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Assessing water quality suitability for shortnose sturgeon in the Roanoke River, North Carolina, USA with an *in situ* bioassay approach

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Summary

The aim of this study was to determine the suitability of water quality in the Roanoke River of North Carolina for supporting shortnose sturgeon *Acipenser brevirostrum*, an endangered species in the United States. Fathead minnows *Pimephales promelas* were also evaluated alongside the sturgeon as a comparative species to measure potential differences in fish survival, growth, contaminant accumulation, and histopathology in a 28-day *in situ* toxicity test. Captively propagated juvenile shortnose sturgeon (total length 49 ± 8 mm, mean ± SD) and fathead minnows (total length 39 ± 3 mm, mean ± SD) were used in the test and their outcomes were compared to simultaneous measurements of water quality (temperature, dissolved oxygen, pH, conductivity, total ammonia nitrogen, hardness, alkalinity, turbidity) and contaminant chemistry (metals, polycyclic aromatic hydrocarbons, organochlorine pesticides, current use pesticides, polychlorinated biphenyls) in river water and sediment. In the *in situ* test, there were three non-riverine control sites and eight riverine test sites with three replicate cages (25 × 15-cm (OD) clear plexiglass with 200-μm tear-resistant Nitex® screen over each end) of 20 shortnose sturgeon per cage at each site. There was a single cage of fathead minnows also deployed at each site alongside the sturgeon cages. Survival of caged shortnose sturgeon among the riverine sites averaged 9% (range 1.7–25%) on day 22 of the 28-day study, whereas sturgeon survival at the non-riverine control sites averaged 64% (range 33–98%). In contrast to sturgeon, only one riverine deployed fathead minnow died (average 99.4% survival) over the 28-day test period and none of the control fathead minnows died. Although chemical analyses revealed the presence of retene (7-isopropyl-1-methylphenanthrene), a pulp and paper mill derived compound with known dioxin-like toxicity to early life stages of fish, in significant quantities in the water (251–603 ng L^{-1}) and sediment (up to 5000 ng g^{-1} dry weight) at several river sites, no correlation was detected of adverse water quality conditions or measured contaminant concentrations to the poor survival of sturgeon among riverine test sites. Histopathology analysis determined that the mortality of the river deployed shortnose sturgeon was likely due to liver and kidney lesions from an unknown agent(s). Given the poor survival of shortnose sturgeon (9%) and high survival of fathead minnows (99.4%) at the riverine test sites, our study indicates that conditions in the Roanoke River are incongruous with the needs of juvenile shortnose sturgeon and that fathead minnows, commonly used standard toxicity test organisms, do not adequately predict the sensitivity of shortnose sturgeon. Therefore, additional research is needed to help identify specific limiting factors and management actions for the enhancement and recovery of this imperiled fish species.

Introduction

The shortnose sturgeon *Acipenser brevirostrum* is the smallest of the sturgeon species occurring in eastern North America, living 50–60 years, attaining maximum lengths of about 120 cm and weights of 24 kg (Dadswell et al., 1984). The species was listed by the United States Government as endangered in 1967, and it remains imperiled throughout its range (NMFS, 1998). Currently, 19 population segments are known between Florida and Canada, and two of these occur in coastal North Carolina (NMFS, 1998; Armstrong, 1999). An extant population of fewer than 50 individuals exists in the Cape Fear River (Ross et al., 1988; Moser and Ross, 1995). The species was regarded as extirpated from the Roanoke River/Albemarle Sound region prior to the capture of a single adult in 1998 (Armstrong, 1999). The only other published record of the species in the Albemarle Sound region is a juvenile museum specimen collected in 1881 (Vladykov and Greeley, 1963).

When extirpated from systems of historic occurrence, the reintroduction of cultured shortnose sturgeon may be a viable recovery option (NMFS, 1998). The Roanoke River population could be a candidate for restoration efforts by state and federal management agencies, however water quality and habitat suitability for the shortnose sturgeon are unknown at present. The Roanoke River is known for having contaminant problems, in terms of historic loadings and ongoing permitted point-source discharges. Release of contaminants associated with production of wood, lumber, and paper products into the lower Roanoke River and Welch Creek, a tributary, began around 1937 and continues to the present (NOAA, 2004; NCDENR, 2006). Uptake of available contaminants by fish led the State of North Carolina to issue fish consumption advisories in the region for dioxins in 1991 and mercury in

1997 (NCDENR, 2006). Pollutants are recognized as a principal threat to the shortnose sturgeon (NMFS, 1998).

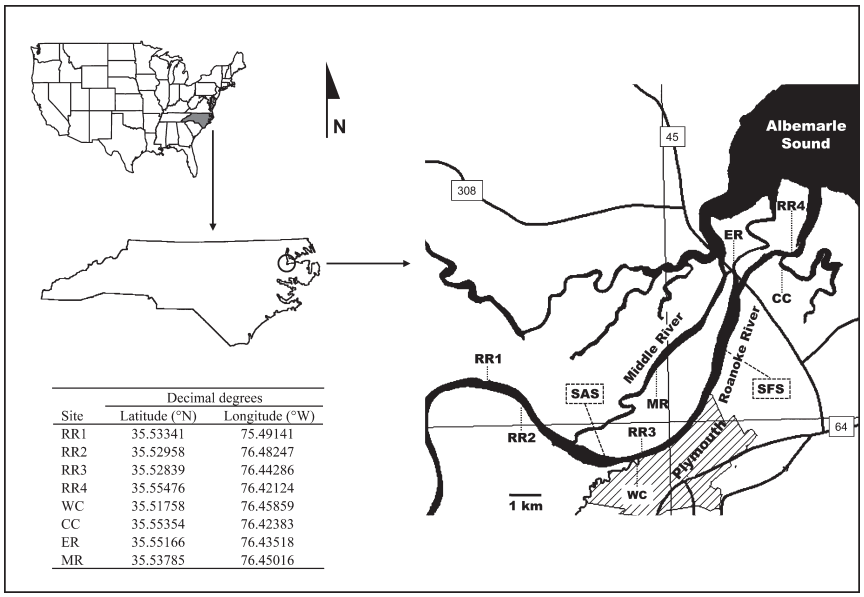
The *in situ* toxicity test has become a powerful tool for evaluating the suitability of environmental conditions for species of interest or their surrogates (Hall et al., 1985; Pereira et al., 2000; Hewitt et al., 2006). *In situ* toxicity tests integrate complex site-specific conditions, such as oxygen, pH, and temperature that can alter the bioavailability and toxicity of contaminants and sensitivity of biota to perturbation. Laboratory toxicity tests have shown that the shortnose sturgeon was one of the top four most sensitive species out of 18 tested against a suite of individual pollutants, whereas the fathead minnow *Pimephales promelas*, a standard toxicity test organism, was among the most tolerant (number 16 of 18) (Dwyer et al., 2005a). Likewise, in subsequent testing with industrial effluents, shortnose sturgeon demonstrated greater sensitivity relative to fathead minnows (Dwyer et al., 2005b). Because sensitivity to contaminants varies among fish species (Dwyer et al., 2005a), results of tests with surrogate species like fathead minnows may not always be applicable to protect a particular species of interest. Thus, the aim of our study was to evaluate the suitability of water and sediment conditions in the lower Roanoke River for naturally occurring or reintroduced shortnose sturgeon with an *in situ* testing approach.

Materials and methods

Study area and test design

We assessed eight riverine test sites (Fig. 1) and three non-riverine control sites (used to evaluate handling and transport stress, potential stress due to changes in water chemistry conditions, and feeding ability of test fish) during the 28-day *in situ* toxicity test conducted from 4 May to 1 June 2005. The riverine test sites were selected based on the range of chemical and physical conditions that occur in the lower Roanoke River, as well as locations where juvenile shortnose sturgeon would likely reside in this portion of the river. For example, it is known that adult, subadult, and juvenile shortnose sturgeon largely reside in the lower riverine reaches of their natal systems near the fresh-brackish water interface (Kynard, 1997; Collins et al., 2003), which accurately describes the area of the lower Roanoke River studied here. Several of the sites were located in proximity to known historic sites of contamination and present point source outflows (Fig. 1) from permitted dischargers (NCDENR, 2006).

The three non-riverine control sites were all located at US Fish and Wildlife Service (USFWS) National Fish Hatcheries. One control site was the Bears Bluff National Fish Hatchery (BBNFH) in Wadmalaw Island, South Carolina, where the juvenile shortnose sturgeon used in this study were produced. A subsample of fish from the total test fish population used in the study were randomly chosen and retained in cages at BBNFH and were considered a nontransport control; these fish were also used to assess potential cage and feeding effects. Two additional control sites were near the Roanoke River test sites (644 km from the BBNFH source population) at the Edenton National Fish Hatchery (ENFH) in Edenton, North Carolina: one in indoor tanks at the hatchery that simulated the holding and feeding conditions at the BBNFH, but with water chemistry similar to the Roanoke River; and one in an outdoor rearing pond that simulated the water chemistry of the Roanoke River and the lack of a human-provided feeding regime, similar to the situation for the riverine caged fish. Together, these two control sites assessed the potential effects of handling and transport stress, potential stress due to changes in water chemistry conditions, and, at the ENFH pond, the ability of test fish to feed independently on natural food sources.



Site	Decimal degrees	
	Latitude (°N)	Longitude (°W)
RR1	35.53341	75.49141
RR2	35.52958	76.48247
RR3	35.52839	76.44286
RR4	35.55476	76.42124
WC	35.51758	76.45859
CC	35.55354	76.42383
ER	35.55166	76.43518
MR	35.53785	76.45016

Fig. 1. Map of study sites, lower Roanoke River, during *in situ* toxicity test with shortnose sturgeon and fathead minnows, 4 May–1 June 2005. Test site and abbreviations: Roanoke River main stem, RR1 (reference site upstream of Town of Plymouth, NC), RR2 (reference site upstream of Town of Plymouth, NC), RR3 (impacted site downstream of permitted effluent dischargers), RR4 (impacted site downstream of permitted effluent dischargers); Eastmost River, ER (non-impacted site in a connecting channel of the Roanoke River that receives water from main stem of the river upstream of Plymouth, NC); Middle River, MR (non-impacted site in a connecting channel of Roanoke River that receives water from main stem of the river upstream of Plymouth, NC); Welch Creek, WC (impacted site downstream of an historic contaminant effluent); and Conaby Creek, CC (non-impacted comparative reference site for the WC site; SAS = US Environmental Protection Agency (USEPA) Superfund Alternative site; SFS = USEPA Superfund site

Fish transport, deployment and cage description

All sturgeon used in the study (except those randomly selected as BBNFH non-transport controls) were transported from the BBNFH to the test sites near Plymouth, North Carolina and to the ENFH in Edenton, North Carolina (about 650 km and 8 h transit time) by BBNFH staff in a specialized fish transport truck containing re-circulating water and a compressed oxygen tank that provided aeration for the sturgeon during the trip. The temperature and dissolved oxygen (DO) concentration of water in the sturgeon transport tank were measured hourly throughout the trip and averaged 17.1°C (range 16.7–17.5°C) and 11.7 mg L^{-1} (range 10.2–14.6 mg L^{-1} °C), respectively. None of the sturgeon died during transport. Upon arrival at the test site distribution point on the Roanoke River (North Carolina Highway 45 North boat ramp), the surface water temperature of the river was 17.5°C and DO concentration 7.8 mg L^{-1}. Because the water temperature in the sturgeon transport tank at the time of arrival at the site (17.0°C) was nearly identical to the river water temperature (17.5°C), a lengthy period for temperature acclimation of the sturgeon was not necessary. However, to ensure their adequate acclimation, a 1-h adjustment period from BBNFH transport water to Roanoke River water was conducted with sequential additions of river water every 15 min throughout the hour, after which the sturgeon were deployed to their cages at the sites.

The fathead minnows used in the study were purchased from Chesapeake Cultures, Inc., in Hayes, Virginia and transported overnight via commercial courier to the field location. No mortality of fathead minnows occurred during transport. Water temperature in the transport container at the time of arrival at the site (17.7°C) was nearly identical to the river water temperature (17.5°C); therefore, a temperature acclimation was not necessary. A 1-h adjustment period (similar to that used for sturgeon) from shipping water to Roanoke River water was conducted with sequential additions of river water every 15 min throughout the hour, after which the fathead minnows were deployed to their cages at the sites.

The shortnose sturgeon used in the study were 50-day old at the time of test initiation. Before deployment to cages, a subsample of 120 shortnose sturgeon was randomly selected from the overall test population (720 fish) to determine the beginning size [total length (TL, mm) and wet weight (g)], condition factor, and contaminant burden of sturgeon for statistical comparison to these same metrics of caged sturgeon at the end of the 28-day study. These subsampled fish were denoted as the baseline sample. The baseline shortnose sturgeon ranged from 30 to 74 (mean ± SD, 49 ± 8) mm total length, 0.2–1.3 (0.5 ± 0.3) g wet weight, and 2.89–5.60 (4.37 ± 0.71) condition. For the baseline contaminant burden analysis, these 120 sturgeon were randomly allocated into four composite samples of 30 fish each, weighed, and stored frozen at −20°C until analysis. The cumulative wet weight of each of the four composite baseline sturgeon samples ranged from 11.4 to 14.8 g.

The fathead minnows used in the study were 75-day old at the time of test initiation. As with the sturgeon, a subsample of 54 fathead minnows was randomly selected from the overall test population (275 fish) before allocation to cages to determine the beginning size [total length (TL, mm) and wet weight (g)], condition factor, and contaminant burden for statistical comparison to these same metrics at the end of the 28-day study. These sub-sampled fathead minnows were denoted as the baseline sample, which ranged from 30 to 48 (mean ± SD, 39 ± 3) mm in total length with wet weights between 0.3 and 1.1 (0.7 ± 0.2) g, and indices of condition between 8.71 and 16.33 (10.79 ± 1.50). For the baseline contaminant burden analysis, these 54 fathead minnows were randomly allocated into three composite samples of 18 fish each, weighed, and stored frozen at −20°C until analysis; the cumulative wet weight of each of the three composite baseline samples ranged from 10.3 to 13.8 g. Fish condition (C) was quantified using the Fulton condition factor $C = (\text{weight}/TL^3)$ and 1 000 000 used as a scaling factor (Anderson and Neumann, 1996).

For deployment of sturgeon at the riverine and control test sites, there were three replicate cages of 20 shortnose sturgeon per cage deployed at each site. A single cage of 20 fathead minnows was also deployed at each site alongside the three sturgeon cages, totaling 32 cages deployed to riverine sites and an additional 11 cages at the three sites designated experimental controls. Only shortnose sturgeon were held in cages in the indoor tank at the BBNFH because of potential disease transmission concerns to other sturgeon at the hatchery.

All test sturgeon and fathead minnows were held at a site in cage-type enclosures previously described and used by Hewitt et al. (2006) in their *in situ* study with another endangered fish species. The groups of test fish were held within a 25 × 15-cm (OD) clear plexiglass cage by 200-micron tear-resistant Nitex® screen clamped over each end. The plexiglass cages deployed at the riverine sites and at the outdoor pond at the ENFH were loosely surrounded by an additional outer cage of plastic mesh (5-mm openings) to increase security against potential damage by floating debris. The fish cages and outer covering cages were secured by nylon rope and hose clamp to rigid PVC pipe (schedule 40; 3.30-m × 8.25-cm OD) that had been driven 1 m into the river bottom at each of the sites prior to fish deployment. The cages deployed to the outdoor pond at the ENFH were suspended from a water-level control structure rather than a PVC pipe driven into the pond bottom for ease of access and to alleviate concerns about harming the integrity of the pond bottom. At all sites, cages were suspended at a depth of 0.8–1.2 m beneath the water surface, a depth that ensured adequate flow of oxygenated water through the cages, ensured no direct contact of cages with the bottom sediment, and ensured that the distance of cages above the sediment-water interface was consistent among all sites. The river gauge height, a measure of flow conditions and discharge through the study area was obtained from a United States Geological Survey gauging station near the sturgeon and fathead minnow distribution point on the Roanoke River (North Carolina Highway 45 North boat ramp). Gauge height averaged 0.42 m during the test period (May 2005) and ranged from 0.25 to 0.44 m throughout 2005, indicating relatively high flow conditions at the test sites during the study. Observations on all caged fish were made every 72 h by bringing the cage onboard the boat in a water-filled 20-L bucket. Emersion of test fish did not occur during this process. The cages were checked for damage, the mesh over the ends was cleaned of algal and other biofilm growth to prevent clogging and reduced water flow, and any dead fish were removed from the cages, cataloged by date, site, cage, and stored frozen at −20°C. As in the study of Hewitt et al. (2006), the cages exhibited excellent durability and had no adverse issues with excessive biofouling. Caged fish at the river test sites and at the ENFH pond were not fed during the study, but relied on natural food items passing through and/or colonizing the cages. The fathead minnows and shortnose sturgeon held in cages in indoor tanks at the

ENFH and BBNFH were fed a commercial fish food diet (Zeigler Bros., Inc., Gardners, PA) once daily.

Sample collection, processing, and analysis

At the conclusion of the study (day 22 for sturgeon due to unanticipated poor survival and day 28 for fathead minnows), all surviving fish were removed from the cages, cataloged by date, test site, and cage replicate, and measured individually for size. Afterward, only fathead minnows were partitioned into composite samples for analysis of contaminants by site (too few shortnose sturgeon survived at a site to provide sufficient mass for contaminant analysis), where 5 g was the minimum cumulative wet weight for a group. Fathead minnows held for contaminant analyses were enfolded within baked aluminum foil, sealed in plastic bags, and placed on ice upon removal from the cages, and then stored frozen at −20°C until analysis.

Water temperature (°C), DO (mg L^{-1}), pH, and conductivity (μS cm^{-1}) were measured with a calibrated multiprobe (YSI Model 556; Yellow Springs Instruments, Yellow Springs, OH) at each of the test sites every 24 h. Grab water samples were collected by opening and resealing a 1-L container submersed 0.5–1.0 m beneath the water surface at each of the test sites every 72 h. These samples were promptly placed on ice and analyzed following standard methods (APHA et al., 1995), to determine total ammonia nitrogen (TAN in mg L^{-1}), hardness (mg L^{-1} as $CaCO_3$), alkalinity (mg L^{-1} as $CaCO_3$), and turbidity (NTU), within 24 h of collection.

The presence of organic and inorganic contaminants at the test sites was assessed by analysis of sediment and passive sampling devices (PSDs) (Hewitt et al., 2006) that provided time-integrated samples of hydrophobic waterborne organic contaminants, in addition to the caged fathead minnow samples. Surficial (top 5 cm) sediment samples were collected at each site with a small ponar dredge and stored frozen (−20°C) until analysis. The relative composition of sediment samples among sites was evaluated by the analysis of total organic carbon (APHA et al., 1995). The PSDs were deployed for 28 day in a fashion similar that of the fish cages. A plastic mesh cage (5-mm openings) containing two PSDs was secured to the PVC pipe that held the fish cages and was held suspended in the water column. The PSDs were 7.5 × 30 cm, 10-mil (~275 μm) low-density polyethylene (LDPE) sheeting (Brentwood Plastics, Inc., St. Louis, MO) that had been extracted with hexane for 24 h prior to deployment (Hewitt et al., 2006). At the conclusion of the test, the PSDs from each site were combined into a single sample, wrapped in baked aluminum foil, enclosed in plastic bags, cataloged, and stored frozen at −20°C until analysis.

Inorganic analyses of sediment and fathead minnow tissue evaluated a suite of metals and metalloids: antimony (Sb), arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), selenium (Se), silver (Ag), thallium (Tl), and zinc (Zn). These analyses were conducted by Environmental Conservation Laboratories, Inc., a certified analytical chemistry laboratory in Cary, North Carolina. Analyses of fathead minnows, sediment, and PSDs for organic contaminants were performed at the North Carolina State University, Analytical Toxicology Laboratory, in Raleigh, North Carolina. Sediment and PSD samples were analyzed for 48 polycyclic aromatic hydrocarbons (PAHs) and related heterocyclic compounds, 26 organochlorine (OC) pesticides, chlorpyrifos and other current use pesticides (CUPs), and 20 polychlorinated biphenyl (PCB) congeners. The fathead minnow samples were analyzed similarly except that the 48 PAHs were not analyzed in fish tissues because of rapid *in vivo* metabolism of compounds. The validity of all analytical chemistry data generated in this study was demonstrated with a rigorous quality assurance program, which along with detailed analytical procedures and instrumentation used in the analyses, were previously described by Kwak et al. (2007).

For histopathology analysis, sturgeon surviving on day 22 were euthanized by immersion in ice water, examined grossly, then the tails were removed just caudal to the anal pore and a ventral slit made into the coelomic cavity to allow penetration of fixative. Fish specimens were fixed in 10% neutral buffered formalin for 48 h, demineralized in 10% formic acid for 48 h, routinely processed, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Sections were analyzed and photographed via light microscopy by a single pathologist.

To screen for possible viral infection, sections of liver were de-waxed, post-fixed in osmium tetroxide, routinely processed and sectioned for transmission electron microscopy. Thin sections were viewed with a Philips EM-208S transmission electron microscope.

Statistical analysis

Differences in response variables among the test sites were evaluated with analysis of variance (ANOVA). Evidence of significant variation was followed with comparisons among means. Measures of water quality variables and fish size and condition were analyzed with the omnibus F-test in least squares, generalized linear models. The Brown-Forsythe test was used to test the hypothesis of homogeneity of variance (Brown and Forsythe, 1974). When data failed to meet underlying assumptions, the validity of statistical tests (P-values) were evaluated with randomization tests (10 000 repetitions). The Ryan-Einot-Gabriel-Welsch multiple range test was used to identify significant differences among site means, thereby controlling the experiment-wise error rates in the comparisons. Differences in shortnose sturgeon survival functions among test sites were evaluated with product-limit (K-M) estimators (Kaplan and Meier, 1958) using the log-rank test. A pairwise correlation matrix (Pearson correlation coefficient) was used to identify significant relationships between shortnose sturgeon survival and environmental conditions (mean water quality parameter and contaminant concentration measurements) at each riverine site. Statistical significance was judged using a Type I error (α) of 0.05. The Statistical Analysis Software package (SAS/STAT) for PC (version 9.1.3; Cary, NC) was used for analyses.

Results

Shortnose sturgeon

At all river test sites over time, average survival of shortnose sturgeon was 100% on day 1 and declined to 83% at the conclusion of the 1st interval (1–3 day), 65% over the 2nd (3–6 day), 47% over the 3rd (6–9 day), 32% over the 4th (9–12 day), 20% over the 5th (12–15 day), 11% over the 6th (15–18 day), and 9% over the 7th (18–21 day; Fig. 2). There were statistically significant differences in the K-M survival functions among the test sites (χ^2 = 53.4973; d.f. = 7, $P < 0.0001$). The mean (and median) survival times (in

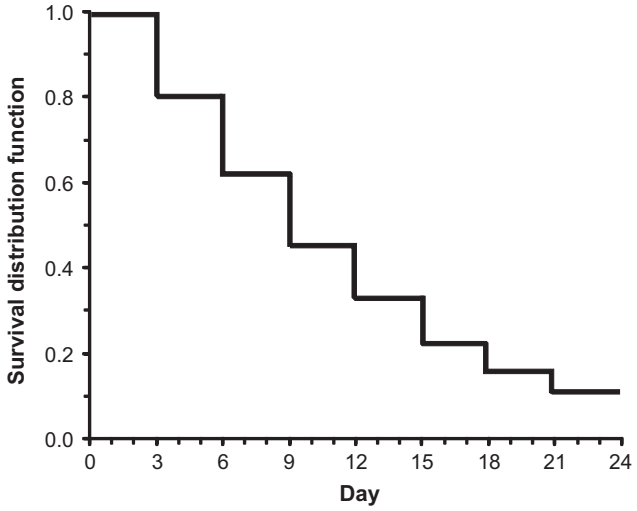


Fig. 2. Product-limit survival distribution function for shortnose sturgeon at all sites, Roanoke River, during *in situ* toxicity test 4 May–1 June 2005

intervals) of shortnose sturgeon at the test sites listed in order of increasing mean survival were: RR3, 3.0 (3.6); RR4, 3.3 (4); WC, 3.3 (3); ER, 3.5 (3); RR1, 3.5 (2); CC, 3.6 (3); MR, 3.7 (4); RR2, 4.2 (4). Pair-wise testing for heterogeneity in the survival functions between test sites indicated statistically significant differences between: RR1 and RR2 ($\chi^2 = 13.87$; d.f. = 1, P = 0.0002); RR2 and RR3 ($\chi^2 = 26.23$; d.f. = 1, P < 0.00001); RR2 and RR4 ($\chi^2 = 27.01$; d.f. = 1, P < 0.0001); and, MR and ER ($\chi^2 = 4.29$; d.f. = 1, P = 0.04). There was no evidence of statistically significant heterogeneity in K-M survival functions between: RR1 and RR3 ($\chi^2 = 0.48$; d.f. = 1, P = 0.49), RR1 and RR4 ($\chi^2 = 3.11$; d.f. = 1, P = 0.08), RR3 and RR4 ($\chi^2 = 2.19$; d.f. = 1, P = 0.14), or WC and CC ($\chi^2 = 1.94$; d.f. = 1, P = 0.16). Among the eight riverine test sites, survival of caged shortnose sturgeon averaged 9% (range 1.7–25%) on day 22 of the 28-day study, whereas sturgeon survival at the three non-riverine control sites averaged 64% (range 33–98%; Fig. 3). Among the individual river test sites on day 22, sturgeon survival was greatest at the RR2 site and least at the ER site (Fig. 3). Control sturgeon in cages in indoor tanks at the ENFH and BBNFH survived at greater proportions (62 and 98%, respectively) relative to those at the ENFH pond (33%). Sturgeon survival among all control sites, even in the ENFH pond with no water flow, was greater than that for any of the river test sites (Fig. 3).

Mean total length and wet weight of shortnose sturgeon recovered from cages at the three control sites on day 22 were BBNFH, 60 ± 7 mm, 0.8 ± 3 g; ENFH-tank, 62 ± 9 mm, 1.0 ± 4 g; ENFH-pond, 54 ± 6 mm, 0.7 ± 0.2 g. There was statistically significant variation in mean length (F3/224, 63.90; P < 0.0001) and weight (F3/224, 64.22; P < 0.0001) of sturgeon among the control sites and the baseline samples. Pair-wise comparisons indicated significant sturgeon growth at the control sites, relative to baseline samples. The shortnose sturgeon from the indoor tanks at BBNFH and ENFH were significantly larger than those recovered from the ENFH pond at the conclusion of the experiment and although the ENFH pond was not the optimal parallel control site in terms of matching environmental conditions (e.g. water flow) at the river test sites and the indoor tanks at the ENFH and BBNFH, we detected no significant variation in mean condition factor (BBNFH-tank, 4.1 ± 0.4; ENFH-tank, 4.0 ± 0.5; ENFH-pond, 4.2 ± 0.5; Baseline, 4.2 ± 319 0.6) among the groups of sturgeon (F3/224, 0.78; P = 0.4517). Because there were so few surviving sturgeon at each of the river test sites on day 22 (only 1–15 per site out of the initial 60), length, weight, and condition factors of these sturgeon were not taken for comparison to baseline samples due to small sample sizes and lack of suitable replication. Therefore, only histopathology analysis of sturgeon surviving on day 22 was performed in an attempt to discern the cause(s) of the mortality.

The histopathology analysis on a sample (n = 34 total) of surviving sturgeon revealed that the fish in cages held in the

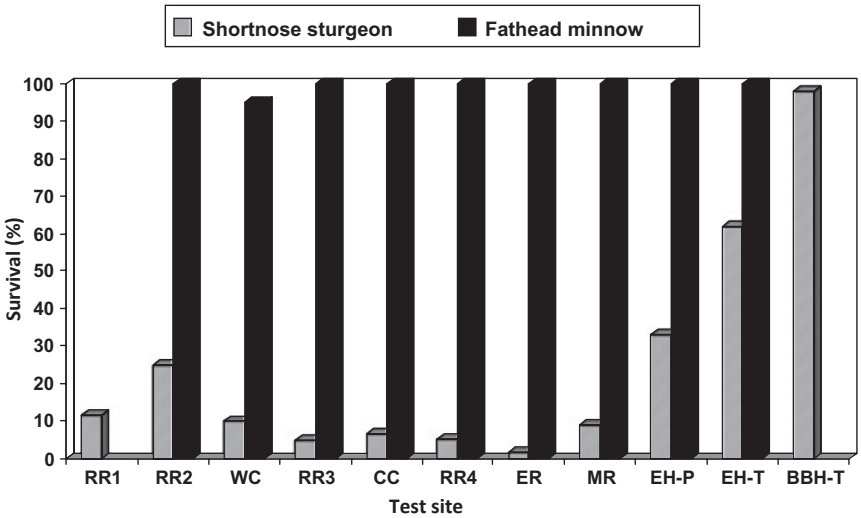


Fig. 3. Survival of shortnose sturgeon and fathead minnows at each Roanoke River and control test site on day 22 of *in situ* toxicity test 4 May–1 June 2005. Riverine test sites and abbreviations: Roanoke River main stem, RR1, RR2, RR3, RR4; Eastmost River, ER; Middle River, MR; Welch Creek, WC; Conaby Creek, CC. Control test sites and abbreviations: Edenton National Fish Hatchery tank (EH-T) or pond (EH-P) and Bears Bluff National Fish Hatchery tank (BBH-T). Data not shown for RR1 fathead minnow sample because of a single wind-driven water fluctuation that caused dessication; no other sites or cages were affected similarly during this high wind event. Data not shown for BBH-T fathead minnow sample because fathead minnows were not permitted to be kept at the hatchery due to potential disease transmission concerns to sturgeon at the hatchery

outdoor pond at the ENFH were all well nourished, had no remarkable microscopic abnormalities, and could be considered relatively normal controls. Their gastrointestinal (GI) tracts were filled with various invertebrate species, indicating that sturgeon were able to feed on natural prey while held in the test cages. Likewise, sturgeon from the riverine sites did not have empty GI tracts or fatty livers (an indicator of lack of feeding).

Sturgeon from RR1 and ER had no remarkable lesions. However, in sturgeon from the remaining river test sites, the liver of most individuals had microscopic changes that were of two primary types; hepatocellular necrosis – loss of hepatocytes with atrophy of the remaining hepatic parenchyma (a few of these fish also had multifocal necrosis of the renal tubules) and neutrophilic hepatitis, characterized by infiltration of variable numbers of mainly neutrophilic granulocytes. In some sturgeon, these two changes occurred together, but in most specimens one lesion type predominated over the other. In addition, the nuclei of scattered swollen and injured hepatocytes in the liver of 9 of 23 sturgeon from the riverine sites were greatly expanded by large, basophilic inclusions. The specific nature of these inclusion bodies was not further discerned by transmission electron microscopy, and no viral particles were found.

As stated previously, the poor survival of shortnose sturgeon at the river sites precipitated the early termination of sturgeon cages on day 22 of 28, and even at that early termination date, precluded having adequate mass of sturgeon tissue for contaminant analyses. Therefore, there are no contaminant bioaccumulation data available for shortnose sturgeon from the *in situ* test, as originally planned.

Fathead minnows

In contrast to the poor survival of shortnose sturgeon, fathead minnow survival averaged 99.4% at all test sites over the 28-day test period (Fig. 3). The only mortality was the death of a single fathead minnow on day 3 at the WC site. Omnibus F tests revealed statistically significant differences in mean length ($F_{11/225}$, 3.76; $P < 0.0001$), wet weight ($F_{11/225}$, 8.70; $P < 0.0001$), and condition ($F_{11/225}$, 7.24; $P < 0.0001$) among fathead minnows from the baseline samples and those recovered from riverine test sites and control sites on day 28 (Fig. 4). Experiment-wise multiple comparison demonstrated that mean length and mean weight were significantly greater in fathead minnows recovered from the ENFH-tank ($P < 0.05$), but differences could not be distinguished among the other groups of fish ($P > 0.05$). When applied to mean condition, multiple comparison procedures showed significantly greater mean condition for fathead minnows recovered from the ENFH-tank, and significantly lower mean condition for fish recovered from the WC exposure site ($P > 0.05$; Fig. 4), compared to the other sites. Point estimates of lipid content (%) were lower in groups of fathead minnows recovered from control and test sites at the conclusion of the study, compared to those used to establish baselines (baseline, 3.18%; RR2, 1.05%; WC, 0.87%; RR3, 0.61%; CC, 1.35%; RR4, 0.78%; ER, 0.59%; and MR, 0.97%; ENFH-tank, 2.31%; ENFH-pond, 0.86%).

Organic contaminant residues (OC pesticides and their metabolites, CUPs, and PCBs) in tissues of fathead minnows used to establish baselines were below detection limits (PCBs and OC pesticides, 0.2 ng g^{-1} dry weight; CUPs, 1.0 ng g^{-1} dry weight). No PCBs, OC pesticides, or CUPs were detected in the tissue of fathead minnows recovered from the *in situ* test sites on day 28, with detection limits at 1 ng g^{-1} dry weight and below. We did not analyze for PAHs in the tissues of fathead minnows because vertebrates rapidly metabolize and eliminate PAHs. Overall, the organic chemicals analyzed did not accumulate appreciably in the tissues of the fathead

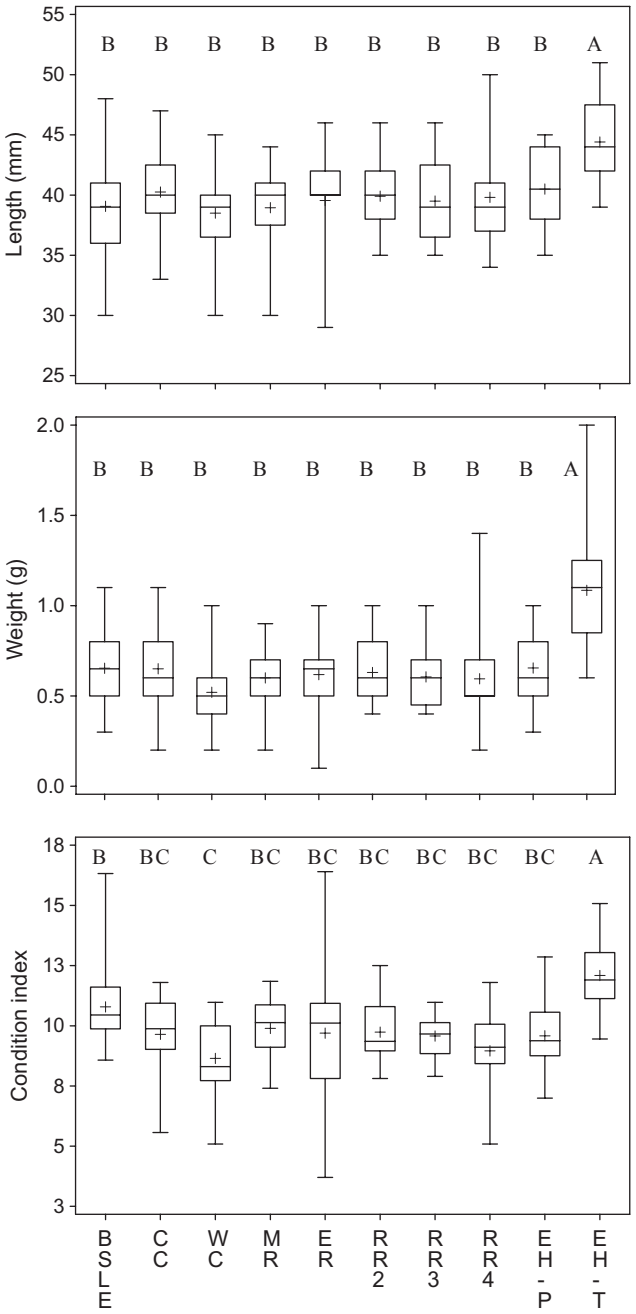


Fig. 4. Total length (mm), wet weight (g), and Fulton-type condition index of fathead minnows from the baseline (BSLE) sample and those recovered from cages at test sites in the Roanoke River or in the Edenton National Fish Hatchery tank (EH-T) or pond (EH-P), designated control sites on day 28 of *in situ* toxicity test 4 May–1 June 2005. Riverine test sites and abbreviations: Roanoke River main stem, RR1, RR2, RR3, RR4; Eastmost River, ER; Middle River, MR; Welch Creek, WC; Conaby Creek, CC. Data not shown for RR1 fathead minnow sample because of a single wind-driven water fluctuation that caused dessication—no other sites or cages were affected similarly during this high wind event. Sites having the same letter = no significant difference ($P > 0.05$) in experiment-wise comparisons

Table 1
Concentrations (mg kg^{-1} dry weight) of metals measured in whole fathead minnow samples before (baseline, BSLE) and after deployment in lower Roanoke River during 28-day *in situ* toxicity test, 4 May–1 June 2005

	Test sites								Control sites	
Metal	BSLE	RR2	WC	RR3	CC	RR4	ER	MR	ENFH-tank	ENFH-pond
Copper	7.4	7.6	26.4	10.7	22.3	10.7	12.0	11.1	16.5	12.0
Lead	2.6	–[a]	–	–	–	–	–	–	–	–
Mercury	0.18	0.29	0.38	0.37	0.32	0.34	0.32	0.31	0.06	0.13
Selenium	–	–	3.3	–	–	–	2.9	–	2.8	–
Zinc	167	200	235	222	204	168	201	193	139	170

RR, Roanoke River mainstem; ER, Eastmost River; MR, Middle River; WC, Welch Creek; CC, Conaby Creek and two sites at US Fish and Wildlife Service, Edenton National Fish Hatchery (ENFH) as controls (ENFH-tank, ENFH-Pond).
[a]Concentration < minimum reporting level (MRL); lead and selenium MRL = 2.5 mg kg^{-1}.

minnows over the 28-day exposure. In contrast, several metals accumulated in the fathead minnow tissue during the 28-day test (Table 1). Specifically, Cu, Hg, and Zn were measured in fish from each of the sites, whereas Pb and Se were detected at concentrations greater than the minimum reporting level at only several of the sites. The greatest measurable concentrations of all metals detected in fish tissue occurred at the WC site.

Water

The mean values of water quality variables for the test sites were: temperature, 19.3–19.6°C; DO, 5.2–6.9 mg L^{-1}; pH, 6.9–7.3; conductivity, 107–136 μS cm^{-1}; alkalinity as $CaCO_3$, 34.4–40.6 mg L^{-1}; hardness as $CaCO_3$, 33.0–36.0 mg L^{-1}; TAN, 0.04–0.07 mg L^{-1}; and turbidity, 10.2–19.2 NTU (Table 2). There was no statistically significant among-site variation in the mean values of water hardness, alkalinity, pH, turbidity, or water temperature. There were, however, statistically significant differences in the means of TAN ($F_{7/56}$, 3.44; $P = 0.0039$), DO ($F_{7/189}$, 5.28; $P < 0.0001$), and water conductivity ($F_{7/194}$, 37; $P < 0.0001$) values, among test sites (Table 2).

Analyses of the PSDs did not reveal the presence of PCBs or current use pesticides (CUPs) in the water at the test sites. In analyses for OC pesticides, only chlordane-related chemicals were detected, and they were all below 1 ng L^{-1}. The sum of the 16 United States Environmental Protection Agency (US EPA) Priority Pollutant PAHs (PP PAHs) were generally very low, ranging from 5.9 ng L^{-1} at the ENFH pond to 203 ng L^{-1} at the WC site and total PAHs from 42 ng L^{-1} at the ENFH pond to 699 ng L^{-1} at the RR4 site. The WC site was the only location that had a significant contribution from pyrogenic (combustion-related) PAHs at 210 ng L^{-1}. The PSDs from the RR3, RR4, CC, and ER sites, which were located downstream of the primary permitted discharger in the sub-basin, were dominated by extraordinarily high concentrations of retene (7-isopropyl-1-methylphenanthrene), ranging from 251 ng L^{-1} (at site CC) to 603 ng L^{-1} (at site RR4) at these four sites. All other test sites had concentrations of retene that ranged from 1.4 to 19.0 ng L^{-1}.

Sediment

The total organic carbon content of sediment among seven of eight river test sites was similar (mean 2.5%, range 1.8–3.3%) with only the WC site having a slightly greater proportion (9.4%) of total organic carbon than the others, indicating relative consistency among sites for sediment composition. Analyses of sediment collected from test sites did not show the presence of any CUPs. Concentrations of PCBs in sediment were low or non-detectable. The primary PCB congeners that were detected were 101, 118, 138, and 153. Low concentrations of 4, 4′-DDE (the primary persistent aerobic metabolite of DDT) were detected at half of the exposure sites. Sediment sampled from RR2 contained a higher concentration of the parent 4, 4′-DDT, though the concentration was quite low (10.7 ng g^{-1} dry weight). PAH concentrations in the sediment were generally low and exhibited contributions from both petrogenic (petroleum-related) and pyrogenic sources (Table 3). The major exception to the relatively low PAH concentrations in sediment was for the WC test site (Table 3). The sum of the 16 US EPA PP PAHs at the WC test site was 3–5 times higher than that at other sites. The sum of all PAH measured in sediments from WC was 10–20 times higher than other sites. The WC station also contained very high concentrations of petrogenic PAHs relative to the other sites and moderately higher pyrogenic PAH. Retene was extraordinarily high at this site (Table 3). The concentration of retene at the WC site was nearly 5000 ng g^{-1} dry weight, indicating a very strong influence from pulp and paper mill effluent. Retene is often included in the sum of pyrogenic PAH, because its most common source is wood smoke; however, we did not include retene in the sum of pyrogenic PAH, because the primary source in this area is likely the mill effluent. In contrast to the sediment, the PSDs deployed at WC did not have particularly high retene concentrations. Several metals were detected in sediments from all test sites (Table 4). Measured concentrations of Sb, Be, Cr, Cu, Pb, Hg, Ni, and Zn were all relatively consistent among most of the sites, with the notable exception of the WC site, which had the greatest concentrations of each of the metals measured.

Shortnose sturgeon survival and environmental conditions

A pairwise correlation matrix of shortnose sturgeon survival (Fig. 3) and environmental parameters (physicochemical variables, Table 2; Sum of PAH, Table 3; metal concentrations Table 4) measured at riverine sites failed to detect any significant correlation between site specific fish survival and ambient contaminant concentrations ($P > 0.05$ for PAH sum and metals), but among physicochemical variables, water hardness and conductivity were significantly correlated with survival ($P < 0.05$). There is no biological basis to support causal mechanisms for these fish survival relationships with water ionic content, which suggests that the variably low

Table 2
Mean physicochemical variables (standard deviation in parentheses) of water at study sites during 28-day *in situ* toxicity test. 4 May–1 June 2005

Site	Alkalinity[a] (mg L^{-1} $CaCO_3$)		pH[a]		Turbidity[a] (NTU)		Temperature[b] (°C)	Hardness[a] (mg L^{-1} $CaCO_3$)		DO[b] (mg L^{-1})		Conductivity[b] (μS cm^{-1})		Total ammonia[a] nitrogen (mg L^{-1})	
CC	34.4	$(13.2)^{A}$	7.1	$(0.2)^{A}$	13.2	$(3.9)^{A, B}$	19.6	35.5	$(6.3)^{A}$	6.5	$(1.3)^{A}$	136	$(10)^{A}$	0.04	$(0.02)^{B}$
ER	40.4	$(11.4)^{A}$	7.3	$(0.2)^{A}$	14.0	$(3.8)^{A, B}$	19.6	35.7	$(8.1)^{A}$	6.7	$(0.9)^{A}$	135	$(10)^{A}$	0.07	$(0.03)^{A}$
MR	35.7	$(9.9)^{A}$	7.2	$(0.2)^{A}$	19.2	$(6.4)^{A}$	19.6	35.0	$(6.4)^{A}$	6.8	$(1.1)^{A}$	109	$(10)^{C}$	0.04	$(0.01)^{B}$
RR1	34.7	$(7.2)^{A}$	7.2	$(0.2)^{A}$	10.8	$(2.8)^{B}$	19.3	34.3	$(6.2)^{A}$	6.8	$(1.1)^{A}$	107	$(4)^{C}$	0.05	$(0.01)^{A, B}$
RR2	34.6	$(7.4)^{A}$	7.2	$(0.1)^{A}$	13.3	$(7.0)^{A, B}$	19.4	33.0	$(6.5)^{A}$	6.7	$(1.3)^{A}$	107	$(5)^{C}$	0.05	$(0.01)^{A, B}$
RR3	37.8	$(13.3)^{A}$	7.2	$(0.3)^{A}$	14.7	$(3.3)^{A, B}$	19.6	35.6	$(6.5)^{A}$	6.9	$(1.1)^{A}$	135	$(11)^{A}$	0.06	$(0.01)^{A, B}$
RR4	40.6	$(9.9)^{A}$	7.2	$(0.1)^{A}$	10.2	$(1.7)^{A, B}$	19.6	36.0	$(6.9)^{A}$	6.5	$(1.1)^{A}$	135	$(10)^{A}$	0.07	$(0.01)^{A, B}$
WC	35.7	$(14.8)^{A}$	6.9	$(0.4)^{A}$	14.8	$(10.0)^{A, B}$	19.6	35.8	$(9.7)^{A}$	5.2	$(1.3)^{B}$	121	$(21.4)^{B}$	0.06	$(0.03)^{A, B}$

RR, Roanoke River main stem; ER, Eastmost River; MR, Middle River; WC, Welch Creek; CC, Conaby Creek. Values with same letter not significantly different among sites.
[a]measured every 72 h.
[b]measured every 24 h.

shortnose sturgeon survival we observed cannot be attributed to any single measured parameter. Furthermore, virtually all of the pairwise comparisons among contaminant concentrations (i.e. PAH sum and eight metals) were significant ($P < 0.05$), with the exception of that between beryllium and zinc ($r = 0.683$, $P = 0.062$). This multicollinearity among measured contaminants at riverine sites describes a gradient in overall site contamination that is strongly influenced by high contaminant concentrations measured at the Welch Creek site.

Discussion

The aim of our study was to determine the suitability of present conditions in the lower Roanoke River for supporting shortnose sturgeon – whether naturally occurring or stocked. Given the poor survival of shortnose sturgeon at the riverine test sites, greater survival of sturgeon at the non-riverine control sites (even at the sub-optimal ENFH pond), and the high survival of fathead minnows at the riverine sites alongside the sturgeon, our results indicate that conditions in the lower Roanoke River are incongruous with the needs of juvenile shortnose sturgeon. These findings are especially important because the *in situ* test was conducted at a time of year (May-June) and location in the river (lower segment, 3.5–11 river km upstream of Batchelor Bay, Albemarle Sound) in which, if present, juvenile shortnose sturgeon would be expected to reside (Vladykov and Greeley, 1963; Armstrong, 1999). For example, studies of shortnose sturgeon in the Cooper River system of South Carolina found that after spawning (between March 4 and 25 when water temperatures were between 10 and 16°C; Duncan et al., 2004), larvae emerge and migrate downstream to nursery areas of low salinity near the fresh-brackish water interface (Collins et al., 2003). Therefore, if a viable and reproducing population of shortnose sturgeon were to exist in the Roanoke River, they would presumably spawn within the same general time frame (March) as the South Carolina population (below the first dam 151 km upstream of Albemarle Sound) and after hatching, would likely migrate downstream and develop in or near the section of river tested in this study.

The survival, habitat use, and movement of shortnose sturgeon within the section of the river studied here, whether natural or stocked populations, is influenced by various chemical and physical water quality conditions such as DO, temperature, salinity, and toxic contaminants, among others, many of which were measured in this study. Therefore, the potential for these measured variables to influence sturgeon survival and condition can be directly assessed by comparison to published values or criteria. For example, Campbell and Goodman (2004) evaluated the acute sensitivity of juvenile (77–134 day old) shortnose sturgeon to low DO concentrations over a range of water temperature (21.8–29.2°C) and salinity (2.0–4.5 ‰) in laboratory tests and found that the 24-h median lethal concentration (LC50) for DO ranged from 2.2 to 3.1 mg L^{-1}. The average DO concentrations and water temperatures at our study sites ranged from 5.2 to 6.9 mg L^{-1} and 19.3–19.6°C. The test sturgeon in our study were 50 day old at the time of deployment, and salinity concentrations at the study sites averaged 0.1‰. Thus, based on fish age, DO, temperature, and salinity conditions in our study compared to the findings of Campbell and Goodman (2004), it is unlikely that these variables alone had a substantial influence on the poor survival of shortnose sturgeon in our test. The US EPA recently evaluated the data of Campbell and Goodman (2004)

Table 3
Concentrations of PAHs in sediments at study sites, lower Roanoke River during 28-day *in situ* toxicity test, 4 May–1 June 2005

Contaminant	Blank	Test sites							
		RR1	RR2	WC	RR3	CC	RR4	ER	MR
Moisture (%) in sample		49.7	54.8	67.3	55.4	45.7	51.8	57.0	52.5
PAHs in sediment (ng g^{-1} dry weight)									
Naphthalene	0.0	2.7	0.0	28.6	3.5	2.1	0.0	2.4	3.3
2-Methylnaphthalene	0.0	3.6	3.1	107.1	4.1	1.4	2.7	3.6	3.8
1-Methylnaphthalene	0.0	2.0	2.3	68.7	2.9	1.0	2.4	2.5	2.7
Biphenyl	0.0	1.8	1.5	22.7	1.7	1.0	1.1	1.2	1.4
2,6-Dimethylnaphthalene	0.0	0.0	0.0	120.8	0.0	0.0	0.0	0.0	0.0
Acenaphthylene	0.0	16.6	6.4	42.0	11.5	1.4	10.1	7.9	7.0
Acenaphthene	0.0	1.2	0.8	26.0	2.3	0.0	1.4	1.3	1.1
Dibenzofuran	0.0	2.1	1.8	48.1	2.0	1.2	1.8	1.8	1.6
2,3,5-Trimethylnaphthalene	0.0	1.2	1.3	53.9	2.3	0.5	1.6	1.3	1.2
C1 – Naphthalenes	0.0	5.8	5.6	174.8	7.3	2.6	4.9	6.0	6.3
C2 – Naphthalenes	0.0	13.4	13.7	447.8	17.9	6.0	14.1	14.4	13.4
C3 – Naphthalenes	0.0	15.1	19.1	585.4	28.0	8.0	18.7	17.3	18.0
C4 – Naphthalenes	0.0	10.7	13.1	341.7	19.5	5.5	12.3	11.2	11.2
Fluorene	0.0	4.3	2.9	62.1	4.3	1.2	3.3	3.2	3.0
1-Methylfluorene	0.0	1.7	1.4	46.6	2.3	0.7	1.7	1.4	1.5
C1 – Fluorenes	0.0	6.2	5.8	104.0	6.9	2.4	5.0	4.9	5.3
C2 – Fluorenes	0.0	17.8	0.0	230.4	15.1	1.4	12.8	11.9	0.0
C3 – Fluorenes	0.0	0.0	0.0	245.8	17.2	0.0	14.8	17.0	0.0
Dibenzothiophene	0.0	2.0	1.6	39.4	1.9	0.6	1.7	1.7	1.5
C1 – Dibenzothiophene	0.0	5.1	4.4	104.3	5.1	1.3	4.0	3.7	3.4
C2 – Dibenzothiophene	0.0	9.1	9.4	181.5	10.8	2.7	7.6	7.0	8.3
C3 – Dibenzothiophene	0.0	10.9	0.0	0.0	18.0	3.4	10.3	8.6	0.0
Phenanthrene	0.0	24.2	15.5	239.7	16.7	5.5	20.4	19.8	13.6
Anthracene	0.0	12.1	6.3	83.6	8.2	1.3	11.1	6.9	5.9
1-Methylphenanthrene	0.0	7.5	5.6	110.1	6.9	2.1	6.8	6.7	4.3
C1 – Phenanthrenes/Anthracenes	0.0	45.3	33.3	411.0	34.2	9.1	31.7	31.9	26.8
C2 – Phenanthrenes/Anthracenes	0.0	43.6	29.5	433.0	40.4	9.7	28.6	29.0	30.3
C3 – Phenanthrenes/Anthracenes	0.0	32.0	27.7	780.6	38.6	10.9	28.4	37.1	23.0
C4 – Phenanthrenes/Anthracenes	0.0	29.3	21.3	238.8	28.6	9.4	25.0	19.2	20.6
Fluoranthene	0.0	60.7	41.1	625.7	50.6	15.3	71.8	52.3	40.5
Pyrene	1.8	78.7	49.1	449.7	65.2	16.2	67.9	56.7	46.3
C1 – Fluoranthenes/Pyrenes	0.0	85.0	41.4	321.0	57.5	12.3	52.4	43.4	38.6
Retene	4.6	39.5	59.2	4910	108	37.4	101	206	37.4
Benz[a]anthracene	0.0	42.2	25.9	123.0	34.2	9.0	43.9	30.0	24.7
Chrysene	0.0	42.4	27.6	209.1	34.5	11.2	48.3	33.6	26.7
C1 – Chrysenes	0.0	37.8	21.6	95.6	30.7	6.6	23.4	22.5	19.4
C2 – Chrysenes	0.0	24.1	15.3	156.0	23.3	5.4	15.2	16.8	14.6
C3 – Chrysenes	0.0	10.2	8.6	80.0	10.6	2.9	6.3	7.4	7.6
C4 – Chrysenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Benzo[b]fluoranthene	0.0	79.8	56.5	185.1	62.7	21.9	75.0	60.0	50.1
Benzo[k]fluoranthene	0.0	21.3	14.1	39.0	16.2	5.5	20.5	15.5	12.8
Benzo[e]pyrene	0.0	39.1	25.6	85.6	29.1	9.5	32.5	27.0	23.0
Benzo[a]pyrene	0.0	38.0	23.6	36.9	32.5	0.0	31.1	20.8	18.5
Perylene	0.0	292	300	206	284	330	158	112	339
Indeno[1,2,3-cd]pyrene	0.0	62.1	36.8	36.6	44.4	15.0	43.4	41.9	32.8
Dibenz[a,h,]anthracene	0.0	11.5	6.6	7.1	8.2	2.8	8.6	7.6	6.2
Benzo[g,h,i]perylene	0.0	38.4	24.0	27.2	28.0	10.2	28.4	26.0	21.6
Coronene	0.0	12.2	6.6	3.6	7.7	2.8	6.9	6.7	5.4
Sum of PAH	6.4	1343	1018	12972	1286	606	1120	1068	984
Sum of 16 PP	1.8	786	612	2305	673	440	600	469	628
Sum of Petrogenic PAH	0.0	423	282	5182	427	106	331	327	261
Sum of Pyrogenic PAH	1.8	568	359	2100	444	125	511	404	329

Blank, procedural analytical blank; RR, Roanoke River main stem; ER, Eastmost River; MR, Middle River; WC, Welch Creek; CC, Conaby Creek.

with regard to the issuance of ambient water quality criteria to protect shortnose sturgeon in the Chesapeake Bay and its tidal tributaries (US EPA, 2003). They determined that DO criteria protective of sturgeon could be developed on the basis of non-stressful temperatures (22–26°C) and stressful temperatures (≥29°C). Under conditions of non-stressful temperatures, the US EPA calculated that a DO concentration of 3.2 mg L^{-1} should be protective of sturgeon and that during stressful temperatures, a DO of 4.3 mg L^{-1} would be needed to protect sturgeon. Even if these more restrictive criteria were applied to the conditions measured during our test, the fish would have experienced no impairment of DO concentrations. Thus, it can be reasonably deduced that the interaction of DO and temperature during our tests was not a likely cause of the observed poor survival of sturgeon.

Ammonia is another water quality variable with the potential to influence survival of test fish in our study for which shortnose sturgeon-specific data are available. Mean measured concentrations of TAN during the test ranged from 0.04 to 0.07 mg L^{-1}. These concentrations are well below the

Table 4
Concentrations (mg kg^{-1} dry weight) of metals measured in surficial sediment samples, lower Roanoke River during 28-day *in situ* toxicity test, 4 May–1 June 2005

	Sediment quality guidelines[a]		Test sites							
Metal	TEC	PEC	RR1	RR2	WC	RR3	CC	RR4	ER	MR
Antimony	–	–	0.7	0.8	1.4	0.5	0.6	0.5	0.5	0.9
Beryllium	–	–	0.15	0.17	0.31	0.13	0.22	0.16	0.17	0.15
Chromium	43.4	111	20.1	20.6	96.7	18.1	20.4	19.5	20.1	21.4
Copper	31.6	149	3.9	4.8	11.7	2.1	3.7	3.9	3.1	4.1
Lead	35.8	128	10.9	9.7	15.4	8.5	8.1	9.7	9.9	9.9
Mercury	0.18	1.06	0.05	0.09	1.76	0.08	0.07	0.06	0.07	0.08
Nickel	22.7	48.6	7.2	7.6	11.2	7.0	7.7	7.5	7.8	7.8
Zinc	121	459	58.3	55.2	92.8	51.4	39.8	55.6	56.9	58.7

RR1, RR2, RR3, RR4, Roanoke River mainstem; ER, Eastmost River; MR, Middle River; WC, Welch Creek; CC, Conaby Creek.
[a]Consensus-based sediment quality guideline from MacDonald et al. (2000); TEC is the threshold-effects concentration and PEC is the probable-effects concentration.

96-h LC 50 of 149.8 mg L^{-1} TAN for fingerling shortnose sturgeon (Fontenot et al., 1998). Therefore, concentrations of TAN did not likely contribute to the reduced survival of shortnose sturgeon in our test.

Historically, dioxin, mercury, and other contaminants from wood pulp and paper production and mercury-based chlorine production have been documented in water, sediment, and biota of the lower Roanoke River (RMT, Inc., 2003; NOAA, 2004; NCDENR, 2006). Our analysis of toxic contaminants in water and sediment that may have influenced the survival and condition of shortnose sturgeon and fathead minnows in this study indicated a relatively uncontaminated environment with regard to PCBs, OC pesticides, and CUPs, but with low to moderate contamination by PAHs and certain metals. For example, analysis of sediments for organic contaminants at the test sites revealed low or non-detectable concentrations of PCBs, and specific congeners. Concentrations of 4, 4′-DDE (the primary persistent aerobic metabolite of DDT) were detected at half of the exposure sites. The parent 4, 4′-DDT was found at the RR2 site at 10.7 ng g^{-1} dry weight. Although the concentration at RR2 does not warrant concern regarding toxicity, this is a relatively recent source of DDT of unknown origin. The sediments at the WC test site showed a strong influence from mill-effluent, as expected given the history of the site. The sum of the 16 US EPA priority pollutant PAHs was 3–5 times higher in the sediments of WC than at the other sites (Table 3). Furthermore, the sum of all PAHs in the sediments at WC was 10–20 times greater relative to other test sites, with very high concentrations of petrogenic PAHs, and moderately higher pyrogenic PAHs. The concentrations of total PAHs measured in sediment at all test sites, except for the WC site, were less than the threshold-effects concentration (TEC) of 1610 μg kg^{-1} dry weight for total PAHs (MacDonald et al., 2000), the value below which effects to sensitive aquatic organisms are not expected to occur. The total PAHs measured at the WC site (12 972 ng g^{-1} dry weight) were greater than the TEC, but less than the probable-effects concentration (PEC) of 22 800 μg kg^{-1} dry weight (MacDonald et al., 2000), a value above which effects to sensitive aquatic organisms may be expected. However, given that shortnose sturgeon tend to associate strongly with bottom sediments (Collins et al., 2002), exposure to PAHs may be of toxicological concern for sturgeon in this area. For example, Kocan et al. (1996), who studied the toxicity of sediment-derived weathered coal tar PAHs to shortnose sturgeon embryos and larvae, found that PAH uptake and subsequent toxicity was due largely to direct contact of the embryos with contaminated sediment rather than from exposure to soluble (waterborne) PAHs. Moreover, for the 10 parent PAH compounds for which sediment quality guidelines have been derived (MacDonald et al., 2000), the sediments at the WC site exceeded the TEC for six (phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, and chrysene), but were not greater than their respective PEC for these PAHs. Interestingly, the single fathead minnow that died during the 28-day study occurred at the WC site. The mean condition of fathead minnows recovered from the WC site at the end of the study was the lowest, but not significantly less ($P > 0.05$) than that of fathead minnows from the other test sites (Fig. 4).

The PAH retene [bleached-kraft pulp mill (BKME) derived], was measured at extraordinarily high concentrations in the sediments of Welch Creek and other nearby test sites in our study. Retene is dioxin-like in its toxicity to early life stages of fish and is formed through two pathways, the thermal degradation of abietic resin acids during the combustion of coniferous woods (Ramdahl, 1983; Mazurek and Cass, 1991; Hodson et al., 2007) and during the anaerobic microbial degradation of dehydroabietic acid (DHAA) during the bleached-kraft pulp mill process. Thus, retene is often used as a molecular tracer of wood smoke, but residues have been found in sediments receiving pulp and paper mill effluents (Judd et al., 1995, 1996; Koistinen et al., 1998). Background concentrations of retene in freshwater and estuarine sediments of eastern North Carolina are typically between 10 and 50 ng g^{-1} dry weight (D. Shea, North Carolina State University, Raleigh, North Carolina, unpubl. data) and have been reported to range from 10 to 100 ng g^{-1} in other coastal sediments (Bouloubassi and Saliot, 1993). Concentrations of retene up to 3300 μg g^{-1} dry weight have been measured in aquatic sediments near a pulp mill in Finland (Leppänen and Oikari, 1999). The concentration of retene at the test site on Welch Creek was nearly 5000 ng g^{-1} dry weight, indicating a very strong influence from the mill effluent. In contrast to the sediments, the PSDs deployed in the water column at Welch Creek did not demonstrate high retene concentrations (an expected result because the current discharge of mill effluent enters directly into the mainstem of the Roanoke River rather than into Welch Creek). However, the PSDs from test sites RR3, RR4, CC, and ER, which were downstream of the mill, the current primary permitted discharger in the basin, were

dominated by extraordinarily high concentrations of retene (251–603 ng L^{-1}).

The toxicological consequences of retene exposure to various fish species have been evaluated, but not specifically with shortnose sturgeon. In rainbow trout, *Oncorhynchus mykiss*, retene binds to aryl hydrocarbon receptors, induces cytochrome P450 monooxygenase 1A (CYP1A), and is excreted in the bile (Fragoso et al., 1998, 1999; Hodson et al., 2007). Increased metabolism and excretion suggest that toxicity results from retene metabolites (Hodson et al., 2007). Alternatively, toxicity is a consequence of oxidative stress, similar to that observed in exposures to dioxin (Hawkins et al., 2002). Retene has been shown as teratogenic to fish embryos at low concentrations in water (10–32 $\mu g\ L^{-1}$), and fish eggs in contact with retene contaminated sediments are at risk for toxicity (Oikari et al., 2002). Induction of liver cytochrome P450 monooxygenase enzymes is characteristic of retene's bioactivity (0–320 $\mu g\ L^{-1}$ exposure range, Hodson et al., 2007). Retene metabolites have been detected in the bile of roach, *Rutilus rutilus,* a benthic fish residing in areas associated with kraft-mill effluent (Leppänen and Oikari, 1999). The concentrations and effects of retene in the water and sediments measured at sites in our study warrant further investigation. Metals were also measured in sediments and fathead minnow tissue from the test sites, with a pattern similar to that previously described for the organic contaminants. The concentrations of metals measured in sediment at all test sites, except for the WC site, were less than the TECs (MacDonald et al., 2000) of the respective metals (Table 4). At the WC site, the measured concentration of chromium (96.7 mg kg^{-1} dry weight) was greater than the TEC of 43.4 mg kg^{-1} dry weight and the measured concentration of mercury (1.76 mg kg^{-1} dry weight) at this site greatly exceeded both the TEC (0.18 mg kg^{-1}) and PEC (1.06 mg kg^{-1}). Thus, adverse effects to sensitive sediment-dwelling or sediment-associated aquatic organisms may be expected from mercury exposure at this site. The relation to potential chronic toxicity to fish at this and the other test sites remains uncertain.

Our correlational attempts to identify relationships between shortnose sturgeon survival and environmental conditions and comparisons with published water quality and contaminant criteria implicated no one single factor to explain the variably low fish survival we observed, despite the occurrence of a gradient of overall contamination among riverine sites. In the absence of direct chemical, physical, or environmental conditions to unequivocally explain the mortality of shortnose sturgeon in our study, combined with the lack of mortality in fathead minnows deployed alongside the sturgeon, we attribute the rapid and almost complete mortality of sturgeon in 22 days to the liver and kidney lesions observed during histopathology analysis as the proximate cause of mortality. At the time of sampling, the lesions were of two main types, namely, necrosis and loss of liver tissue (and kidney in a few fish), and mild to marked inflammation. Because these two main lesion types occurred together in some fish, it is most likely that these two presentations represent a continuum of the same disease process. While our histological findings clearly reveal the proximate factors leading to mortality, the ultimate environmental or disease stressors remain unclear even after our intensive field investigation. A viral or other similar disease remains a potential cause of the mortality because viral diseases are often species-specific, affecting the sturgeon in this study, but not the fathead minnows. Several viral diseases have been reported in sturgeon (Hedrick et al., 1992; Watson et al., 1995; Georgiadis et al., 2001). However, with electron microscopy, we found no evidence of viral infection. The isolation of a virus as a causative agent would require additional research beyond the scope of this study. Moreover, the fact that the shortnose sturgeon at the riverine test sites with unexpected mortalities did not have empty GI tracts, nor were their livers vacuolated, suggests that these fish fed adequately, did not die of starvation, and that any 'cage effect' due to location was minimal. The cause of death of these fish remains unresolved, but at least two possibilities exist. First, there may be a specific stressor (e.g. toxicant or disease) or combination of stressors unmeasured in our study leading to mortality; or second, the cumulative or synergistic effects of several stressors of marginal detriment alone may combine to cause the observed fish mortality.

Nonetheless, our results suggest that the conditions in the lower Roanoke River and its tributaries were not suitable to support juvenile shortnose sturgeon at the time of the study. We identified several toxicant or disease factors that may prove critical in additional directed research to further elucidate such parameters and mechanisms. In addition, our findings with *in situ* testing in the natural river environment support the sensitivity of shortnose sturgeon relative to fathead minnows reported by Dwyer et al. (2005a,b) in laboratory studies and point to the strong utility and applicability of *in situ* toxicity testing for evaluating site-specific conditions for protecting and managing a species of interest, rather than relying on surrogate test species like the fathead minnow. Clearly, additional research is needed to identify specific limiting factors and management actions for the enhancement and recovery of this imperiled fish species in the Roanoke River of North Carolina.

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