held for 3 d 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 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 (these fish were not used in the bioassay). In addition, five composite samples of 10 fish each from this group of 190 fish were 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 (length, 25 cm; outer diameter, 15 cm) covered on each end with tear-resistant Nitex® mesh (mesh size, 2.0 mm; Wildlife Supply, Buffalo, NY, USA) 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 (length, 39.5 cm; width, 19.0 cm; height, 19.5 cm) 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 historical reports) with suitable depth and velocity. The three cages at each site were oriented in the stream parallel to the direction of flow to ensure that water and any associated fine particles passed through the cages. Depths of cages 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 a cage was tied to a shoreline structure (e.g., tree or rock) using nylon rope.

Sample collection and processing

Fish were monitored every 4 d throughout the 28-d exposure period for mortality, and any dead fish were removed. At each 4-d interval, temperature, dissolved oxygen (model 58 meter; Yellow Springs Instrument, Yellow Springs, OH, USA), pH (model Φ110 ISFET meter; Beckman, Fullerton, CA, USA), and conductivity (model CO150 meter; Hach, Loveland, CO, USA) were measured at each site. Water samples also were collected at each site at that time, held on ice, and analyzed for alkalinity, hardness, and turbidity (model 2100 AN meter; Hach) at the laboratory within 24 h of collection with standard methods [26]. Grab samples of water and surficial sediment (top 5 cm taken with a stainless-steel scoop) were collected at the sites once during the 28-d test and stored for chemical contaminant (organic and inorganic) analysis. Water samples for inorganic constituents were preserved to pH less than 2 with concentrated HNO_3 and stored refrigerated (4°C) until analysis, and sediment samples were stored frozen at −20°C until analysis. A set of two passive sampling devices (PSDs), similar to semipermeable membrane devices [27–29], was deployed alongside 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 (~275 μm), virgin (with no additives), low-density polyethylene tubing (Brentwood Plastics, St. Louis, MO, USA) as described by Luellen and Shea [28]. The low-density polyethylene tubing was extracted with hexane for 48 h before use. After the 28-d deployment, the two PSDs (width, 7.5 cm; length, 30 cm) were combined to form a single composite sample from each site, placed in aluminum foil, sealed in a plastic bag, and stored frozen (−20°C) 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 (−80°C) for contaminant analysis. At the time of processing, fish samples were removed from the freezer, lyophilized (less than −50°C, <145 mTorr) 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, and a suite of 20 metals and metalloids. Water samples were analyzed only for the suite of 20 metals and metalloids, and the PSD samples were analyzed only for PAHs, PCBs, and OCs. The suite of inorganic and organic contaminants analyzed in the present study were chosen based on comprehensiveness for all known and suspected common contaminants in the three rivers, balancing funding agency information needs, and the cost per sample for analytical chemistry. Although the suite of contaminants analyzed in the present study was quite comprehensive, potential contaminants and other physicochemical variables remain unmeasured that may have influenced the survival and growth of the test fish deployed in the streams.

Sample preparation, analysis, and quality assurance

All inorganic chemical analyses were performed by the Midwest Research Institute (Kansas City, MO, USA) or the Trace Element Research Laboratory (Texas A&M University, College Station, TX, USA) through contracts with the U.S. Fish and Wildlife Service, Patuxent Analytical Control Facility (Laurel, MD, USA). All organic chemical analyses were performed by the Analytical Toxicology Laboratory at North Carolina State University. All of these chemical analyses were conducted according to established standard procedures and protocols. The detailed analytical procedures and instrumentation used in all chemical analyses have been described previously by Kwak et al. [25].

The validity of the analytical chemistry data generated in the present study was demonstrated with a rigorous quality-assurance program. For the analysis of inorganic constituents, the accuracy of all determinations was assessed by analyzing one or more standard reference materials (SRMs) 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, 20% of samples were analyzed in triplicate, and analyses included certified reference materials from Spex CertiPrep (Metuchen, NJ, USA). 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 less than 5% below the certified range. The relative standard deviation, estimated from analyses of 18 triplicate samples of river water, averaged 6% (range, 3–13%).

For analyses of Cape Fear shiners, the National Research Council of Canada (NRCC; Ottawa, ON) SRM, 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 percentage difference from duplicate fish samples was 16% (range, 0–70%). The U.S. National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) Buffalo River sediment (SRM 8704), Tennessee River sediment (SRM 8406), and NRCC MESS-3 (marine sediment) were analyzed with sediment samples and yielded concentrations within the certified range for all analytes. The mean analyte recovery from spiked-sediment samples was 93% (range, 68–118%). The percentage 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.

For the organic constituents, 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 the blanks were extremely low. No PCBs or OC pesticides were detected, and only small amounts (<1 ng/g) of several PAHs were detected. Recoveries of surrogate internal standards ranged from 40 to 120% for all analytes except several samples in which naphthalene-d_8 was between 30 and 40%. The lower recoveries for naphthalene most likely resulted from evaporative losses during the solvent exchange step required for the silica column cleanup. Data were not corrected for surrogate recoveries. Matrix spike recoveries also were within the range of 40 to 120%, but with several exceptions of higher recoveries for analytes that were not detected in any environmental samples. The percentage difference between matrix spike and spike duplicates, and duplicate sample analyses, was usually less than 10% and always less than 30%.

Statistical analyses

Statistical analyses were performed with PC SAS® Version 8.1 [30]. Variation among sites in mean survival, growth, and contaminant concentrations in fish, sediment, water, and PSDs was evaluated with the general linear model 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 before 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 (α) of 0.05 was used to judge statistical significance.

RESULTS

The mean physicochemical characteristics of river water measured every 4 d during the 28-d bioassay at the 10 test sites ranged from 25.1 to 28.9°C for temperature, 5.8 to 12.5 mg/L for dissolved oxygen, 7.6 to 9.0 for pH, 121 to 617 μS/cm for conductivity, 37 to 59 mg/L as $CaCO_3$ for alkalinity, 40 to 128 mg/L as $CaCO_3$ for hardness, and 2 to 41 NTU for turbidity (Table 2).

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, averaged 21 mm (range, 14–33 mm). The mean wet weight of test fish before deployment was 0.080 g (range, 0.022–0.283 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 4 of the 10 sites (Fig. 2). One was in the Rocky

Table 2. Mean physicochemical characteristics of river water (standard error in parentheses) measured at each site every 4 d during the 28-d bioassay with Cape Fear shiners ($n = 7$ samples analyzed per constituent) in the Haw, Rocky, and Deep rivers of North Carolina, USA

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)[a]
Haw River							
HR1	26.1 (0.8)	7.5 (0.3)	8.1 (0.1)	246 (38)	41 (4)	42 (4)	20 (7)
HR2	26.5 (0.8)	8.0 (0.2)	8.3 (0.1)	238 (37)	42 (3)	40 (2)	20 (7)
Rocky River							
RR1	27.2 (0.9)	6.1 (0.7)	7.6 (0.1)	121 (8)	43 (2)	43 (3)	7 (1)
RR2	25.1 (0.7)	7.1 (0.4)	7.8 (0.1)	617 (40)	59 (3)	128 (7)	6 (1)
RR3	25.3 (0.7)	8.5 (0.3)	8.0 (0.1)	445 (12)	56 (2)	94 (2)	2 (0)
RR4	28.9 (0.8)	12.5 (0.6)	9.0 (0.2)	194 (10)	37 (2)	46 (1)	3 (1)
Deep River							
DR1	26.4 (0.7)	8.0 (0.6)	7.9 (0.1)	214 (59)	45 (7)	48 (5)	38 (20)
DR2	28.1 (1.0)	8.7 (0.6)	8.2 (0.2)	316 (42)	49 (7)	51 (4)	41 (34)
DR3	27.9 (0.6)	5.9 (0.6)	7.6 (0.1)	230 (35)	41 (2)	40 (3)	4 (1)
DR4	27.7 (0.8)	5.8 (0.4)	7.6 (0.1)	223 (34)	40 (1)	41 (2)	6 (1)

[a] NTU = nephelometric turbidity units.

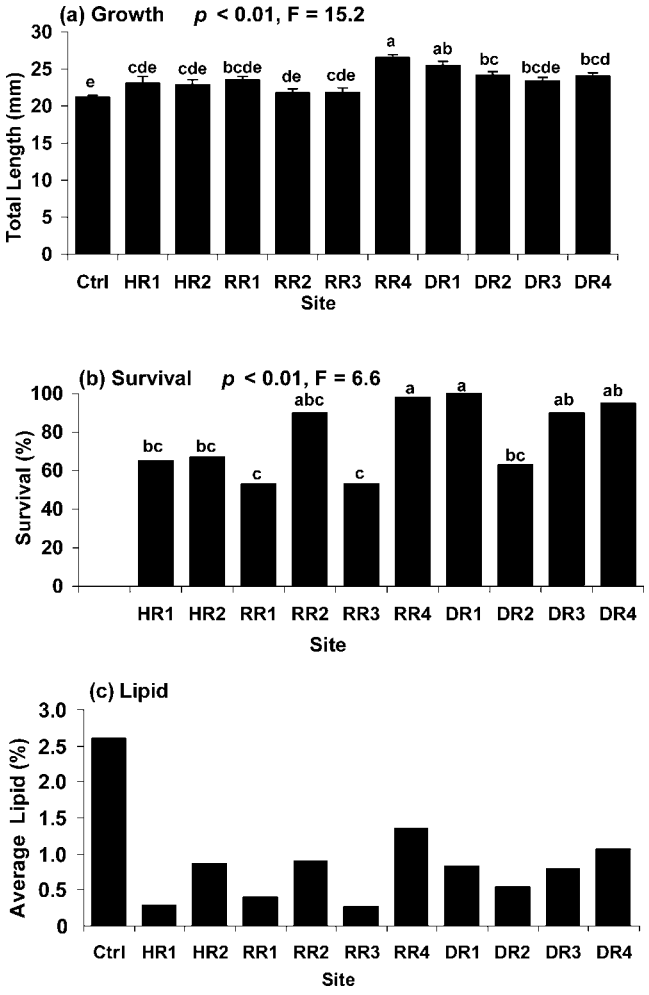

Fig. 2. Mean growth (**a**), survival (**b**), and lipid concentration (**c**) of Cape Fear shiners after the 28-d bioassay (Ctrl = baseline control sample on day 0 of the test) at sites in the Haw River (HR), Rocky River (RR), and Deep River (DR) of North Carolina, USA. Sites accompanied by the same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

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% (range, 53–100%). 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 and HR2], two in the Rocky River [RR1 and RR3], and one in the Deep River [DR2]) had fish with significantly reduced survival (Fig. 2). The surviving fish at HR1, HR2, RR1, and RR3, which had reduced survival rates, also had no detectable growth (as measured by an increase in length) over the duration of the test (Fig. 2). However, mean survival and growth of fish were not significantly related ($r = 0.60$, $p = 0.06$) among all sites, but a potential trend (demonstrated by the p value of 0.06) was indicated. At the six extant sites, survival of fish averaged 80% and was 74% at the four extirpated sites. Significant growth was observed at three of the extant sites and at one (DR1) of the extirpated 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 (Fig. 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 a wide range of inorganic and organic contaminants over the 28-d exposure (Table 3). Unexpectedly, we also detected some of the more persistent, bioaccumulative contaminants (e.g., Cd, Hg, PCBs, chlordanes, and DDT and its metabolites [DDTs]) in our baseline control fish. These persistent contaminants presumably originated in the test fish through dietary and aqueous exposure at the hatchery. Although detected in the baseline control fish, the presence of these contaminants did not hamper the comparison of relative concentrations between control and exposed fish.

No apparent relations were found between exposure to, or accumulation of, a specific contaminant and reduced growth or survival of fish among all the sites. However, certain sites exhibited trends in cumulative contaminant presence with re-

Table 3. Measured concentrations of selected contaminants in Cape Fear shiner (fish) tissue (ng/g wet wt), river water (ng/L), and sediment (ng/g dry wt) from sites in the Haw River (HR), Rocky River (RR), and Deep River (DR), of North Carolina, USA, and the baseline control fish samples in the 28-d Cape Fear shiner in situ bioassay[a]

		River and site									
Analyte	Control	HR1	HR2	RR1	RR2	RR3	RR4	DR1	DR2	DR3	DR4
Cd											
Fish	54	31	26	15	18	26	6	9	54	16	9
Water	—	250	110	ND	ND	ND	ND	ND	ND	ND	ND
Sediment	—	160	280	220	330	40	380	130	140	90	210
Cu											
Fish	1,050	1,150	1,090	750	1,470	1,160	780	970	1,710	950	950
Water	—	ND	ND	ND	7,000	ND	ND	ND	ND	ND	ND
Sediment	—	4,100	6,400	4,900	8,700	16,500	6,300	4,000	3,600	2,000	9,300
Hg											
Fish	30	36	24	36	28	31	23	21	25	39	55
Water	—	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sediment	—	9	14	11	19	24	16	19	10	16	178
Pb											
Fish	70	242	155	89	257	163	50	104	850	162	282
Water	—	ND	ND	ND	ND	ND	ND	3,000	ND	ND	ND
Sediment	—	4,300	7,700	6,700	9,400	11,500	9,700	3,900	4,300	2,800	6,500
Zn											
Fish	53,600	98,700	86,200	86,800	101,500	99,100	53,900	61,900	93,500	81,100	74,000
Water	—	27,000	16,000	ND	17,000	ND	ND	7,000	6,000	ND	ND
Sediment	—	24,300	37,600	21,900	43,000	52,700	33,000	13,300	16,400	10,300	24,600
PCBs											
Fish	5.2	9.7	4.2	6.3	2.7	7.8	5.2	6.3	2.0	ND	1.9
Water	—	0.6	0.3	ND	0.2	ND	ND	0.1	0.1	ND	ND
Sediment	—	0.1	ND	ND	ND	0.8	ND	0.2	0.2	0.1	0.7
PAHs											
Fish	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
Water	—	45	106	13	24	6	9	33	18	15	39
Sediment	—	62	683	58	45	444	45	114	39	22	443
Chlordanes											
Fish	34	36	19	34	16	44	23	35	21	12	9.7
Water	—	1.6	0.66	0.03	0.13	0.06	ND	2.4	0.77	ND	0.16
Sediment	—	ND	ND	ND	ND	0.4	ND	0.4	3.0	ND	1.1
DDTs											
Fish	6.9	1.8	0.9	1.1	0.7	ND	2.5	2.0	2.9	0.7	0.5
Water	—	0.28	0.34	0.06	0.15	0.08	0.05	0.27	0.10	0.08	0.02
Sediment	—	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a] DDTs = DDT and its metabolites; ND = not detected (value < analytical detection limit); NM = not measured; PAHs = polycyclic aromatic hydrocarbon; PCBs = polychlorinated biphenyls; — = not applicable.

duced fish survival and growth. For example, Cd, Cu, Hg, Pb, and Zn were detected in fish, water, and sediment samples from all sites. The accumulation of Cd, Cu, and Pb in Cape Fear shiners was greatest at DR2, which also had significantly reduced fish survival. Concentrations of Cu, Pb, and Zn in sediment were greatest at RR3, another site with significantly reduced survival and no growth of fish. Accumulation of Zn in fish tissue was greatest at RR2, a site with no significant growth. Mercury was greatest in both fish tissue and sediment at 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. Again, several of the sites with reduced survival and growth of Cape Fear shiners (e.g., DR2 and 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 high relative concentrations of certain organics in fish, sediment, and water at HR1 and HR2, which also had reduced survival and growth of fish.

Because of the variation in measured contaminant concentrations among 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 assessment was based on ranking the three highest measured concentrations for a given analyte and media at a site (Table 4). Through this analysis, certain sites and rivers could be identified as having pervasive contamination, which generally corresponded to those that exhibited decreased survival and growth of Cape Fear shiners during the 28-d in situ bioassay. For example, the metals Cd and Zn and the organic contaminants PCBs, chlordanes, and DDTs contribute to the overall degraded water quality in the Haw River (Table 4). The upper

Table 4. Summary of generalized hazard assessment for selected inorganic and organic contaminants among sites during the 28-d in situ bioassay with Cape Fear shiners in the Haw, Rocky, and Deep rivers of North Carolina, USA[a]

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

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

Haw River Basin is affected by point- and nonpoint-source discharges, and six streams from that basin were rated as poor or poor/fair in a recent basinwide report [11]. From sites on the Rocky River, Cu and Zn 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 (a tributary of the Rocky River), and were among the highest concentrations measured at any of the sites during the study. Organic contaminants, such as PAHs and PCBs, also were 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 4) and clearly represents some of the best remaining water quality for Cape Fear shiners. However, chlordane was surprisingly prevalent at Deep River sites and was among the three highest analytes measured in water and sediments at three of the four sites.

To assess whether any individual chemicals measured at the sites represented a potential hazard to aquatic life, we compared our results to existing water-quality, sediment-quality, and toxicity criteria and guidelines. The majority of our results for contaminants in water were not above the U.S. Environmental Protection Agency (U.S. EPA) freshwater criterion continuous concentration (FW CCC) [31] (http://www.epa.gov/wqsdatabase/reports_inter.html), with only several exceptions. Site RR2 had a Cu concentration of 7,000 ng/L; this value approaches the U.S. EPA FW CCC of 9,000 ng/L at a hardness of 100 mg/L as $CaCO_3$. Site RR2 is downstream of the Siler City WWTP and, as a result, has a recurrent problem with elevated levels of Cu. Total Pb concentration in water at DR1 was 3,000 ng/L, which is slightly greater than the dissolved U.S. EPA FW CCC of 2,500 ng/L at a hardness of 100 mg/L as $CaCO_3$. However, the mean measured hardness at DR1 was 48 mg/L as $CaCO_3$; thus, the adjusted dissolved U.S. EPA FW CCC would be near 1,200 ng/L, resulting in a measured total Pb value greater than the criterion.

Chemical residues measured in PSDs were converted to estimates of time-weighted average concentrations in water over the period of deployment using the method described by Luellen and Shea [28]. This method uses a laboratory calibration to establish effective sampling rates (L/d) for the PSD and a linear uptake model to convert measured PSD residues (ng) to concentrations in water (ng/L). Sampling rates for PAHs are from Luellen and Shea [28]; sampling rates for PCBs and pesticides are from Heltsley [29]. Although this is an indirect measurement, previous studies have demonstrated approximately twofold agreement between PSDs and multiple time-point sampling (see, e.g., [28]). Polycyclic aromatic hydrocarbons were detected in all PSD samples, but estimated PAH concentrations in water were relatively low with respect to thresholds for toxicity to aquatic species [31]. Concentrations of PCBs in PSD samples generally were low or undetectable, and all estimated PCB concentrations in water were less than 1 ng/L at all sites, well below the U.S. EPA numeric criteria of 14 ng/L. Chlordanes were detected in all PSDs except those at RR4 and DR3. Estimated concentrations of chlordanes in water ranged from 0.03 to 2.4 ng/L, with the greatest concentration occurring at DR1. Concentrations greater than 1 ng/L can cause adverse effects in aquatic organisms,

but concentrations known to affect fish generally are much greater (e.g., 200 ng/L) [32].

Polycyclic aromatic hydrocarbons also were detected in all sediment samples; however, all concentrations were extremely low compared to Canadian Sediment-Quality Guidelines for the Protection of Aquatic Life (SQGPAL) [33] (http://www.ec.gc.ca/ceqg-rcqe/English/ceqg/sediment/default.cfm), U.S. EPA sediment benchmarks [34], and other benchmarks [35]. Concentrations of PCBs in sediment were extremely low (<1 ng/g dry wt) at the sites, and all were less than the probable effect level of 277 ng/g dry weight set by the Canadian SQGPAL [33] and other benchmarks [35]. Chlordanes, which were detected in four sediment samples (RR3, DR1, DR2, and DR4), were less than the Canadian interim freshwater sediment-quality (4.5 ng/g dry wt) guidelines [33] and other benchmarks [35].

Cadmium was detected in sediment samples from all 10 sites. However, all measured concentrations were less than 400 ng/g, which is below the protective level of 600 ng/g established by the Canadian SQGPAL. Copper and Pb also were detected in sediments from all sites; their greatest concentrations were less than 16,500 and 11,500 ng/g, respectively—well below the Canadian SQGPAL protective levels. Mercury was detected in sediment at six sites (RR2, RR3, RR4, DR1, DR3, and DR4). The Hg concentration measured at DR4 was 178 ng/g dry weight, which was slightly greater than the Canadian SQGPAL; the other five sites were less than the criterion of 170 ng/g dry weight. Zinc was detected in all sediment samples, but concentrations were less than the Canadian interim SQGPAL level of 123,000 ng/g. However, Zn concentrations at RR2 and RR3 were elevated relative to concentrations at the other sites.

Although only a few of the existing chemical-specific criteria for water and sediment were exceeded among sites during the present study, the generalized hazard assessment (Table 4) 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

Comparison of contaminant availability among sites

Results of the in situ bioassay indicate that water quality may be a limiting factor for the Cape Fear shiner in the Haw River. The two Haw River sites, HR1 and HR2, are considered to be two of the five remaining populations of the Cape Fear shiner. However, population densities are extremely low at these sites, and these fish could be prone to extirpation [9]. At HR1 and HR2, survival of caged fish was 65 and 67%, respectively. Fish survival at these two sites was statistically reduced compared to that at the Rocky River reference site (RR4), where survival was 98%. Also, surviving fish at both Haw River sites did not differ significantly from the pretest control fish in terms of 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 higher concentrations of Zn and Pb relative to those of control fish. Measured concentrations of Zn in whole Cape Fear shiners from HR1 were 20-fold greater than controls after only 28 d of exposure. 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 [36].

The DDTs are highly persistent and toxic compounds. Evidence for reproductive toxicity and adrenotoxicity in birds and mammals is prevalent, and growing evidence indicates adverse effects on the adrenal and reproductive systems in many fish [37,38]. The estimated concentrations of DDTs in water were greatest at HR1 and HR2. The levels of DDTs in the water were approximately one-third of the U.S. EPA FW CCC for DDTs, and the metabolite 4,4′-dichlorodiphenyldichloroethylene (4,4′-DDE) accounted for 64% of the total detected. Fish tissue, including the background sample, contained concentrations of 4,4′-DDE. All fish likely were exposed to 4,4′-DDE before deployment through their diet, but after adjusting for lipid lost during the exposure, fish at HR1 had much greater concentrations of DDT compared with 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 be affecting the Cape Fear shiner in those reaches.

Interestingly, DDT metabolites were not detected in sediments. Sediment often serves as a reservoir for OC pesticides and can act as a method of transport [39–41]. Other studies have shown a positive relationship between DDT metabolites in soil and fish tissue [41]. However, the lack of DDT metabolites in sediment suggests that the source of DDT in the Haw River may be from nonpoint-source pollution and not from a reservoir of the chemical. Possible nonpoint sources should be investigated to identify the source of contamination.

Polychlorinated biphenyls and PAHs were found at low concentrations in fish tissue, water, and sediment and likely are not a threat to Cape Fear shiners at these sites. 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 accumulated chlordanes from the river. The concentration of chlordanes in water at all sites was less than the level (4.3 ng/L) known to cause adverse effects on aquatic organisms [31].

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

Fish survival at RR1 was statistically less relative to the reference site (RR4), and fish length was not significantly different from the pretest control sample. Site RR1 is located in the upper Rocky River, where the species has never been collected. It is possible that Cape Fear shiners already were extirpated from this reach when they were 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 similar to those in the control. Zinc concentrations were elevated in fish tissue with respect to the control, so accumulation presumably occurred in fish during the test. Although laboratory tests with other fish species at comparable concentrations had no or little effect [36], it is possible that Cape Fear shiner sensitivities to Zn are greater than those of other species. Low-flow conditions at this site during the test also could have contributed to fish stress and reduced survival and growth. Water quality in this reach is comparable to that of the reference site, but lack of

adequate flow, affected by the Siler City drinking-water reservoir immediately upstream, may have contributed to low survival.

Survival of fish at RR2 did not differ significantly from that of fish at the reference site, but fish length was not significantly different from the pretest control sample. The Zn concentration in fish tissue at this site was the highest relative to other sites and twice as high as background controls. This site is immediately downstream of the mouth of Love's Creek, a tributary where point-source discharge from the Siler City WWTP is released. Portions of Love's Creek are on the state's list of impaired waters [11]. State monitoring at site RR2 in 1998 yielded a good/fair rating [11]; however, the relatively high water conductivity at this site reflects an ongoing impact of the wastewater effluent. Physicochemical characteristics, such as conductivity, can influence the toxicity of contaminants; therefore, this site should continue to be monitored for effects of the upstream WWTP on the biological community. Conditions at this site clearly are degraded because of influences from the upstream urban areas; thus, combinations of site-specific interactions (including Zn and chlordane uptake) may be responsible for the corresponding lack of fish growth.

Lack of fish growth, poor survival, and contaminant residues suggest that water quality is limiting at RR3, where Cape Fear shiners have been extirpated. This site is considered to be a potential area for reintroduction of the species. Survival of test fish was only 53% (significantly different from the reference site) at this site, and mean fish length was not significantly different from the pretest controls. Both Zn and chlordanes were accumulated in fish tissue, and concentrations of Zn, Cu, and Pb in water and sediment were the greatest among all sites. This site is downstream of RR2 and is influenced by upstream urban areas, but the immediate area is directly affected by agricultural practices.

Our results confirm that water quality is not limiting for the Cape Fear shiner at RR4. This site was considered to be the best reference site for the present study based on existing habitat and fish population data. Moreover, this site historically has had good water quality [11], and it supports the most abundant population of Cape Fear shiners. Survival of test fish at this site was high (98%), and fish growth was significantly different from the pretest controls. All concentrations of metals and chlordanes in fish tissue were similar to controls, and concentrations in water and sediment generally were low and not of concern.

Site DR1 is in a reach of the Deep River where the Cape Fear shiner has been extirpated above Coleridge Dam, and our results suggest that water quality may not have been a limiting factor in this reach at the time of the test. It also is considered to be a potential site for reintroduction of the species. Fish survival at this site was the highest (100%) measured during the present study, and fish growth was highly significant. Metal concentrations in fish tissue were similar to background controls. Total Pb concentration in water was above the adjusted U.S. EPA FW CCC (for dissolved metal), although Pb concentration in fish was similar to the control. Therefore, site-specific conditions may have affected the availability of Pb. Despite significant fish growth and high survival, concentration of chlordanes in water (2.4 ng/L) was more than double the concentration known to cause adverse effects in some aquatic species. Water-quality problems have been documented in the upper Deep River during the past two decades, but conditions have continuously improved [11]. Overall, water quality does not appear to be limiting for the Cape Fear shiner in this reach of the Deep River.

Site DR2 represents the uppermost population of the Cape Fear shiner in the Deep River, and this river section is classified as high-quality waters [11]. However, our results suggest that water quality may be impaired for the Cape Fear shiner in this reach. Fish growth was significant, but survival (63%) was significantly less than at the reference site. Fish accumulated Zn, and Zn concentrations in water were near the U.S. EPA FW CCC [31]. 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 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 buffer, and it may be affected by current-use pesticides that were not part of the present assessment. Although fish growth was not affected, survival was low relative to the reference site and may be the result of a local combination of water-quality factors.

Our results show that water quality at DR3 in the mainstem of the Deep River is not a limiting factor to the Cape Fear shiner. Site DR3 is in the extant reach of Cape Fear shiners on the Deep River downstream of Carbonton Dam, and it represents the strongest remaining population. This area supports a single abundant metapopulation located in the Rocky River below the Rocky River Hydroelectric Dam and near the confluence with the Deep River and connected sections of the Deep River (i.e., sites RR4, DR3, and DR4 combined). Survival of fish was high (90%) at this site, but there was no statistically significant difference in fish growth. Concentrations of chlordane in fish, sediment, and water were all low with respect to critical threshold levels. Although Zn 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 Pb, but not at levels known to cause adverse effects [36]. 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 [11]. In general, water quality improves in the downstream portion of this river. However, these tributaries have been affected by local agricultural practices that have led to streambank erosion and degraded instream habitat.

Site DR4, like site DR3, represents the range of the strongest population in the lower Rocky and Deep rivers. Survival of fish at this site was high (95%), and growth was significant. The Hg concentration in sediment at this site was the highest measured among all 10 sites (178 ng/g) and was above the standard for quality sediment set by the Canadian SQGPAL. However, the Hg concentration in fish did not indicate accumulation from the local environment. Fish accumulated Pb and Zn, but concentrations were not high enough for concern to aquatic species. The surrounding watershed has high numbers of certified agricultural 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 [11]. Despite declining water quality at this site and from upstream river reaches, our results, including significant fish growth and high survival, indicate that water quality is not 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 likely resulted from differences in current and historical land-use patterns and degree of urbanization. The pesticides and organic contaminants detected in the present 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; concentrations of these contaminants should continue to decline. Sites varied in the composition of contaminants; therefore, potential effects on fish survival and growth were difficult to assess and predict. Water quality in the reaches of extant Cape Fear shiner populations supported fish growth and 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 among river reaches where the fish is extant, extirpated, and rare [25]. Past acute poor water-quality events, combined with loss of riverine instream physical habitat and fragmentation of populations by dams, which prevent recolonization, have produced the isolated and increasingly rare metapopulations of the Cape Fear shiner that exist today.

Overall recovery of the Cape Fear shiner is focused on restoration of the physical habitat, including dam removals. Recommendations for restoration and management of Cape Fear shiners related to water quality are to improve water quality in the lower Haw River, where the species is vulnerable to extirpation, and to improve water quality and the flow regime in the upper Rocky River, where the fish has been extirpated. The potential reintroduction site in the Rocky River (RR3) contains physical instream habitat similar to that in the lower Rocky River [25], but water quality most likely would hinder any reintroduction efforts at RR3 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) 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 reaches where the fish is extant are present. Water quality in that reach appears to be suitable for reintroduction of the species in the near future.

The in situ bioassay approach with caged fish has been successful in the present and other studies for evaluating water quality and the effects of local contaminants [22,42], and the rare opportunity to conduct a study of this type with an endangered species in its native streams has yielded valuable information for its future conservation and protection. The sustainability of Cape Fear shiner populations depends on the protection and preservation of extant populations and habitats. Pressure from urban development and increasing demands of the human population for water resources may confound and impede restoration efforts. The present study identified riverine areas of concern that may require restoration; it also helped to identify those areas most suitable for reintroduction of the species or population augmentation. This information can now be used to improve the management of aquatic resources that are necessary to ensure the long-term survival of the Cape Fear shiner.

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