Annual Report

Culture Techniques and Ecological Studies of the Robust Redhorse *Moxostoma robustum*

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Introduction

Robust redhorse *Moxostoma robustum* are large (maximum size about 760 mm TL) riverine fish belonging to the family catostomidae (suckers) that were re-discovered in the Oconee River, Georgia, during the summer of 1991 by personnel from Georgia Department of Natural Resources (GA DNR). Currently, the species is known only by the population in the Oconee River; but, there are anecdotal reports of large redhorse suckers occasionally being caught in portions of the Pee Dee River in North Carolina and the Savannah, Ogeechee, and Ohoopee Rivers in Georgia. Despite these reports, attempts by GA DNR to locate other populations in the fish's historic range (i.e., Atlantic Slope drainages from the Pee Dee River in North Carolina to the Altamaha River system in Georgia) have been unsuccessful. Ichthyologists and fisheries biologists working with this species agree that the species is threatened with extinction, probably within the next decade, and efforts to recover the species should begin immediately. These experts also agree that this recovery effort should be made, if possible, without having to rely on federal protection (i.e., listing on the Federal Endangered Species List), for which the species may qualify.

Toward this end, GA DNR, in conjunction with fisheries biologist from the U.S. Fish and Wildlife Service, the National Biological Service, and the University of Georgia, established the Robust Redhorse Conservation Committee. This committee compiled a prioritized list of research needs necessary to recover the species. Of these, culture techniques and ecological studies have the highest priority ranking. We therefore, proposed to conduct research on specific issues related to 1) artificial propagation (i.e., induced spawning with hormones), dietary requirements of post-larval and juveniles, and performance (i.e., survival, growth, and feed conversion) of fingerlings fed different commercial diets under intensive aquaculture conditions, and 2) ecological studies, including habitat requirements for various life stages (effects of instream flow, turbulence and siltation; and substrate, depth and temperature preferences). The original proposal contained nine tasks, each addressing a separate identified in Items 1 and 2 above. Georgia Power Company provided financial support for four (hormone-induced spawning, spawning behavior, reproductive and recruitment success, and additional population surveys) of the nine tasks in the original proposals. Work on these projects began during April 1995, as water temperatures in the stretch of river where these fish occur reached 17 °C. The results of 1995 spawning season are outlined in the following subsections, each detailing the outcome of work performed on the respective task.

Task 1. **Hormone induced ovulation of robust redhorse *Moxostoma robustum***

Efforts to bring about the recovery of the Oconee River population of the robust redhorse depend heavily on the development of reliable and efficient techniques for propagation of this species. The first effort to culture this species was in 1992 when six individuals (two males and four females) were collected by GA DNR and taken to the U.S. Fish and Wildlife Service hatchery at Warm Springs, GA, but this effort to spawn these fish was unsuccessful. Attempts to spawn robust redhorse continued in 1993 and 1994, but very few of the adult females collected and examined would release viable eggs. Some fingerlings were produced in 1993 and 1994 from females that were "running-ripe" at the time of collection. During the 1994 spawning season 85 adult females were collected and examined, but only six were "running-ripe" and ready for immediate egg collection. Attempts to induce ovulation through the use of hormone injections were also made during the 1994 spawning season, but no viable offspring were produced. By this time it was clear that the development of a hormone treatment regime for artificial spawning was vital to the success of the recovery program. Hormone-induced ovulation would mean much less disturbance of adult fish due to broodstock collection during spawning season, would facilitate the establishment of a captive breeding population, and would assure genetic diversity was optimized during future spawning efforts.

During spring 1995, a formal research project was initiated to develop an effective hormone treatment regime for spawning robust redhorse. Cooperators in this work included fisheries biologists with the University of Georgia, the U. S. Fish and Wildlife Service, and GA DNR. The objectives for this task were to: 1) evaluate the effectiveness of five different hormones in inducing robust redhorse to ovulate, 2) determine the optimum treatment dosage for the most effective hormones or hormone combination, and 3) determine if robust redhorse spawn intermittently.

Materials and Methods

In April of 1995, a temporary hatching facility was constructed next to the boat landing at Beaverdam Wildlife Management Area, Wilkinson-Laurens Counties, Georgia. This site was selected because of its close proximity to the area of brood fish collection. This meant that stress to brood fish due to transport and holding was minimized. Site preparation, including leveling and the addition of a gravel base was conducted by GA DNR personnel. The spawning facility included a broodfish holding system consisting of nine circular tanks that were supplied with a continuous flow of river water via a pump, and a continuous source of aeration via a blower. The pump and blower were powered by a gasoline generator.

Broodfish were collected with boat-mounted electrofishing gear from May 1 to May 3, 1995. Female fish were transported to the spawning facility and distributed among six of the tanks in the brood fish holding system. The condition of each female was checked at the time of collection and again prior to treatment. Any female found to be running ripe was not used in the study, but was spawned, and the fertilized eggs transported to a hatching facility for incubation. Any fish found to be over ripe was returned to the river. A total of 33 females were captured; four were over ripe and were returned to the river; eight females released their eggs naturally during anesthesia and were not used in the study. The remaining 21 females were randomly assigned to one of six treatments. A total of 58 adult males were collected during this period. All males were observed to have free flowing milt. Seventeen of the males collected were transferred to holding tanks. As females ovulated, either naturally or through hormone induction, these males were used for egg fertilization.

Five hormone treatments, administered by intramuscular injection, were selected for this study because of their known effectiveness in inducing ovulation in other fish species. A group of control fish was injected with sterile saline solution as a placebo. Dosage rates were based on a review of scientific literature, personal communication with other researchers, and product information provided by manufacturers. Table 1 details the hormone dosage and regime for each treatment.

Given the imperiled status of robust redhorse, and the fact that the sex ratio of the one known population is skewed towards males, the number of females used in this study was kept

Table 1. Hormones and treatment regimes used during May 1995 to induce robust redhorse to ovulate.

Hormone(s)	**Treatment regime**
Ovaprim	1 dose of 20 µg/kg
Carp pituitary extract (CPE)	3 doses of 15 mg/kg at 12 hr intervals
Human chorionic gonadotropin (HCG)	1000 IU/kg daily for 4 days
Carp pituitary extract + resolving dose of HCG	15 mg/kg CPE + 1000 IU/kg HCG administered 24 hr later
Luteinizing hormone-releasing hormone analog (LH-RHa)	20 µg/kg
Control*	placebo

* control group consisting of non-flowing females receiving an injection of sterile saline solution.

to a minimum. The initial proposal plan called for using four females in each treatment group, but the actual number in each group varied between 3-4 females because: 1) flow constraints and the conditions necessary for brood fish capture where only available for a limited period of time, 2) minimal holding times were required to ensure that egg maturation was not affected by holding time prior to onset of hormone treatment, and 3) captive females whose eggs had matured naturally did release them until the fish were anesthetized, which then meant that the actual number of fish in each tank that could be used in the study was not known until the treatment process was initiated.

The hormone treatment process involved transfer of a female from a holding tank to an anesthesia chamber containing aerated water which had been treated with methomidate hydrochloride at a rate of 10 mg/liter. When the fish was anesthetized, the vent was covered to prevent egg spillage; next, the fish was removed, dried with a towel, and held over an egg collection container. If ovulation occurred, the dry eggs were collected and the fish was returned to the holding tank. A male was then anesthetized and dried, then milt was extracted and applied directly to the eggs. The male was then returned to the holding tank for recovery from anesthesia. Water was then added to eggs to activate fertilization, the fertilized eggs were then washed and allowed to water harden for a minimum of one hour. The total volume of eggs was then measured, and the eggs, in clean water, were transferred to plastic bags and placed in styrofoam fish-transport boxes. Plastic bags were filled with approximately one-third fertilized eggs in water and two-thirds pure oxygen by volume. Fertilized eggs were then transported to one of three hatching facilities for incubation. Travel time ranged from 4 to 6 hours.

Once hormone treatments were initiated, each female was checked for ovulation every twelve hours. Females were held for three days from the last hormone injection or until ovulation occurred, whichever came first. After three days, the fish were returned to the river.

Results and Discussion

Nine of the 17 fish injected with hormones ovulated, but none of the fish in the control group ovulated during the study period (Table 2). Several of the ovulating females released additional eggs 24 h after initial egg collection. This suggests that female robust redhorse are capable of intermittent spawning. The total number of eggs collected per spawning female (i.e., fecundity) ranged from 1,485 to 86,295[1]. Based on this initial trial, it appears that Ovaprim, Carp Pituitary Extract and Human Chorionic Gonadotropin were effective in inducing ovulation and increasing spawning success of robust redhorse. However, questions remain about the amount of these hormones needed (i.e., dosages) and treatment schedules (i.e., dosage intervals) needed to maximize eggs production by broodstock. Further, it should be noted that our results are based on one trial, conducted under specific conditions, and has not been repeated for verification. Therefore, although we obtained positive results this year, the lack of repeated trials limits our ability to assess the utility of these hormones for inducing robust redhorse to spawn under different climatic regimes For example, water temperatures

[1]The 1,485 eggs per females came from the treatments that were not very successful in inducing ovulation, whereas the 86,295 eggs per females came from treatments that successfully induced the female to ovulate. Also, robust redhorse are intermittent spawners, and may not have released all their eggs by the time they were returned to the river.

Table 2. Number of female robust redhorse ovulating after being injected with ovulation-inducing hormones (experimental treatment) or sterile saline solution (control group) during May 1995.

Treatment	No. Females treated	No. Females ovulating
Control	4	0
HCG	3	2
Combination	3	1
Ovaprim	4	4
CPE	3	2
LH-RHa	4	0

during the period prior to spawning will determine the rate of egg maturation in female robust redhorse, and fluctuations in river level and air temperatures can make it difficult to schedule brood fish collection to coincide with optimum egg maturation in females. As conditions vary from year to year, different dosages of hormones, or different treatment intervals may be required. Further study is needed to evaluate the effectiveness of the treatment regimes showing promise in this initial trial.

Approximately 800,000 fertilized eggs were produced during this study by females that either spawned naturally or were induced to spawn. These eggs were shipped to McDuffie Fish Hatchery (GA DNR), Warm Springs Fish Hatchery (USFWS) and Whitehall Fisheries Lab (UGA) for incubation. From these eggs, about 71,000 fry were produced and subsequently shipped to designated state and federal facilities to be grown out in ponds. These phase-one robust redhorse fingerlings were inventoried on November 30 - December 1, 1995. This inventory indicated that 56% (about 40,000) of the fry had survived to fingerlings stage by the end of 1995. These fish are currently distributed between Bo Ginn National Fish Hatchery, Walton Hatchery (GA DNR), McDuffie Hatchery (GA DNR) and McKinney Lake National Fish Hatchery. Plans are to rear these fingerlings until September 1996 when they will be released at suitable riverine and pond sites in Georgia, within drainages where historical records of this species exist.

Task 2. Spawning behavior of robust redhorse in the Oconee River, Georgia

Observations and documentation of spawning behavior for all species of redhorse suckers are scarce or lacking. Observations for some species (e.g., golden redhorse *Moxostoma erythrurum* and black redhorse *M. duquesnei*), suggest that two males and one female are usually involved in the spawning act. Golden redhorse and black redhorse do not construct nests or gravel redds. Conversely, published and unpublished reports of observed spawning activity in the river redhorse *M. carinatum* suggest elaborate courtship and nest construction are preparatory to the actual spawning event (Hackney et al. 1968). Field observations of potential nests or redds, the recovery of eggs from these suspected gravel redds, and subsequent capture of nearby robust redhorse in spawning condition suggest the possibility of redd construction by this species. This behavior, however, has not been conclusively documented. Knowledge of whether robust redhorse actually construct redds or simply spawn on or in the substrate is necessary to answer questions about the effects of unstable peaking flows on the recruitment of robust redhorse in the Oconee River.

Therefore, we investigated the behavioral spawning repertoire of the robust redhorse in the Oconee River, GA. Specific objectives of this task were to document spawning-related behavior prior to and during the spawning act, and to identifying specific habitat conditions associated with spawning sites.

Materials and Methods

Three preliminary surveys for spawning robust redhorse were conducted in the Oconee River during May 1995 (Figure 1). Surveys were conducted by boat and focused on known gravel areas shallow enough to permit either direct observation or remote observation with an underwater camera. The surveys were conducted in each of three reaches that contained areas suspected of being spawning areas for robust redhorse, and also included explorations for

Figure 1. Oconee River, from Sinclair Reservoir to downstream of Dublin, GA. Reaches sampled for spawning robust redhorse are depicted by brackets and numbers. River mile tics are 5 miles apart. The symbol ★ show the location of the 1995 spawning site adjacent Avant Mine boat ramp.

previously-unknown areas within each reach that contained suitable spawning habitat. Reach One extended from Georgia Highway 57 upstream to the Central of Georgia Railroad Trestle (Figure 1) or river mile (RM) 98.4 to 104.4. Reach Two ran from near the mouth of Big Sandy Creek downstream to the vicinity of the junction of Deep Creek and the Oconee River. Reach three extended from near the mouth of Big Sandy Creek (RM 90.9) upstream to the vicinity of the Avant Kaolin Mine (RM 120).

Observations of spawning robust redhorse were recorded as verbal descriptions and on either video (8 and HI-8 mm) or 35 mm still film. Video footage of spawning robust redhorse was recorded via several methods. An underwater-remote black and white camera (Furhman Diversified) was deployed in deep and shallow areas to record spawning activities from as near as possible. The underwater camera was attached to an aluminum pole and lowered to near the bottom of the river in shoal areas and video footage was recorded. Also, a handheld 8 mm and a HI-8 mm camera equipped with a polarizing filter were used to record spawning behavior from several vantage points. A time-code was recorded on master tapes and these were used to produce VHS tapes for analysis of various behaviors. Still photography of spawning activities and habitat was recorded with a 35 mm camera equipped with either a 100 mm macro lens or a 35-105 mm zoom lens. A circular polarizer was used to reduce glare and improve visibility.

Field sketch maps were prepared for future analysis. These maps depicted spawning location, substratum type and location, and the location of water current velocity transects. Water temperature was recorded with a mercury thermometer. Water velocities were determined with a Marsh-McBirney Model 2000 current meter attached to a wading rod, and were measured in several zones of the spawning riffle, from the pool above downstream through the tail.

Results and Discussion

Spawning habitat

Reach One had meanders and adult robust redhorse were common in the bends in this reach (Figure 1). However, the spawning site survey conducted in this was not productive, primarily because of high flows and turbidity during the survey period. This reach, which was surveyed last during 1992, had suitable spawning substrates on which robust redhorse are suspected of spawning. However, the river was too high and turbid to relocate this site, and other suitable gravel bars were not exposed during the survey period. A large cobble and bridge-rubble spoil island, located downstream of the Central of Georgia RR crossing, appeared to be too coarse and compacted to allow for spawning.

Reach Two, the southern-most of the study reaches, did not contain as many meanders as the other two reaches. Instead, this reach consisted mainly of extensive pools that were hundreds of meters long. Two exposed gravel bars were located within this reach: one mid-channel bar was about 70 m^2 (i.e., 7 X 10 m) in area, and had suitable substratum and current conditions; but, robust redhorse were not observed spawning there.

Reach Three was similar to Reach One in sinuosity, but the only suitable spawning habitat located within Reach Three was near the Avant Mine site at about river mile 120 (Figure 1). During a May 1992 survey of this reach, three possible robust redhorse spawning "redds" (i.e., areas with disturbed gravel) were located at river mile 103 in the Oconee River, downstream of the Avant Mine site. Males of the closely-related river redhorse *M. carinatum* have been observed constructing redds in gravel prior to spawning (Hackney et al. 1967). Congenerics such as golden redhorse *M. erythrurum* (Page and Johnson 1990, Kwak and Skelly 1992) and shorthead redhorse *M. macrolepidotum* (Burr and Morris 1970) spawned over depressions in the gravel (Table 3).

None of these researchers witnessed any maintenance or construction of the depressions and theorized that such depressions may form as a result of spawning activity and egg burying, and not from purposeful construction. The "redds" encountered during the 1992 Oconee River survey were located in a mid-channel gravel bar; water depth ranged from 48 to 73 cm, and current velocity was estimated as 50 cm s^{-1}. They could be described as pits arranged obliquely to the current flow approximately 1.2 m long with a spoil ridge at the downstream portion. Two eggs were recovered from the site by disturbing the gravel and capturing the drift with a 1/8" seine. The eggs were preserved in 10% formalin solution but were not positively identified.

Circumstantial evidence indicates that these depressions are associated with robust redhorse spawning. The eggs were similar in size and appearance to robust redhorse eggs. The late spawning date, large egg size (about 3 mm dia.), and overall dimensions of the depressions precluded all other known Oconee River species as the candidates responsible for the observed "redds". Boatshocking in an adjacent pool the next day yielded four robust redhorse, three of which escaped. The specimen captured was a mature male freely-flowing milt. Redd construction at this site contradicts observed spawning behavior at the Avant Mine site, but does not eliminate this locale as a potential robust redhorse spawning site.

Spawning behavior

We observed robust redhorses spawning May 14-22, 1995, at the Avant Mine site. Local fishermen reported that the fish at that site, which they identified as "carp", had been spawning for two weeks prior to our observations. We observed a minimum of fifteen individuals (13 males

Table 3. Literature reported spawning behaviors for various species of *Moxostoma*. Codes are as follows: Y=yes, N=no, U=unknown, R=Redds, D=depression in the substrate.

Species	Territorial	Dominance	Modified Substratum	Author

M. carinatum	Y	U	R	Hackney et al.
M. duquesnei	Y	Y	N	Bowman
M. duquesnei	N	N	N	Kwak and Skelly
M. erythrurum	Y	Y	D	Page and Johnson
M. erythrurum	Y	Y	D	Kwak and Skelly
M. macrolepidotum	N	N	D	Burr and Morris
M. robustum	Y	Y	D	This Report
M. valenciennesi	N	N	N	Jenkins and Jenkins

and 2 females) spawning from dawn to dusk. Spawning observations were not made at night. Spawning occurred over a loose substratum of small- to medium-sized gravel. Local current velocity taken at the standard 60% depth ranged from 35 to 60 cm s^{-1} and daytime water temperature fluctuated around 25 □C for the duration of the observations. The fish spawned on the gravel bar at the margin between riffle and pool habitats. These parameters are consistent with spawning habitat use for other redhorse suckers (Hackney et al. 1967, Burr and Morris 1970, Page and Johnson 1990, Kwak and Skelly 1992).

Males formed two parallel rows perpendicular to the current across the upstream margin of a transverse gravel bar (Figure 2). Males in both rows faced upstream. The first row contained three males at the tail of the pool. The water in this area was slightly deeper and slower than that of the main spawning area; depths ranged from 36 to 60 cm and current velocities ranged from 5 to 18 cm s^{-1}. Males in the pool were excluded by the males in the second row and were not observed to spawn. Similar male dominance has been observed for golden redhorse (Page and Johnson 1990) (Table 3). The 10 males of the second row spaced themselves from 0.6 to 1.0 m apart and actively defended their positions against neighboring males. The gravel substratum in this area was cleaned of silt and algae and appeared in sharp contrast to the rest of the brownish gravel of the bar and in the pool. Redds were not present, although a slight depression was observed. Redd construction or gravel cleaning behavior was not observed. The clean gravel and the depression probably resulted from spawning activity rather than intentional preparation.

Females gathered in the pool upstream of the gravel bar. When ready to spawn, females entered the spawning area head- or tail-first and settled to the substratum facing upstream

Figure 2. Sketch map of robust redhorse spawning aggregation during May 1995 at the Avant Mine site, showing the location of males (♂) and females (♀), and the spacing between dominant and subdominant males.

between two males. The males positioned themselves on each side of the female and pressed against her sides. The trio arched their backs and quivered energetically, releasing gametes as their anal and caudal fins plowed into the substratum (Figure 3). Eggs were buried in the gravel as they were fertilized. This behavior is consistent with observations for other species of the genus *Moxostoma* (Hackney et al. 1967, Burr and Morris 1970, Jenkins and Jenkins 1980, Page and Johnson 1990, Kwak and Skelly 1992).

Spawning lasted from 2-5 seconds with an average duration of about 3 seconds. Occasionally, one or two neighboring males joined the spawning triad and may have released gametes at the periphery of the spawners. One cluster of spawning fish included 3 males and two females. As observed for golden redhorse (Page and Johnson 1990), non-participating males commonly disrupted spawning triads. These males approached the spawning group laterally and used their tuberculate snouts to forcefully butt the flanks of the participating males (Figure 4). This behavior was violent and many of the males had large contusions and appeared visibly battered. Females did not appear to preferentially spawn with any one pair of males. Rather they visited many of the territories of the dominant males on the second row with equal frequency.

Conclusions

The lower Oconee River below Sinclair Reservoir has at least one active spawning population and suitable habitat to support other breeding groups. Several aspects of robust redhorse spawning behavior are common for other redhorse suckers. They are non-guarding, brood-hiding lithophils (egg buriers), and spawn in triads comprised of two males and one female. Spawning habitat is moderate to swift, shallow water over loose gravel substratum.

Figure 3. Triad of robust redhorse spawning over shallow gravel in the Oconee River adjacent to the Avant Mine boat ramp. The female (center) is flanked by two males--with one on each side.

Figure 4. Triad of spawning robust redhorse being disrupted by a non-participating male charging in from the right side of the frame.

Task 3. Reproductive and recruitment success of robust redhorse in the Oconee River, GA

Robust redhorse in the Oconee River, GA have been observed exhibiting spawning behavior during late spring, but the scarcity of juveniles suggests reproductive or recruitment failure in the recent past. Further, data on nest success, distribution and abundance of larvae, and estimates of larval growth and mortality are lacking. These data are needed to help determine if the scarcity of larval and juvenile robust redhorse is related to recruitment failure or to other non-biological factors. Our objectives for this task were to: 1) document spawning activity, 2) determine reproductive success (i.e., abundance), and 3) determine the growth and mortality of larval life stages sampled from the Oconee River.

Material and Methods

Larval and juvenile fishes were sampled from about an 51-km reach of the Oconee River that runs from Avant's Landing, adjacent to the Thiele Kaolin mine (at the Baldwin-Washington County line) downstream to the Beaverdam Wildlife Management Area (Wilkinson-Laurens Counties). Sampling was begun on May 10 and continued to December 8, 1995. Initially, pushnets (505 µm) and D-rings nets (800 µm) were used to sample ichthyoplantkon drift from surface and epibenthic areas in the mainstem of the river. About two weeks later, light traps were added to the sampling protocol, and were used to sample ichthyoplankton from slack-water habitats. Finally, a seine (800 µm) was used to sample larval and juvenile fishes from sandbar and oxbow areas. The dates and duration of sampling with each gear are given in Figure 5, and the sampling frequency and number of replicates with each gear are given in Table 4. Environmental conditions (e.g., depth, flow, turbidity, water temperature, and dissolved oxygen) at each station

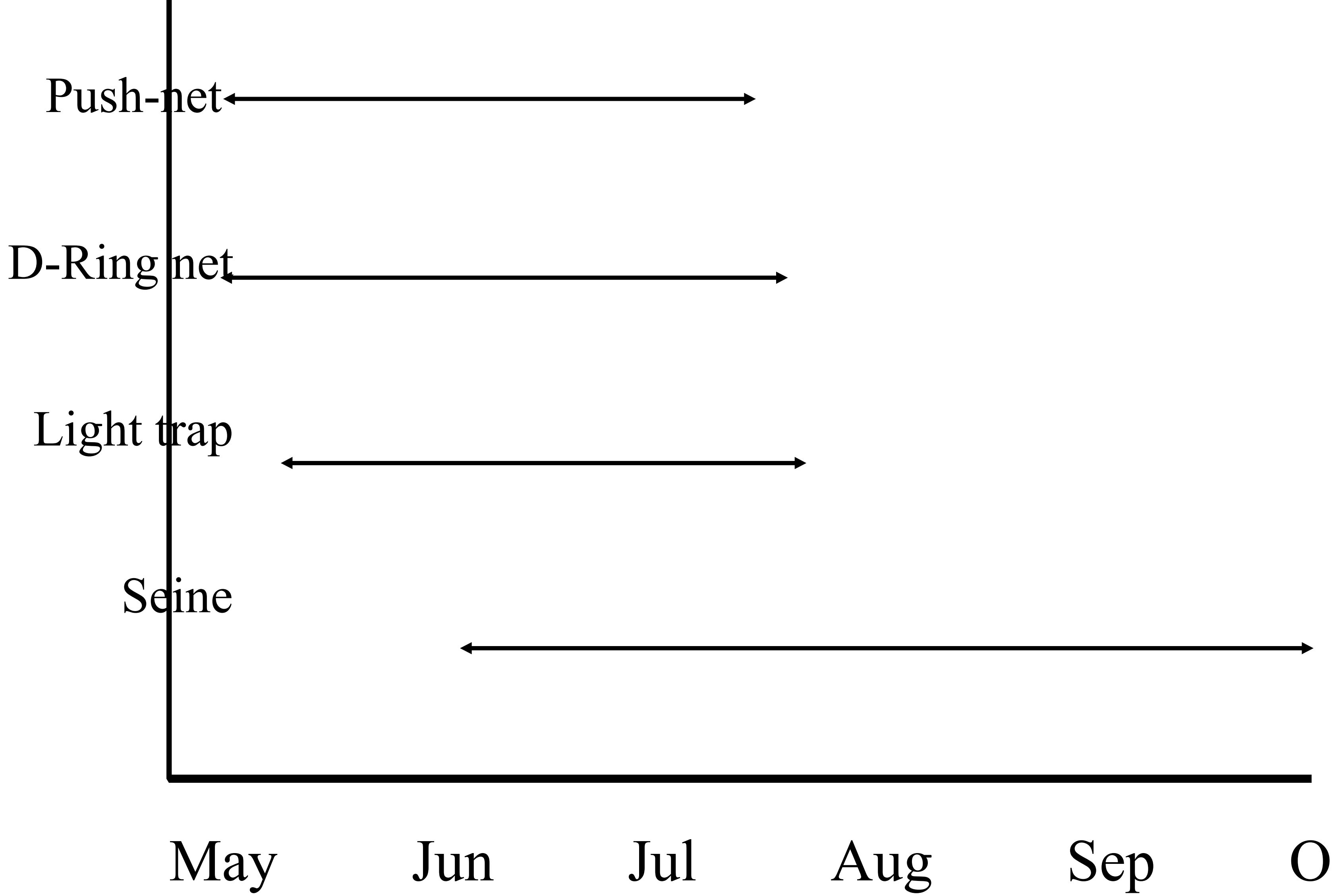

Figure 5. Sequence and duration of four gear types used to sample larval and juvenile fishes from the Oconee River, GA between Avant's Landing (Washington Co.) and Beaverdam Wildlife Management Area (Laurens Co.) during 1995.

Table 4. Gear type used to sample larval and post-larval fishes in the Oconee River, Georgia between May 10, and December 8, 1995.

Gear (mesh µm)	Sampling freq. (No./week)	Stations sampled	Replicates per station	Mean volume water sampled (m^3)
Push-net (505)	2	2	3	100
D-Ring net (800)	2	2	3	100
Light trap (N/A)	2	2[2]	2	N/A[3]
Seine (800)	1	3	3	N/A[4]

[2]Three sites were sampled on occasions when light traps were the only gear being fished.

[3]Light traps were fished for about 4-6 h in slack-water areas.

[4]On average, a seine tow was 20 m.

was recorded at the time of each sample. Depth was measured with a Lowrance boat-mounted transducer; flow was measured with a General Oceanics Flow meter, turbidity was measured with a Hach Model 2100P Turbidimeter, temperature and dissolved oxygen were measured with a YSI Model 57 Temperature-Dissolved meter. Pushnet, D-ring, light trap, and seine samples were preserved in 10% buffered formalin and returned to the laboratory where they were examined for the presence of larval and juvenile robust redhorse.

Fish larvae were extracted from the formalin-preserved samples, enumerated, and placed in vials for later identification. Extraction was begun on July 14, 1995 and continued through February 5, 1996. Extraction efficiency, estimated by re-examining 20% of the sample residues, averaged 99.5 % (s.d. = 3.1). Identification of larval fish, begun in September 1995 and still ongoing, is based on morphometric descriptions and meristic counts of larval fishes, as presented in numerous taxonomic keys (e.g., Wallus et al. 1990, Kay et al. 1994).

Results and Discussion

Six hundred twenty two samples containing a total of 45,698 larval and juvenile fishes were taken from the study reach of the Oconee River. The fishes in about two thirds of the samples (367 of 622) have been identified and enumerated. These samples, taken between May 10 and July 19, 1995, contained a total of 6,978 fishes representing 11 families, including 30 severely-damaged fishes that could not be identified to family (Table 5). Cyprinidae (minnows), Clupeidae (shads), Catostomidae (suckers), and Centrarchidae (sunfishes) were the families with at least one very-abundant species; other families with at least one moderately-abundant species included Atherinidae (silversides) and Ictaluridae (catfishes) (Table 5).

The large number of larval and juvenile fishes taken in the different gear-types used to sample ichthyoplantkon from the study reach indicates that these gear effectively sampled the species available when each gear was being used. More samples and higher catches of fish were collected with light traps and seines than with the pushnet and D-ring net (Table 6). Whereas these gear have all proven to be effective for sampling larval fish in the Oconee River, each seemed to work best under different flow conditions. For example, the pushnet and D-ring worked best under moderate flow (i.e., about 0.3-0.4 m/sec), but such conditions were unsuitable for sampling with light traps, which required slack water to work effectively. However, finding slack-water areas in the study reach that were suitable for light trap deployment was especially difficult during periods with increasing or decreasing flow. Decreasing flows often left light traps resting on shore with most or all of the trap out of water, and rapidly-increasing flows resulted in too-swift a current for effective light trap sampling. In one instance, rapidly-increasing flow resulted in three light traps being destroyed at or breaking loose from their mooring and being swept downstream. Nonetheless, most of the gear worked well most of the time, as indicated by the abundance and diversity of larval and juvenile fishes collected (Table 5).

Larval and juvenile suckers were relatively abundant in our samples (Table 5); but, as of July 19, only 6 larval robust redhorse had been caught (Figure 6; Table 7), and they were collected in late May. Larval robust redhorse were caught in two of the four gear-types used to sample ichthyoplankton, but most (5 of 6) were caught in pushnets (Table 7). Density estimates of robust redhorse larvae, available for May only, ranged from 0.0 to 13.4 per 1000 m^3 of water sampled (Table 8). Juvenile robust redhorse have not been identified in samples taken on or before July 19th; but, five silver redhorse *Moxostoma anisurm*, a close relative of robust

Table 5. Number of larval and post-larval fishes (n=6,978), by taxonomic family, collected in the Oconee River, Georgia from May 10 - July 19, 1995 for the first 367 samples processed.

Family	Number collected
Lepisosteidae	4
Clupeidae	981
Cyprinidae	4,774
Catostomidae	367
Ictaluridae	95
Belonidae	17
Poeciliidae	68
Atherinidae	203
Centrarchidae	382
Percidae	56
Soleidae	1
Unknown	30

Table 6. Total number of samples (n=622)and fishes (n=45,698) taken, by gear, from the study

reach of the Oconee River during the period May 10 - July 19, 1995.

Gear	Number of Samples	Number of Fishes
Pushnet	136	1,641
D-ring net	114	624
Light traps	206	7,649
Seine	166	35,784

Figure 6. Ten-day old, hatchery-produced, larval robust redhorse *Moxostoma robustum* (about 14 mm total length)

Table 7. Number of larval and post-larval catostomids (n=255) collected, by gear, in the first 367 samples taken from the Oconee River, Georgia from May 10 - July 19, 1995.

	Gear			
Species	Push-net	D-Ring net	Light Trap	Seine
robust redhorse	5	1	0	0
silver redhorse	0	0	1	4
carpsucker[5]	156	0	47	4
spotted sucker	2	3	1	0
unknown	23	6	2	0

[5]Two undescribed species of carpsucker, one related to the quillback *Carpiodes cyprinus* and the other related to the highfin carpsucker *C. velifer*, occur in the Oconee River where we sampled.

Table 8. Average total length (mm) and estimated density of larval robust redhorse *Moxostoma robustum* at specific reaches in the Oconee River between Milledgevill and Dublin, GA, during May 1995.

Date (1995)	Location (Lat/Long)	Time	Water temp (°C)	Number / mean length (mm)	Estimated Density (larvae·1000 m^3)
May 10	‡	‡	‡	0 / --	0.0
May 19	32°45' 82°57'	1355	24.4	1 / 13.7	3.3
May 24	32°49' 82°58'	0145	25.8	2 / 13.4	8.5
May 27	32°49' 82°58'	0100	26.3	3 / 13.7	13.4
May	‡	‡	‡	0 / --	0.0

‡ Catch of larval robust redhorse was zero at all sampling locations (except as noted in the this table), during day and night, and at water temperatures ranging from 17 to >27 °C.

redhorse, were caught during this period. Silver redhorse begin spawning in late January or early February when water temperature is 13 °C (Jenkins 1979) and complete their spawning before robust redhorse begin spawning in late April or early May when water temperature is 17 °C. However, the vulnerability of juvenile silver redhorse to our gear suggests that juvenile robust redhorse (≥39 mm TL) also may be vulnerable to our gear later in the summer. Carpsuckers *Carpoides* spp. were the most abundant suckers in the samples taken on or before July 19th (Table 5; Table 7).

On May 22, 1995, robust redhorse were observed spawning in the Oconee River over a shallow gravel bar in the river adjacent to the boat ramp at the Avant Mine site (see Task 2 for account). A D-ring net was deployed nine different times about 1-3 m directly downstream of spawning fish. Four of these net sets did not contain eggs; the other five net sets contained 81, 1, 1, 9, and 2 eggs (avg. = 19, S.D. = 35). Considering the fecundity of these fish (see Task 1), the relatively few eggs collected directly downstream of spawning fish suggest that the fertilized eggs were being buried in the gravel. Therefore, not many eggs were drifting in the water column, which then suggests that newly-hatched larvae would also not be found in the water column. Instead, newly-hatched larvae probably remain in the interstitial spaces of the gravel beds until they have absorbed most of their yolk material, and then emerge from the gravel to begin exogenous feeding. The six robust redhorse larvae collected were all between 13-14 mm total length (e.g., Figure 6) and five of the six were collected at night, directly above a suspected spawning site. These fish probably had just emerged from the gravel beds. Estimated abundances of larval robust redhorse downstream of a known spawning site and in the vicinity of a second, strongly-suspected site was extremely low.

Assessing the growth and mortality of larval robust redhorse was the third objective of this task. Estimates of growth and mortality were not calculated because insufficient numbers and sizes of larvae were captured. The intended methods (i.e.,formulae) of estimating growth and mortality require more than one size class of larvae, and many individuals per size class to compute meaningful estimates. Unfortunately, only six specimens have been identified thus far, and they were all about the same size. As a result, this task's third objective was not achieved.

At the beginning of this project, many hypotheses were put forth to explain the apparent failure of this population to recruit new members. The mechanisms suspected of causing recruitment failure included reduced number of poor-quality eggs produced by old fish, lack of suitable spawning substrate, lack of suitable rearing habitat, or ineffective sampling gear. One spawning site has been confirmed, and a second site is suspected strongly but not yet confirmed. The successful hatching of fertilized eggs obtained from wild fish at the confirmed spawning site, and fertilized eggs produced under hatchery conditions indicate that lack of spawning habitat or egg viability are not acute problems. The scarcity of robust redhorse larvae in our samples suggest that we may have sampled after the peak larval-emergence period, sampled outside the main spawning area, that emerging larvae (about 14 mm TL) swim well enough to avoid our gear, or that incubation conditions in the gravel bed were not well suited for larval survival to swim-up stage. Consequently, unsuitable incubation substrates, lack of adequate (i.e., reduced-flow) rearing habitat, and ineffective sampling gear cannot be ruled out as the mechanism(s) responsible for the extremely low abundance of larval robust redhorse in the study reach.

Conclusions

Robust redhorse are still spawning in the study reach of the Oconee River, and fertilized eggs from wild and artificially-spawned fish are hatching successfully. These results indicate that some suitable spawning substrate remain in the river, and that the fish are not past reproductive age. Estimate densities of larval robust redhorse were low, and juvenile have not been caught as of July 19, 1995. The mechanism(s) responsible for the low densities are unknown, but plausible hypotheses remain to be tested. Chief among these are 1) unsuitable incubation substrates, 2) inadequate rearing habitat, and 3) ineffective sampling gear. Reliable test of these hypothesis should reject or support one or more of these hypotheses as the mechanism responsible for the extremely low abundance of larval robust redhorse in the study reach, and in turn, may help explain the apparent failure of larval and juvenile to recruit to the adult population.

Task 4. Surveys for additional populations of robust redhorse

Robust redhorse are presently known to only occur in a limited reach of the Oconee River, between Milledgeville and Dublin, Georgia. Ongoing recovery efforts would greatly benefit from the discovery of remnant populations that might be isolated in stream reaches in river systems within the species' former range. Such a discovery would provide additional information about habitat requirements for this species, and possibly aid in the identification of other populations. Additional status surveys would provide information on the occurrence of other populations of robust redhorse, and of the presence of populations of the flathead and blue catfishes, two recently introduced, highly predatory species that are both expanding their range.

Therefore, we surveyed additional river system reaches within the historical range of the robust redhorse in Georgia. Our main objective was to locate other remnant populations of robust redhorse that may still inhabit Georgia rivers.

Materials and Methods

Preliminary surveys for robust redhorse were conducted in the Broad River, and in Brier Creek, two major tributaries of the Savannah River (Figure 7). The Broad River near Madison-Elbert Counties Georgia, in the vicinity of Vineyard Creek, is of a proper size for robust redhorse. Historical collections of robust redhorse are known from the Savannah River near Augusta. Brier Creek joins the Savannah River in the upper coastal plain. Robust redhorse remains were identified from a shell midden located in the floodplain of Brier Creek (Burke Co. GA) approximately 34 river miles upstream from the confluence with the Savannah River. This reach of Brier Creek is known to contain exposed chert gravel, which might be suitable for robust redhorse spawning sites. Preliminary sampling reaches and access points in Brier Creek were identified.

Preliminary surveys for additional populations of robust redhorse were conducted with a boat electrofisher. The boat electrofisher consisted of a 14 foot, heavy-duty aluminum john-boat fitted with a custom electrofishing rigging. Electricity was supplied with a Smith-Root Model 2.5 GPP Electrofisher, which included a pulsator box and a custom-wired generator.

All fishes encountered were collected; small fishes were preserved and large specimens were released, except for vouchers. All catostomids were identified, measured, sexed if possible, and released. Stream reaches sampled were plotted on USGS 7.5 minute maps. Effort expended

Figure 7. Map of sampled reach of Broad River, Elbert-Madison Counties, GA. Small arrows indicate up- and downstream sampling boundaries of the reach. Map location is on the Bowman and Carlton Quads (USGS 7.5 minute series).

in a reach was recorded as the number of seconds the pulsator was used. Additional physical information such as water temperature, stream depth and width, bank conditions, and substratum type were also recorded. The reach of the Broad River sampled was bounded on the upstream end by rapids and riffles that were impassable to our boat electrofisher. This upstream reach was at least six miles long.

Results and Discussion

Eight collections were made in the Broad River in the vicinity of Vineyard Creek (Madison, Elbert County Georgia) during December 1995 and January 1996, but none of our samples contained robust redhorse. However, several other sucker species including silver redhorse *Moxostoma anisurum,* northern hogsucker *Hypentelium nigricans,* and jumprock *Scartomyzon sp.* were captured during these surveys; carpsucker *Carpiodes* sp. were seen but not captured. Silver redhorse were particularly abundant in the surveyed reach; the mean number of individuals captured per hour for all collecting efforts was 21.5. The Asian clam *Corbicula fluminea*, a food item of both robust and silver redhorse (personal observation), was also abundant in the survey area. Flathead catfish *Pylodictus olivaris* were also collected in this area, but they were small (60-325 mm) and appeared to be at a low density within the area; the mean number of individuals per hour for all collecting efforts was 1.2. Flathead catfish were introduced into Clark's Hill Reservoir (the terminus of the Broad River) in 1964 (South Carolina DNR, personal communication). Although flathead catfish have been resident in this system for at least 30 years, their low numbers and small size indicates that they may have had limited success in colonizing the upper Broad River. Because intense predation of juvenile suckers by flathead catfish can negatively affect sucker populations (Marsh and Brooks 1989), the diverse and abundant sucker population in the upper Broad River seems to support our contention that flathead catfish in this reach of the river may have had limited colonization success.

Conclusion

The population of robust redhorse in the Oconee River is the only known population. Preliminary results from sampling on the upper Broad River indicated that proper habitat and a potential forage base are available for robust redhorse. Flathead catfish may pose a future problem, but currently do not appear to be affecting the local population of silver redhorse and the undescribed species of jumprock. Brier Creek has sufficient size to support robust redhorse, has records of a prehistoric robust redhorse population, and has ready access for boat electrofishing equipment. It should be prioritized for future robust redhorse population surveys.

Project Summary

Knowledge about the biology and ecology of robust redhorse in the Oconee River has increased as a direct result of the research conducted during the first year of this project. Before this project, basic questions about the reproductive status of the population went unanswered, and attempts to artificially propagate this species usually failed. Our efforts to date have resulted in methods that show promise in artificially-inducing robust redhorse ovulation. We also now know that although the most of the robust redhorse in the system are older, many are still reproductively active, (i.e., engaging in courtship behavior, and producing viable eggs and sperm). Before this project began, we postulated that robust redhorse constructed spawning redds that served first as incubation sites, and then as early rearing habitat for hatching fry. Project results show otherwise; in fact, robust redhorse bury their eggs in loose gravel, which is were the fertilized eggs incubate and where hatching fry probably spend their first week of life. Further, we have identified and confirmed one spawning site; a second site is suspected strongly, but has not been confirmed visually. Questions about the fate of larval robust redhorse have been partially resolved. For example, larval robust redhorse have been collected from the Oconee River, but only from two sites, and then at very low densities. The reasons for the seemingly limited distribution and low abundance of this life stage are unclear, but plausible hypotheses that may explain our observations remain to be tested.

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