

Flow Preferences for Juvenile Robust Redhorses in an Experimental Mesocosm: Implications for Developing Sampling Protocols

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Abstract.—The robust redhorse *Moxostoma robustum*, originally described by Edward Cope in 1870 from specimens collected from the Yadkin River, North Carolina, apparently went unnoticed until 1991 when it was rediscovered in the lower Oconee River, Georgia. Despite extensive surveys and an ongoing, decade-long restoration program, wild-spawned juveniles 30–410 mm in total length have not been collected. This project experimentally evaluated one hypothesis (flow class use) that seeks to explain the absence of juvenile robust redhorses from the catch. Two experimental mesocosms were used to determine whether juvenile robust redhorses use flow classes in proportion to their availability. Pond-reared juveniles were exposed to four flow-based habitat types (eddies = -0.12 to -0.01 m/s, slow flow = 0.00 – 0.15 m/s, moderate flow = 0.16 – 0.32 m/s, and backwaters) in four 10-d trials, in which 16 pond-reared test fish were used per trial, with replacement. Location data were recorded hourly during daytime hours for each fish in all trials and evaluated with a log-linear, chi-square model. In winter, test fish showed a preference for eddies and backwaters and avoided slow to moderate flows. In early spring, test fish showed a preference for eddies and avoided the moderate flows. Current field sampling for juvenile robust redhorses has not targeted the flow classes used by fish in this experiment; however, collection of wild-caught juveniles may be improved by sampling in eddies and their associated transitional areas.

Robust redhorse *Moxostoma robustum* has been the focus of much research since its rediscovery in 1991 (e.g., Weyers et al. 2003). General background information about this species and its rediscovery are available in Ruetz and Jennings (2000). The robust redhorse population in the Oconee River, Georgia, seems to consist primarily of large adults (Evans 1994); wild juveniles ranging from 30 to 410 mm in total length (TL) have not been collected (Evans 1994; Jennings et al. 1996, 1998, 2005). The absence of juveniles in the Oconee River has been attributed to sampling gear inefficiency, sampling in areas that are not inhabited by juvenile robust redhorses, or an actual low abundance of juvenile robust redhorses. Therefore, whether the observed population structure results from failed recruitment or an inability to detect juvenile robust redhorses is not clear. As a result, the status of the Oconee River population and robust redhorse across its range and how best to manage them also are unclear.

Fossil remains suggest that historically robust redhorses occupied medium to large rivers of the Atlantic slope drainages from the Pee Dee River system in North Carolina to the Altamaha River system in Georgia (Bryant et al. 1996). At present, wild populations of the species have been found in (1) an 85-km stretch of the Oconee River between Milledgeville and Dublin, Georgia, and (2) the Savannah River in the Fall Line Zone around and below Augusta, Georgia, and North Augusta, South Carolina. A few specimens also have been collected from the Ocmulgee River of Georgia and the Pee Dee River of North Carolina (RRCC 2000). Length-frequency data suggest that the Oconee and Savannah River populations are made up mostly of larger individuals (Evans 1994; RRCC 2000).

Reintroduction effort has resulted in the stockings of tens of thousands of juvenile robust redhorses, but none of these stocked fish have been sampled. Inexplicably, stocked robust redhorses less than 385 mm TL or older than 4 years have not been captured in the Broad River (Freeman et al. 2002). What proportion of fish stocked in the Broad River has survived is unknown, but healthy robust redhorses 385 mm TL or greater and at

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FIGURE 1.—Panel (a) shows a top view of the mesocosm in which the habitat preferences of robust redhorses were studied. The backwater environment, located in the upper right-hand corner behind the parabolic-shaped polyurethane divider, is outlined. Access to the backwater is via a window in upper right corner of the bend. Panel (b) shows a top view of the mesocosm with the main-channel environments and meander bend outlined (rectangles and semicircle, respectively).

least 4 years old are captured continuously in Clark Hill Lake, Georgia, downriver from the release sites (C. Jennings, unpublished data). The capture site on Clark Hill Lake is an average of 101 km downstream from all release sites of the hatchery-reared fish (Freeman et al. 2002). Why the fish were not collected for 4 years after their release is unknown and raises questions about (1) whether the sampling methods were capable of capturing robust redhorses of less than 385 mm TL, and (2) whether all environments were sampled sufficiently to detect juvenile robust redhorses.

Given the lack of knowledge about where juvenile robust redhorses live, the goals of this investigation were to gain an understanding of how juvenile robust redhorses use available flow classes and to make

inferences about where and how to sample these fish in the wild. We used two experimental mesocosms to investigate the behavioral patterns of pond-reared juvenile robust redhorses in relation to meanders, straight channels, backwaters, and their corresponding flows. Our objective was to determine whether pond-reared juvenile robust redhorses use available flow classes in proportion to their availability.

Study Site

The experiment was conducted in mesocosms that mimicked the lower Oconee River. The lower Oconee River of central Georgia is a low-gradient, highly sinuous, sand-bedded river located in the southeastern region of the United States. Main-channel environments of the river consist of meanders and straight channels. Though not as abundant, off-channel areas such as tributaries, backwaters, and oxbows are also present (Ligon et al. 1995). These areas potentially provide juvenile robust redhorses with several environmental types for various activities (e.g., foraging, resting, predator avoidance). Off-channel environments can be reduced or lost by the elimination of high flows (Poff et al. 1997). The scarcity of off-channel areas in the Oconee River may reflect the geomorphic changes the river has undergone since the construction of Sinclair Dam in 1953 (Ligon et al. 1995).

Methods

The trials were conducted in two identical experimental tanks, each 4.87 m long, 1.22 m wide, 0.67 m high, and included three main areas. On either end of the tanks, parabolic-shaped bends made of polyurethane plastic simulated meander environments. Behind these structures was the simulated backwater environment, and within the tank were simulated straight channel areas (Figure 1). These three areas are characteristic of environments in the lower Oconee River. The mesocosms were situated under a pole barn adjacent to Whitehall Fish Laboratory at the University of Georgia, where they were exposed to ambient temperatures, artificial light (four 100-W incandescent light bulbs), and some natural light. Gravel of the same type and size (2–36 mm) as that found in the Oconee River was used as substrate in both mesocosms. Chlorine- and fluoride-free water was recirculated in a bioball-filter system for all tanks used in the study.

Water flow was generated by two Minn Kota 18-kg thrust trolling motors positioned at opposite corners on either end of the mesocosm. A grid made of yarn, with 135.5-cm² cells was superimposed on the mesocosm and used as a location reference for recording observations. Current velocity maps for both meso-

cosms were constructed by measuring water velocity with a Marsh-McBurney 2000 flowmeter just below the surface, in the middle of the water column, and just above the substrate within each cell of the superimposed grid. Two viewing platforms were constructed 2.7 m above each tank and used to make observations of the fish. The observation platform was sufficiently far away from the fish that they did not respond to the presence of the observer.

The fish in this experiment were progeny of Oconee River broodstock (2003 year-class) and were pond-reared at the Richmond Hill Fish Hatchery in Richmond Hill, Georgia. The fish were transported from Richmond Hill Fish Hatchery to Whitehall Fish Laboratory on October 26, 2004. The fish ranged from 94 to 142 mm TL and from 6.3 to 27.5 g in weight. One potential drawback of using pond-reared fish is that, having never been exposed to flowing water, they may not behave the same as wild robust redhorses in the mesocosm (Rhodes and Quinn 1998). Therefore, all of the hatchery-reared fish were placed in an acclimation tank and allowed to acclimate in flowing water for at least 6 weeks before the beginning of the experiment. Two weeks of acclimation time is probably sufficient because the hatchery-reared fish are first-generation captive fish and their phenotypes or genotypes are not likely to change (Rhodes and Quinn 1998; Deverill et al. 1999; Larsen and Pedersen 2002; Teel et al. 2003; Weber and Fausch 2003). Longer acclimation times have been suggested for second-generation captive fish because the phenotypes, genotypes, or both, of these fish may be altered (Jonsson et al. 2003; Metcalfe et al. 2003; Petersson and Jarvi 2003; Miyazaki et al. 2004).

The water used in the tanks was treated with uniodized salt (NaCl); Melafix, an antibacterial solution; and Pimafix, an antifungal solution, to prevent pathogen outbreaks. Salt was used at 3.6‰ and both Melafix and Pimafix were used at 2.2‰.

Unique, color-coded tags made of yarn were surgically attached to each fish so that the fish could be observed individually in the mesocosms. Before the tags were attached, the fish were anesthetized with tricaine methanesulfonate (MS-222) at 80 mg/L. Each fish was then measured and weighed, and the tag was surgically attached with a small suture under the skin near the first dorsal spine. After the tag was attached, the fish was placed in a bath of malachite green and formalin solution (East Riding Koi Co.) for 20 min to kill any pathogens on the fish's body (Piper et al. 1982). A few minutes after treatment for pathogens, test fish were placed in the experimental mesocosms and allowed to acclimate for 2 d before the beginning of each trial. During the trials, fish in the mesocosms

were fed frozen bloodworms daily at 1% of their body weight (Allouche and Gaudin 2001). In an effort to prevent biases of flow class selection because of food availability, the appropriate amount of bloodworms was thawed in water, and about half of the bloodworms were poured in the center of each of the straight channels, in both mesocosms. The flow of the water distributed the bloodworms proportionately around the tanks. To further prevent biases of environmental selection because of food availability, the fish were fed after the last observational period every day during each trial. As fish were fed at only 1% of their body weight, food did not accumulate in the tanks.

This study was designed to investigate whether juvenile robust redhorses used a variety of environmental conditions, based on water velocity and channel morphology, in proportion to their availability. The experiment was conducted in four 10-d trials, with eight pond-reared, juvenile robust redhorses used per trial, per mesocosm. Therefore, one trial consisted of 10 d, two mesocosms, and 16 fish. Trial 1 began December 12, 2004, and ended December 21, 2004. Trial 2 began January 11, 2005, and ended January 20, 2005. Trial 3 began February 15, 2005, and ended February 24, 2005. Trial 4 began March 9, 2005, and ended March 18, 2005. On these dates, the water temperatures at which the experiments were conducted were below the optimum for the species but reflected temperatures to which wild fish would be exposed during similar times of the year. Fish locations in the tank were determined via aerial observations. Fish were observed and their locations in the tank were recorded during the first 10 min of each hour between 0800 and 1700 hours each day for all trials. In all trials, water temperature in the tanks and ambient temperature were also recorded every hour between 0800 and 1700 hours.

On the basis of the available literature for flow classification in riverine environments (Jowett and Richardson 1994; Pert and Erman 1994; Beechie et al. 2005) and using the range of velocities that were present in each mesocosm, we divided the available flows in the mesocosm into four benthic flow classes. A detailed description of how flow velocities were mapped and the resulting flow distribution map are given in Mosley (2006). The four flow classes are identical in terms of flow value for each mesocosm. Flow class 1 (–12 to –1 cm/s) corresponds to eddies, flow class 2 (0–15 cm/s) to slow flows, flow class 3 (16–32 cm/s) to moderate flows, and flow class 4 to backwaters. Fast flows (≥ 45 cm/s) were not available in either of the mesocosms. All flow classes represent the benthic layer in the mesocosms. The amount of each flow class was similar between the two mesocosms (Mosley 2006).

Flow and temperature data were tested for normality with the Shapiro–Wilk test (SAS Institute 1999) and for constant variance with the F-Max test (Sokal and Rohlf 1995). If data were not normal or if the variances were not constant, then the data were transformed (\log_{10}). An α of 0.05 was used to evaluate the significance of all statistical tests.

A frequency table of the flows, one for each mesocosm, was created from the flow data collected. The range, mode, and mean of the flows were also determined for each mesocosm (Mosley 2006). We used a *t*-test to evaluate whether the flow distributions in mesocosm 1 and mesocosm 2 were the same (SAS Institute 1999).

An analysis of variance (ANOVA) was used to evaluate the mean temperatures among trials to determine whether there were any differences. A Waller–Duncan means separation test was conducted on the mean temperatures of the four trials to partition any similarities or differences among the temperatures (SAS Institute 1999).

Ten location observations were recorded for each fish each day. Consequently, the locations of the fish were not independent from one hour to the next. Therefore, we determined the modal location reference for each fish within a day. Thus, instead of having 10 location observations per fish per day, there was one location observation per fish per day. The modal location was used for each fish by day to make analyses on the environment used by the fish throughout the trials (J. Reeves, University of Georgia, personal communication). For each trial and mesocosm, a potential of 80 data points were used for the flow-class use analysis. A log-linear model analysis was used to evaluate the fish use data, specifically to determine whether the fish used environments differently between mesocosms, among seasons and flow class, and among all combinations of the three (SAS Institute 1999). A post hoc log-linear model analysis (with flow classes of interest removed) was used to determine if the preference of eddies, the avoidance of moderate flows, or both, contributed to the significance of the model. Average fish movement (i.e., number of changes in location) versus water temperature was determined. We used the Kruskal–Wallis test to compare the average movements among trials (SAS Institute 1999) and ran a *t*-test on the before- and after-trial subsample data to determine whether the flow distributions remained the same within and between trials.

Results

Benthic flows in mesocosm 1 ranged from -12 to $+30$ cm/s and averaged $+7$ cm/s; in mesocosm 2, they

ranged from -11 to $+32$ cm/s and averaged $+6$ cm/s. The resultant flow vectors and current velocities in the tanks were similar to these same factors in the lower Oconee River (T. Rassmussen, University of Georgia, personal communication). Mean flows in the two experimental mesocosms were significantly different ($P < 0.01$), primarily because mesocosm 2 had more cells with negative flows than mesocosm 1. The cells in mesocosm 2 had 30 times as many flows ranging from -7 to -4 cm/s as the cells in mesocosm 1 (Mosley 2006).

The mean water temperatures (Figure 2) were significantly different among trials ($P < 0.001$; SAS Institute 1999). The Waller–Duncan mean separation test grouped the mean temperatures for trials 2–4 together and separated the mean temperature of trial 1 (SAS Institute 1999). Hereafter, trial 1 is referred to as “winter” and trials 2–4 are referred to as “early spring”. Water temperatures were constant among environmental settings in each mesocosm throughout the study (Mosley 2006).

The log-linear model analysis indicated that fish use of flow classes was similar between tanks ($P = 0.97$) and seasons ($P = 0.54$); however, the use of flow classes within each mesocosm was not in proportion to their availability ($P < 0.001$). The preference for eddies and the avoidance of moderate flows contributed to the significance of the model. Flow-class use results were partitioned into winter and early spring. During the winter, fish showed a preference for eddies and backwaters (i.e., without flow) and avoided slow to moderate flows ($P < 0.001$), based on the proportion of their availability. During the early spring, the fish showed a preference for eddies, avoided the moderate flows, and used slow flows and backwaters in proportion to their availability ($P < 0.001$).

The results from the Kruskal–Wallis test for mean movements per trial indicate that there was no significant difference in fish movement among the trials. Generally, however, fish did move more as temperatures increased (Figure 3). The test fish were never observed using the water column. During trial 1, two fish died prematurely and were not replaced.

Discussion

Environmental preference and use by fish change with life stage, season, diel pattern, and specific conditions. In this study, juvenile robust redborses exhibited a preference for certain flows, and this preference was not influenced by food availability or predator presence. Instead, our results suggested that juvenile robust redborses' use of the various flow environments was influenced by flow and some seasonal component(s) such as temperature.

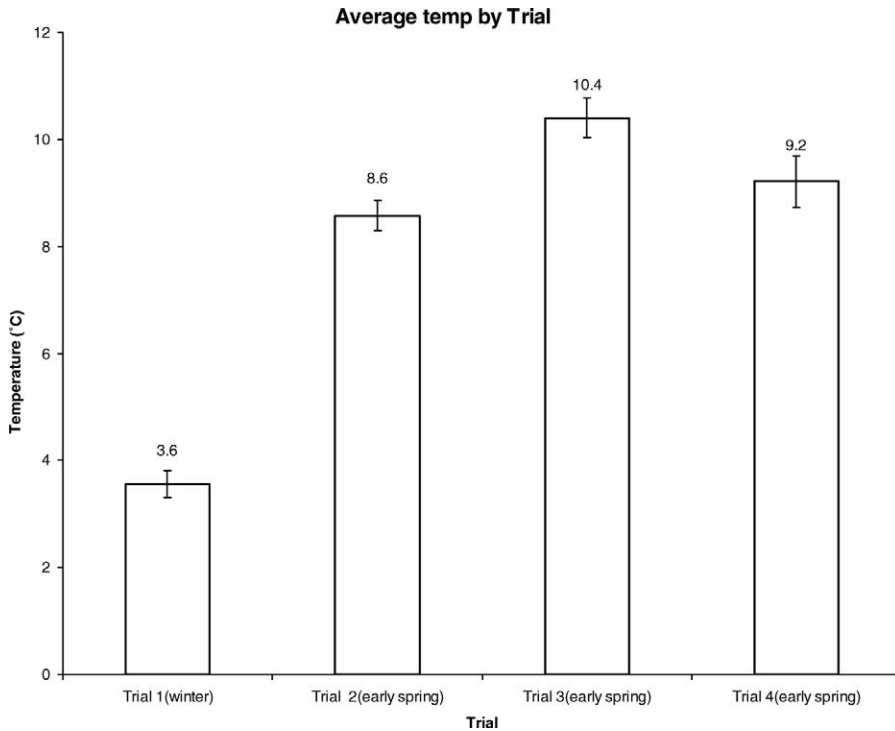


FIGURE 2.—Temperatures during the trials used to determine the flow classes preferred by juvenile robust redhorses in an experimental mesocosm. The bars represent averages, the vertical lines standard deviations.

In this study, pond-reared juvenile robust redhorses showed a preference for eddies and backwaters during winter and eddies alone during early spring. This pattern of which flow classes the fish used is similar to patterns observed for other suckers and riverine fishes in the wild. Recently released pond-reared juvenile robust redhorses may have selected deeper, slow-moving water in the Broad River, Georgia, in October 1997 (Freeman et al. 2002). Deep pools often contain large eddies along their sides (Brown et al. 2001). Adult northern hog suckers *Hypentelium nigricans* use slow deep pools that are flanked by eddies during winter (Matheney and Rabeni 1995). Juvenile bluehead suckers *Catostomus discobolus* also used slow, deep pools during the spring, as did juvenile flannelmouth suckers *C. latipinnis* in the summer (Childs et al. 1998). In the Current River, Missouri, adult northern hog suckers used rocky rapids and riffles in addition to eddies during the winter (Minckley 1963). The use by juvenile bluehead suckers and flannelmouth suckers of eddies near deepwater pools in the Little Colorado River, Arizona, during the spring and summer was hypothesized to be a trade-off between foraging efficiency and predation risk (Childs et al. 1998). A

similar mechanism may have affected environmental use of the fish in the present study.

Many riverine fishes use backwater environments during high discharge in the winter (Brown et al. 2001; Modde et al. 2001; Gurtin et al. 2003). For example, white suckers use backwaters during high-flow events and use runs when discharge and water levels are low in the Grand River of Ontario during the winter (Brown et al. 2001). Off-channel environments may provide some juveniles and small-bodied adults refuge from high flows or predators (Tschaplinski and Hartman 1983; Brown and Hartman 1988; Harvey et al. 1999). Juvenile robust redhorses may use backwaters during high discharge as a velocity shelter or for predator avoidance; however, water levels in the lower Oconee River are usually too low to inundate floodplains and backwater environments during winter.

Many riverine fish species prefer low-velocity environments and avoid flows exceeding 0.15 m/s during the winter and early spring (Mueller et al. 2000; Hesthagen and Heggenes 2003; Schwartz and Herricks 2005). In the present study, test fish avoided moderate flows during both winter and early spring. Similarly, larval and juvenile robust redhorses experienced higher survival and growth when exposed to low-velocity

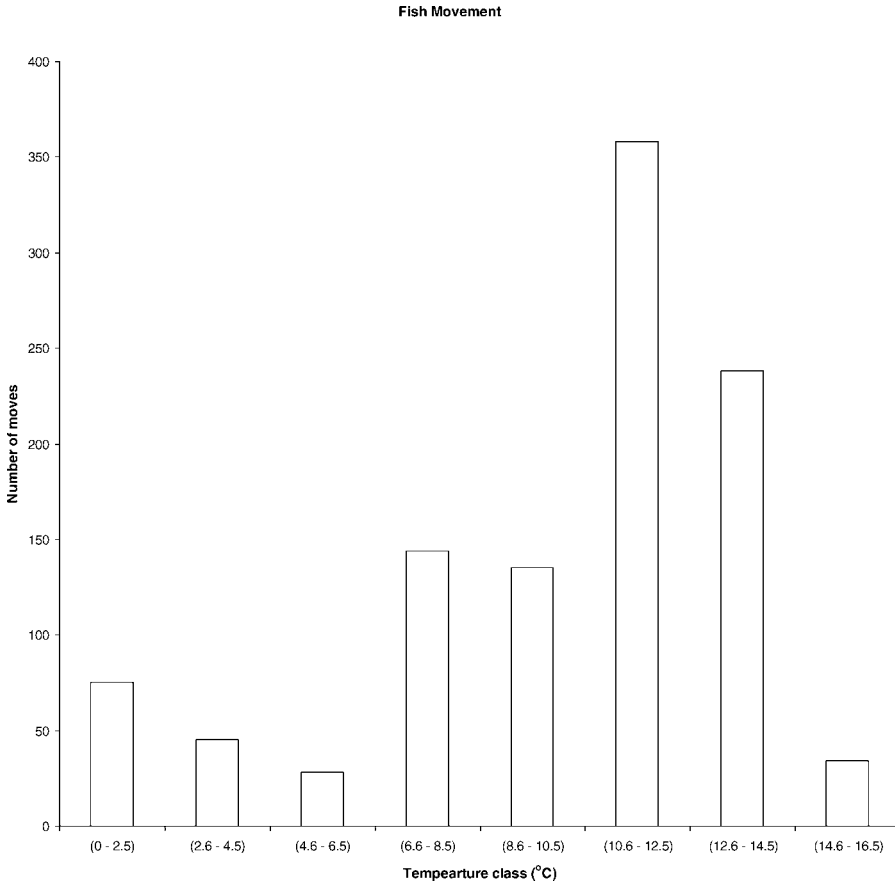


FIGURE 3.—The number of moves by juvenile robust redhorses within temperature classes during winter 2004 and early spring 2005.

water flows versus high-velocity water flows (Weyers et al. 2003). Several studies have shown that fishes use low-velocity environments to conserve energy during the winter and early spring (Cunjak and Power 1986; Chisholm et al. 1987; Baltz et al. 1991; Brown and Mackay 1995; Harvey et al. 1999; Solazzi et al. 2000). In this study, the slow flow-class was avoided during winter conditions.

During the winter, the test fish rarely moved during the observational period. In the spring, the test fish used backwaters and slow flows in proportion to their availability. During the early spring study, the test fish were often observed swimming around the mesocosms in the main channel and backwaters. Usually, one or two of the eight fish (per mesocosm) would remain in the same location throughout a 10-h daily observational period, whereas the other six or seven would switch locations several times during the period. Fish that moved switched among locations from eddies, backwaters, and slow flows. In the present study, the

proportional use of backwaters and slow flows during early spring may have resulted from increases of fish movement because of the increase of water temperatures (Hasan and Quasim 1961) and more than likely reflected their physiological preference of low-velocity environments.

Rivers contain a variety of low-velocity environments, such as backwaters, eddies, and boundary areas between water–sediment interface (slow flows); fish use of these areas may be influenced by differences among the environments. Eddies flow opposite to the flow in the main channel, whereas slow flows travel in the direction of the flow in the main channel (Harding et al. 1998). Slow-flow environments provide low water velocities that fish may use to conserve energy (Hasan and Quasim 1961; Chan et al. 1997; Thurow 1997; Beechie et al. 2005). Eddies provide low water velocities and also are areas of deposition for solid materials, including food; therefore, fish may use eddies both for foraging (Lehane et al. 2002; Beechie et

al. 2005) and as a refuge from strong currents (Harding et al. 1998; Schwartz and Herricks 2005). Food in eddies may result in increased feeding opportunities for fish.

Backwaters are off-channel environments that provide a similar ecological function to fish as eddies and slow water areas. Backwaters typically provide (1) velocity refuge for young fish (Parker 1989; Modde et al. 1996; Tyus et al. 2000), (2) warmer water than that in the main channel (Schlosser 1988; Papoulias and Minckley 1990; Jenkins and Burkhead 1993; Modde 1996), (3) a richer source of food than in the main channel (Schlosser 1988; Papoulias and Minckley 1990; Jenkins and Burkhead 1993; Modde 1996), and (4) more shelter and structure to protect young fish from predators than are available in the main channel (Schlosser 1988; Parker 1989; Modde et al. 1996). Current velocity in the backwater areas was nonexistent; however, some areas in the main channel also were nonflowing. Generally, test fish were seen in close proximity to the walls, crevices, and tight spots within the mesocosm. The backwater areas had all three features and were four times as small as the open main channel. In fact, the test fish showed preference for the backwater with the standpipe (more structure) than for the backwater without the standpipe (less structure). Fish affinity to structure (e.g., woody debris, boulders, stream edge) has been well documented in other studies (Wahle and Steneck 1992; Allouche and Gaudin 2001; Martin 2001), and predation has been hypothesized as the evolutionary process reinforcing this behavior (Johns and Mann 1987).

Most sampling effort for juvenile robust redhorses has focused on the middle to outside portions of meanders and sandbars, with minimal effort made in backwaters (Jennings et al. 1998). Boat electrofishers (Evans 1999; RRCC 2000), gill nets (Jennings et al. 2005), and hoop nets (Jennings et al. 2004a) have been used to sample juvenile robust redhorses around meanders (moderate to fast flows), and seines have been used to sample around sandbars (Jennings et al. 1998, 2004a, 2004b; RRCC 2000). Few attempts have been made with backpack electrofishers to collect juvenile robust redhorses in backwaters (RRCC 2000; Freeman et al. 2002). All attempts to collect wild juvenile robust redhorses from the Oconee River or elsewhere have been unsuccessful. Although different gear types in various environments have been used to sample other juvenile suckers, none has proved to be efficient because only a few individuals have been caught with any type of gear (Mosley 2006). The results of this study support the hypothesis that the absence of juvenile robust redhorses in catch data may be related to sampling in the wrong environments.

However, gear inefficiency and the actual abundance hypotheses also need to be evaluated. Furthermore, scarcity of juvenile suckers has been reported for others species with healthy adult populations (Beal 1967; Hand and Jackson 2003; Morey and Berry 2003).

Juveniles of many suckers, including robust redhorses, have been difficult to collect (Beal 1967; Hand and Jackson 2003; Morey and Berry 2003). Sampling for blue suckers, a federally listed "species of concern" (Williams et al. 1989), failed to capture juveniles in two different regions of the United States (Hand and Jackson 2003; Morey and Berry 2003). Of 4,093 overnight hoop net sets over 10 years, only 264 adult and zero juvenile blue suckers were collected from randomly selected 1-km stream reaches of the upper Yazoo River, Mississippi (Hand and Jackson 2003). Electrofishing and hoop nets used to sample moderate to swift flows and inside bends (which include eddies) for blue suckers in the James River and Big Sioux River, South Dakota, during summer caught 74 adult blue suckers in the James River and 28 adults in the Big Sioux River; however, juveniles were not collected in either river (Morey and Berry 2003). Fish behavior and gear bias were suggested to be the main factors that affected the inability to sample juvenile blue suckers (Beal 1967). The environment (high current velocity) that juvenile blue suckers are suspected to use was also determined to be difficult to sample (Moss et al. 1983). Juveniles are uncommon in samples from healthy populations of other suckers such as notchlip redhorse *M. callapsium* and spotted sucker *Minytrema melanops*. In October 1997, 300 pond-reared robust redhorse fingerlings were released into Hannah Creek, a tributary of the Broad River, Georgia. The creek was sampled with a backpack electrofisher 24 h after the fish were released, but only 29 of the 300 fingerlings were recaptured (Freeman et al. 2002). Therefore, the absence of wild juvenile robust redhorses in capture data may reflect their behavior and the difficulty of sampling all possible environments.

The results of this study suggest that juvenile robust redhorses are likely to be found in eddies and backwaters in the winter and in eddies and slow flows during early spring. The environments implicated as prime ones for these juvenile robust redhorses are the types of areas that wild juvenile robust redhorses are likely to use but that are often overlooked or under-sampled by researchers (R. Jenkins, Roanoke College, personal communication). Of the four different flow classes in the mesocosms, the test fish in this study showed an overall preference for eddies, the flow class that was least abundant, and a secondary preference for backwaters, the second least abundant flow class. Incidentally, little if any sampling effort has been

concentrated in eddies and backwaters, which also are two of the least abundant flow classes in the lower Oconee River and other rivers where robust redhorses are known to exist. Therefore, the current catch data may reflect biases in the current sampling regimen in regard to the type of environment that is sampled and the time of year when sampling for juvenile robust redhorses occurs. The difficulty of sampling juvenile suckers in general, and sampling in the wrong environments for juvenile robust redhorses in particular, may explain why these fish have not been collected in the wild. Targeting future sampling efforts to "preferred" areas identified in this study should help increase the probability of capturing juvenile robust redhorses in the Oconee River and elsewhere. Such captures will improve our ability to make inferences about the status of the population and how best to manage the species throughout its range.

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