# **REPORT OF THE**

# ROBUST REDHORSE CONSERVATION COMMITTEE ANNUAL MEETING

CHARLIE ELLIOTT WILDLIFE CENTER, GEORGIA OCTOBER 11 – 12, 2000



### Video tells the robust redhorse discovery and RRCC recovery story!

Meeting facilitated and report written by T.A. DeMeo under contract with the United States Fish and Wildlife Service

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ACRONYMS & ABBREVIATIONS



CP&L	Carolina Power and Light Company
DPC	Duke Power Company
DWC	Dennis Wildlife Center
GA DNR	Georgia Department of Natural Resources
GPC	Georgia Power Company
GRN	Georgia Rivers Network
GWF	Georgia Wildlife Federation
NC WRC	North Carolina Wildlife Resources Commission
NRCS	Natural Resource Conservation Service
NYU	New York Medical Center
PNWR	Piedmont National Wildlife Refuge
SC DNR	South Carolina Department of Natural Resources
SCA	South Carolina Aquarium
UGA	University of Georgia
USACOE	U.S. Army Corps of Engineers
USFS	U.S. Forest Service
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey (Biological Resources Division)

MOU	Memorandum of Understanding
RRCC	Robust Redhorse Conservation Committee
CCAA	Candidate Conservation Agreement with Assurances for the Ocmulgee River

с	Celcius
cfs	Cubic feet per second
cm	Centimeter
gr	Gram
m	Meter
mm	Millimeter
mg/l	Milligrams per liter
NTU	Nephelometric Turbidity Unit
rkm	River kilometer

### EXECUTIVE SUMMARY



The robust redhorse recovery effort, in its  $6^{th}$  year, encompasses management activities, research and conservation efforts undertaken by members of the Robust Redhorse Conservation Committee (RRCC), university scientists and other affiliates. The RRCC was established by a Memorandum of Understanding (MOU) signed in 1995 to develop and manage a prelisting recovery approach for the robust redhorse (*Moxostoma robustum*), previously a Category 2 candidate for Federal listing under the Endangered Species Act. The effort and expertise applied to the questions of recovery are brought together at the annual meeting of the RRCC to share knowledge and advance recovery of the robust redhorse. This report summarizes the management activities, research findings, conservation efforts and management decisions made at the 2000 RRCC Annual Meeting, held October 11 - 12 at the Charlie Elliott Wildlife Center in Georgia.

During 2000, the TAG made significant progress on or accomplished all of the work items identified at the 1999 Annual Meeting. Major advances in developing new educational enterprises were an especially exciting accomplishment in 2000. The long anticipated robust redhorse video was previewed in a draft form at the meeting and will be ready for release before the end of the year. In addition, a preliminary Internet web site is under construction at the domain name: www.robustredhorse.com and a representative of the South Carolina Aquarium attended the 2000 annual meeting to discuss a possible installation of robust redhorse at the aquarium. As well, the robust redhorse Conservation Strategy was finalized and distributed to RRCC members. Status surveys were begun in North Carolina on the Pee Dee River system resulting in the collection of 1 mature female representing the second verified specimen collected in the Pee Dee since Cope's 1869 description of the species. Status surveys in South Carolina began on the Broad River in the fall of 2000 and surveys in Georgia increased in frequency and distribution to include the Altamaha and Savannah rivers.

Eighty-three robust redhorse were captured during spring 2000 broodfish collection on the Oconee River. Over the 9 years of sampling the Oconee River, no fish have been collected below the 42-cm length. Yet four of the 83 were juveniles (3 wild and 1 stocked) representing the most immature robust redhorse seen or captured during a single sampling effort. Spring 2000 was the first year a breeding protocol was implemented to maximize genetic diversity of spawning resulting in 65 crosses. Although drought conditions seemed to affect the quantity and quality of gamates and sperm, over 320,000 eggs were fertilized. No short-term cryopreservation studies were undertaken in 2000 due to availability of eggs but activities focused on preservation of sperm from 10 Oconee and 2 Savannah river males using previously developed technology. Approximately 80,852 fry from the spring 2000 spawning season were distributed to ponds at 8 hatcheries in Georgia and South Carolina.

The first year of research on movement patterns of juvenile robust redhorse in the Oconee River was begun in 2000. It used external, radio telemetry devices with mortality sensors to help determine why this age group has been underrepresented and nearly absent from sampling efforts. Research to assess the reproductive and recruitment success in the Oconee River was continued and a one-year study of population dynamics to help determine the long-term status and fate of the Oconee River population was conducted in 2000. As well, further study of the

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ratios of strontium to calcium in otoliths was undertaken to evaluate whether the current wild robust redhorse populations are associated with the use of saline environments during some life-history phase.

The Candidate Conservation Agreement with Assurances and application package for the Ocmulgee River has been forwarded to the USFWS regional office for approval, after which, it will appear in the Federal Register for public comment. The fall 2000 and spring 2001 stocking scenario will be somewhat effected by the timing of an approved Conservation Agreement. The anticipated total harvest from all hatcheries in Fall 2000 is approximately 20,000 - 29,000 Phase I and 2,000 - 3,000 Phase II robust redhorse fingerlings, some of which will be stocked in the Ocmulgee if the Agreement is in effect in time for spring releases.

This was the first year that research in supplemental breeding design was conducted to enhance breeding protocols for maximum genetic diversity. Research on the population structure and genetic diversity in robust redhorse from the Oconee, Savannah and Pee Dee rivers was continued. The RRCC engaged in a rich dialogue on the implication of management activities for the maintenance of genetically distinct strains of robust redhorse. Particularly critical to the discussion of management options and the final management decisions is the tension between managing for the survival of the species and managing for the preservation of individual robust redhorse populations.

Members of the Technical Advisory Group (TAG) investigated funding for a permanent robust redhorse position in 2000 and will continue to explore options to secure this position as one of the 12 priority work items identified for 2001. The 2001 RRCC Annual Meeting will be held October 4 and 5 at Clemson University, South Carolina. Of final note, the responsibilities of Chair of the RRCC passed from Mr. Scott Hendricks to Ms. Terry DeMeo at the 2000 Annual Meeting. Scott brought tremendous organizational skills and energy to his duties and increased the overall professionalism of the position and the RRCC during his tenure. He will remain involved in the recovery of the robust redhorse as a member of RRCC and the TAG, but will be difficult to follow as chairman.

### INTRODUCTION



The sixth annual meeting of the RRCC was held October 11 - 12, 2000 at the Charlie Elliott Wildlife Center near Mansfield, Georgia. The RRCC was established by a MOU signed in 1995 to develop and manage a prelisting recovery approach for the robust redhorse (*Moxostoma robustum*), previously a Category 2 candidate for Federal listing under the Endangered Species Act. The robust redhorse was re-discovered in the Oconee River in Georgia in 1991, the first scientifically confirmed sighting since naturalist Edward Cope described the species in 1869. The RRCC is actively committed to the recovery of the imperiled robust redhorse throughout its former range. This report summarizes the management activities, research findings, conservation efforts and management decisions made at the 2000 RRCC Annual Meeting.

Historically, the robust redhorse inhabited Atlantic slope drainages from the Pee Dee River system in North Carolina to the Altamaha River system in Georgia. The species is presently known to exist in a relatively short reach of the Oconee River between Sinclair Dam and Dublin, Georgia and in a short upper Coastal Plain section of the Ocmulgee River in Georgia. Individuals also have been found in the Savannah River (the boundary river between Georgia and South Carolina) in the Augusta Shoals area as well as below the New Savannah River Bluff Lock and Dam. In addition, a single specimen was found in the Pee Dee River below Blewett's Falls Dam in North Carolina in 2000. The robust redhorse appears to inhabit specialized areas of large rivers, which are difficult to sample and regardless of the absence of sightings, small numbers are sometimes found when species-targeted surveys are conducted.

Nevertheless, river impoundments, predation by introduced nonnative species (principally flathead catfish and blue catfish) and significant deterioration of habitat due to sedimentation and water pollution are believed to have contributed to the decline of the species. The complex and diverse problems facing the robust redhorse require an interdisciplinary approach, using a broad spectrum of experience, expertise and management authority to maintain and restore this imperiled species. In addition, it is essential that recovery efforts include a process that works closely with the private sector as well as government agencies potentially impacted by and interested in robust redhorse conservation.

Approximately 30 representatives of the signatory agencies to the MOU, university research affiliates and other interests attended the 2000 RRCC Annual Meeting (Attachment 1 Participants of the 2000 RRCC Annual Meeting). The twelve signatory agencies include: Georgia Department of Natural Resources, South Carolina Department of Natural Resources, North Carolina Wildlife Resources Commission, Georgia Power Company, Carolina Power and Light Company, Duke Power Company, U.S. Fish and Wildlife Service, U.S. Geological Survey (Biological Resources Division), U.S. Forest Service, U.S. Army Corps of Engineers, Georgia Wildlife Federation, and Georgia Rivers Network. University research affiliates include: University of Georgia Warnell School of Forest Resources, University of Georgia Institute of Ecology, University of Georgia Cooperative Fish and Wildlife Research Unit, State University of New York Medical Center, and Roanoke College Department of Biology. In addition, representatives of other concerns with interest in recovery of the robust redhorse include: South Carolina Electric and Gas Company, Santee Cooper Power Company, South Carolina Aquarium and Natural Resource Conservation Service. The success of the recovery effort, to a large

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extent, depends on the willingness of RRCC members and others to participate in the annual meeting and to continue to support recovery throughout the year.

The RRCC identifies priority conservation needs for the robust redhorse and its habitat as well as coordinates implementation of research and management programs for addressing those needs. The annual RRCC meeting satisfies partial requirement for conservation of the species as designated in the MOU. It also represents the only scheduled time for all interests to assess progress and to establish management directions that guide recovery efforts in the upcoming year and beyond. The annual meeting is the occasion to explore the scientific and management implication of research results and new data, to debate philosophical viewpoints and to gather the collective expertise of fisheries and environmental management professionals. This dialogue includes the best available science on the robust redhorse, which forms the basis of the RRCC's recovery and policy decisions. Although a consensus decision-making approach is sought at the annual meeting, consensus is not always possible due to divergent points of view on the interpretation of preliminary findings, differing comfort levels with acceptable risk or to enduring responsibility related to the consequences of certain decisions. Therefore, some decisions may need to be made by representatives of the administrative agencies that have legal purview for the management decision.

The RRCC has formalized roles to increase efficiency in the management of the recovery process including a robust redhorse project manager and a RRCC chair, vice-chair and technical advisory group (TAG). Jimmy Evans, a biologist with the Georgia Wildlife Resources Division of the Department of Natural Resources, is the robust redhorse project manager and served as the first RRCC Chairman. Mr. Evans oversees coordination of hatchery and spawning efforts. Scott Hendricks, a fisheries biologist with Georgia Power Company and second RRCC Chairman, provides overall administration of the recovery approach including coordination of the TAG. Mr. Hendricks' two-year tenure as chairman expired at the end of the 2000 RRCC Annual Meeting at which time he announced the shift from vice-chair to RRCC chair of Ms. Terry DeMeo, an environmental policy analyst with the Carl Vinson Institute of Government, The University of Georgia. A search for a new vice-chair will be conducted by the Nominating Committee and confirmed by the RRCC at the 2001 Annual Meeting. The TAG was formed as a core work team at the direction of the RRCC at the 1997 Annual Meeting. The TAG provides guidance on and assists the day-to-day work of the project manager and chair. It acts as a decision body in lieu of the full RRCC and makes recommendations for the RRCC's consideration.

At previous Annual Meetings, progress on management activities was reported separately from research results. The format of the 2000 Annual Meeting mixed research and recovery updates by topic, emphasizing the complimentary and iterative nature of science and management in the robust redhorse recovery effort. Some of the research and management activities summarized in the following report have been underway for many years, while others are in their first or second year. Each year, the RRCC identifies and prioritizes work items during the Annual Meeting and directs the TAG to work toward their achievement. Scott Hendricks reported significant progress that was made on or completes accomplishment of all of the 1999 work items. Full

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presentations were made on many of the following work items (in Italics) and their summaries can be found in the body of the report:

- 1. *Finalize development and approval of the robust redhorse conservation strategy.* It was finalized in February 1999 and distributed in June 1999 to RRCC members as part of an RRCC update package.
- 2. Facilitate concentrated status surveys for robust redhorse in North Carolina and South Carolina.

Updates on status surveys in North Carolina and South Carolina are in the report. The status surveys in Georgia increased in frequency and distribution; the results of which are also provided in the report.

3. Continue robust redhorse investigations of the Savannah River including status surveys, spawning activities of broodfish, habitat surveys, and investigation of survival of fingerlings stocked in the Broad River.

Investigations found good gravel bed spawning sites downstream of the New Savannah River Bluff Lock and Dam. A spawning aggregation was observed at one of the sites. Further results of sampling are provided in the report.

4. Create a subcommittee to seek funds for a permanent robust redhorse coordinator position to organize recovery efforts.

The subcommittee was formed, a draft of the position duties and responsibilities was prepared and investigation into various funding scenarios was begun, but options were not developed enough to report at the Annual Meeting.

- Establish a robust redhorse Internet site.
   A preliminary site has been established and was reviewed for comment during the meeting; see summary.
- 6. *Pursue installation of robust redhorse at the South Carolina Aquarium in Charleston.* Progress was made on this work item also and a representative of the aquarium attended the meeting to discuss possibilities, summarized in the report.

The 2001 RRCC Annual Meeting will be held either October 4 and 5, 2001 (first choice) or October 11 and 12, 2001. Ross Self, SC DNR and Amanda Hill, USFWS will host the meeting and seek a meeting location in South Carolina.



#### Spring 2000 Broodfish Collection on the Oconee River - Jimmy Evans, GA DNR

Annual broodfish collection on the Oconee River is an integral aspect of the robust redhorse recovery effort and has been the cornerstone of the supplemental breeding activities since 1993. Usually, run-of-the-river flows are too high for broodfish collection and flows must be reduced at Sinclair Dam. In spring 2000, however, low stable flows (approximately 800 cfs) dominated the entire collection period resulting in excellent sampling conditions. High air temperatures and reduced spring rainfall also caused water temperatures to rise rapidly. Broodfish collection was planned to occur over 3 weeks in early to mid May, but efforts lasted only 2 weeks in late April and early May (April 25 - 26 and May 1 - 4).

Eighty-three robust redhorse were collected from 4 sampling areas (Attachment 2 Summary of Robust Redhorse Collection Efforts in 2000). Of the total, 31 were female (37 percent), 52 were male (63 percent), 57 (69 percent) were tagged recaptures, and 1 was a stocked recapture. In addition, 4 of the 83 were immature (3 wild and 1 stocked) representing the most immature robust redhorse seen or captured during a sampling effort.

The length frequency of the collected broodfish, calculated on 78 fish, resulted in a modal length of 66-cm (Attachment 3 Robust Redhorse Length Frequency 2000). When compared to length frequencies from 1992 to 2000, a continuation of characteristics was evident. As in previous years, 1 - 3 fish were collected in the 42 - 44 cm length. Over the 9 years of sampling the Oconee River, no fish have been collected below the 42-cm length. There continues to be a mystery as to where fish below 42 cm may be, although there is evidence from other rivers that juveniles tend to be collected further downstream than where adults are caught. A comparison of 1994 and 2000 length frequencies reveals a size distribution shift toward larger fish indicating an aging population but slightly more smaller fish are beginning to be seen (Attachment 4 Robust Redhorse Length Frequency Comparisons 1994 vs. 2000). This may represent a promising trend toward evidence of recruitment or it may be sampling bias. The growth rate of tagged fish slows after approximately 15 years of age; the 44-cm length represents approximately 3 - 4 years of age.

A comparison of 1993 - 2000 catch rates shows a decline in the number of fish collected per sampling hour until 1996 when catch rates became stable although lower than for the 1993 – 1996 period (Attachment 5 Robust Redhorse Catch Rates 1993 – 2000). The mean and modal length comparison of 1992 – 2000 electrofishing samples shows an increase in the average length over the years, indicating an aging population (Attachment 6 Mean and Median Lengths of Robust Redhorse 1992 – 2000). The mean and median length trend is beginning to flatten when compared to earlier years (Attachment 7 Trends in the Mean and Median Lengths of Robust Redhorse 1992 – 2000). A minimum and maximum length comparison shows that the largest and smallest fish collected is nearly the same every year (Attachment 8 Minimum and Maximum Lengths of Robust Redhorse 1992 – 2000).

The assessment of length frequencies and catch rates raises questions related to population dynamics. There is a very low catch rate of juveniles, a general shift in length frequency to

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larger fish and declining overall catch rates. These may be long-term trends or normal recruitment fluctuations for species such as the robust redhorse, which can live to 25 years.

#### Spring 2000 Spawning Results - Greg Looney, USFWS

Spring 2000 is the second year spawning efforts were undertaken in April rather than May. Due to 2-year drought conditions, the Oconee River water temperature rose rapidly requiring the spawning facility to be erected earlier than usual at the Beaver Dam Wildlife Management Area. The climatic conditions may also have contributed to the overall poor quality of sperm produced by males. In addition, both males and females displayed breeding characteristics inconsistent with the quality and quantity of eggs and sperm usually expected. Some males, just beginning to display breeding tubercles, produced no sperm, while others produced a quantity of sperm that displayed no motility. Females exhibited a full range of breeding characteristics; some were past spawning while others were not yet ready to spawn.

Ovaprim hormone was used according to the spawning protocol developed over previous spawning seasons. Thirteen of the 26 females sent to the spawning facility produced viable eggs; each female was mated to an average of 4.92 males (range 2 - 9). Thirty-one of the 41 males sent to the spawning facility were used; 30 for fry production and 1 for research purposes only. Each male was mated to an average of 2.13 females (range 1 - 6). Sixty-five crosses were made between the 13 females and 31 males resulting in the fertilization of over 320,000 eggs, 13,700 of which were used for research.

Each spawning season has been conducted to produce a large number of crosses, but 2000 was the first year a breeding protocol was implemented to ensure as much diversity as possible. In previous years, the number of crosses averaged between 20 - 25 compared to the 65 crosses in 2000. The protocol involves dividing eggs from individual females into multiple containers and mixing each egg batch with a different male to achieve separate crosses.

#### Robust Redhorse Sperm Cryopreservation — Greg Looney, USFWS

The study of robust redhorse sperm cryogenic techniques has been underway since 1993 to help protect and conserve the species' viability in the event of sudden declines in the wild and to ensure short-term availability of sperm for annual spawning activities. Short-term storage has been successful for up to 14 days with techniques involving refrigeration, or holding on ice, at  $2 - 4^{\circ}$  C. Long-term storage, cryopreservation, can be successful for an indefinite time using liquid nitrogen freezing at about -196° C. In 2000, sperm was collected using the stripping techniques employed in the past with the sperm being stored on ice until needed.

No short-term storage research studies were undertaken this year due to time constraints and availability of eggs. Sperm samples were, however, stored on ice for use in production due to the less than anticipated number of quality males available for production. Some of the sperm samples were stored with a modified Hanks' balanced salt solution (HBSS) extender added. When collecting sperm of marginal quality, the addition of the extender has lengthened the

### SUPPLEMENTAL BREEDING



period that the sperm was suitable for fertilization of eggs. This enabled the spawning crew to effect additional crosses that would not have been possible otherwise.

Activities involving cryopreservation of robust redhorse sperm in 2000 were focused on preservation of sperm using previously developed technology and fertilization of eggs with cryopreserved sperm. Sperm from 10 Oconee River males and 2 Savannah River males were cryopreserved this year. Some of the Oconee River sperm frozen this year was used in a study to produce fry. Some of the Savannah River sperm was used in a test of larger volume freezing in vials with the remainder frozen using current technology for placement in the repository. Samples from all Oconee males were not frozen due to the average poor quality of sperm samples and time constraints. Two hundred and eighty-four fry were produced with the various study treatments of cryopreserved sperm tested. The 2000 sperm samples used for study purposes were frozen for 48 to 72 hours prior to use. Other samples used in the fertilization study had been frozen for up to 3 years. Dimethyl sulfoxide (DMSO) is currently used as the cryoprotectant.

Studies in the future will attempt to refine and simplify the cryopreservation technique. The quality of eggs and of the motility of individual males continues to be a major influence on the variable outcomes of the results. Cryopreserved sperm has not been as good as fresh sperm but fertilization rates are high enough (20 - 35 percent average) to make it useful for improvement of genetic diversity and, in the better cases, for production. Fertilization rates in the study compared to a 71 percent control were between 0 to 33 percent using three treatments starting at 20 percent DMSO (and increasing). Fertilization results are not outstanding but good for recovery and adequate for production.

Future plans for sperm storage experiments include additional fertilization studies with both refrigerated and cryopreserved sperm and refinement of the techniques. Techniques applicable to production have produced viable fertilization rates with long-term stored sperm, but research into fertility rates per length of storage times needs additional study. Future plans also include the continued management of the robust redhorse sperm repository, which currently contains cryopreserved sperm from several males collected since 1997 (Attachment 9 Cyropreserved Sperm on Hand).

#### 2000 Fry Production and Fingerling Distribution - Jay Shelton, UGA

Approximately 80,852 fry from the spring 2000 spawning season were distributed to ponds at 8 hatcheries in Georgia and South Carolina. None of the ponds have been harvested yet this year but growth in all ponds has been similar to the 1999-growing season. Although a few ponds experienced parasitic disease problems that are new to the robust redhorse effort, a harvest similar to previous years is anticipated. Extra controls were put into place at hatcheries to prevent diseases and development of a disease prevention and control protocol for hatchery managers should be targeted as a work item in 2001.

### SUPPLEMENTAL BREEDING



The Hatchery Pond Monitoring study began in 1998, however a research update was not available at the time of the RRCC Annual Meeting. The research technician that has been gathering and analyzing field data unexpectedly resigned for personal reasons just prior to the meeting. Nevertheless, the study will continue with no loss of data or interruptions to the research.

#### Refugial Population at PNWR — Mark Bowers, USFWS

Refugial ponds were established at the PNWR in 1996 to meet the following robust redhorse management objectives:

- 1. provide and maintain a refugial population to conserve the species' viability in the event of sudden declines in the wild;
- 2. provide larger fish for re-introduction to ensure better survival as well as for research purposes; and
- 3. provide a source of adult broodfish.

The first 2 objectives have been realized and progress is being made on the third to the extent that the fish are healthy enough to breed. A few captive fish are approach the age of maturity, but no eggs or sperm have been produced from captive broodfish to date.

When the refugial management effort began, little was understood about growing a large riverine fish in hatchery ponds. Yet survival has been respectable. Best estimates indicate mortality averages approximately 30 percent at initial stocking and ponds experience an additional mortality of approximately 20 percent each summer season. Pond 11A has experienced 2 flood events with an estimated 7 percent survival rate. Another, holding 1997-year class, is the only pond with no competing species problems and these fish are nearly twice the size of the other year classes. More than 13,000 refugial fish have been stocked; the typical catch per unit effort averages 10 fish per one electrofishing hour. There is an estimated 4,000 robust redhorse remaining at PNWR. In 2001, new equipment will come on-line to monitor all of the ponds to help target water quality concerns and to better determine the number of older fish.

The most time and effort has been invested in these older refugial year classes (1995, 1997, and 1998) of robust redhorse, however, each year a percentage dies. There may be a point (perhaps approximately 5 years of age) when the annual mortality eliminates all but a few of each year class. Consequently, it may be more prudent to stock older year classes than to retain them in the ponds.



# Movement Patterns of Juvenile Robust Redhorse in the Oconee River — Jacquie Hilterman, UGA

The pattern of juvenile robust redhorse movement has been a question in the recovery effort for many years as this age has been underrepresented and nearly absent from sampling efforts. This research, in its first year, sought to address habitat use and movement patterns of juvenile robust redhorse and to determine the contribution of juveniles to the adult population. Fish that had been spawned at Beaverdam Wildlife Management Area and raised at McDuffie State Fish Hatchery were tagged with external, mortality-equipped radio telemetry devices and with PIT and Floy tags. These juveniles ranged in total length from 324 to 377 mm (346 mm mean) and ranged in weight from 360 to 600 g (454 g mean). The decision to use the less invasive external tag procedure was based on striped bass studies and on the fact that the juveniles were thought to be too small to survive an internal tag operation. In the laboratory, fish with external dummy tags lived for approximately 2 months before experiments were terminated.

The Oconee River study reach extended from the Milledgeville Boat Ramp (rkm 230) to the Sportsman Club (rkm 110), just south of Interstate 16. Fish were tracked every 1 to 3 weeks from June 1 to September 12, 2000 in 16 boat surveys and 2 aircraft flights. Data on fish identification, latitude and longitude of tag location, and water quality were recorded. Of the 28 tagged fish, 27 were relocated and 20 were relocated more than 1 time. A total of 64 contacts were made before mortality signals were received from all tags. Nineteen fish moved greater than 1 rkm from the release site; the mean distance moved was 15 rkm (Attachment 10 Movement Patterns of Juvenile Robust Redhorse in the Oconee River). Water temperature ranged from 22.9° to 31.1° C, turbidity was relatively low and ranged from 2.4 to 12.1 NTU; the daily mean water discharge was low averaging below 1000 cfs until late September (Attachment 11 Daily Mean Water Discharge 1950 – 1998 vs. 2000).

All of the tagged fish remained within the study area displaying a distribution pattern similar to adults. Mortality signals were attributed to the death of the fish, a shed tag, or transmitter malfunction. It was impossible to determine if all the mortality signals were triggered by actual fish deaths or by shed tags and transmitter malfunction. Some of the tags may have continued to emit signals as they moved in the current after being shed. Two tags were recovered from the river. Neither tag was attached to a fish at the time of recovery nor was there evidence of a fish nearby. Telemetry remains an imperfect science; Advanced Telemetry Systems allows 5 percent malfunction rate in their manufactured transmitters. The potential role of the drought conditions (low flows, low turbidities and high water temperatures) on the mortality of the juveniles is unknown.

Future directions for this research should include trials during non-drought years, and investigation of seasonal and adult movement patterns. Research results show that small juveniles inhabit the same area as adults and little upward migration was exhibited. However, the high mortality rate of tagged fish indicates that post-stocking stress may have biased movement patterns. Further research is needed on preferred methods of radio-tagging juvenile robust redhorse. There may be an entire suite of life history strategies that are dependent on a



variety of environmental factors affecting seasonal movements and high or low water migrations.

#### Reproduction and Recruitment in the Oconee River — Cecil Jennings, USGS

This research, ongoing since 1995, aims to assess the reproductive and recruitment success of the Oconee River robust redhorse by estimating the abundance and distribution of larval and postlarval fish in response to flow regimes. Sampling occurs during April – September of each year. Investigators are currently analyzing the 2000 data, therefore results reported here are preliminary. Every year this research has modified its methods to more effectively sample. Sampling gear includes seine nets (SN), light traps (LT), benthic light traps (BT) (new this year) and push nets (PN). In 1999, 69,754 fish were collected during 418 samples (Table 1).

Table 1. 1999 Catch Data

	SN	LT	PN	Total
Samples	288	69	61	418
Fishes	68,908	348	498	69,754

Thirteen families of fishes were collected in 1999 including 63,158 Cyprinidae (91 percent), 3,551 Catostomidae (5 percent), and 1,461 Centrarchidae (2 percent). Seventeen larval Catostomidae were collected (Table 2).

	SN	LT	PN
Redhorse	1,144	6	17
Spotted sucker	15	39	62
Carpsucker	2,243	0	1
Northern hogsucker	0	0	1
Total	3,402	45	81

Table 2. 1999 Larval Catostomidae

In 2000, the sampling effort included 275 seine net hauls, 50 light trap sets, 12 benthic light trap sets and 12 push net hauls. Preliminary catch data for 2000 include 359 samples, of which 250 have been sorted. Forty-four samples have been analyzed resulting in 144 identified silver and robust redhorse larvae.

This study has seen strong year classes of redhorse larvae in years of low flows, 1995 - 11, 1996 - 37, 1997 - 35, 1998 - 8, and 1999 - 1179. Past larvae collections of all species averaged 30,000 - 35,000 compared to 69,754 collected in 1999 representing the most ever collected. Preliminary data indicate that for many species in the Oconee River, stable low flows promote high spawning success and better recruitment success. There are limitations in the length-frequency histograms for species identification during years of low flow when the silver and robust redhorse spawning seasons overlap (Attachment 12 Larval Redhorse Collected in 1999). This points to the need to develop genetic markers for species identification of robust versus silver redhorse larvae.



#### Population Dynamics of Robust Redhorse in the Oconee River — Brent Hess, UGA

This one-year research effort was begun in 1999 to study population characteristics as they relate to management concerns, specifically conservation strategies for the current population and reintroductions into the native range. Study objectives included assessing population parameters such as population size, survival, capture probability, and recruitment to determine the potential long-term fate of the Oconee River population.

Georgia DNR capture data records (1994 - 1999), UGA fish sampling results (October 1999 – February 2000), and water quality were examined for the study reach, which extended from the Georgia Highway 22/24 near Milledgeville to the Shady Field Landing in Lowery. The UGA 1999 – 2000 sampling resulted in the capture of 26 robust redhorse (0.33 fish/hours), 12 (46 percent) of which were recaptures. Total length ranged from 417 to 688 mm and weight ranged from 1100 to 4900 g (Attachment 13 Length Frequency of Robust Redhorse 1999 - 2000). The 417-mm fish represents the smallest robust redhorse ever caught from the Oconee River. Water quality parameters included water temperature of  $7.3 - 19.9^{\circ}$  C, dissolved oxygen at 6.6 - 10.5 mg/l, and turbidity measures of 4 - 53 NTU.

The Jolly-Seber Model was used to develop an estimated population size based on capturerecapture data. Population estimates for robust redhorse apply only to the reach of the Oconee River sampled and to the size of fish sampled (> 416 mm total length). The model estimates (averages during the study) the Oconee River robust redhorse population (in the limited reach) at approximately 484 individuals, with an annual survival of 61 percent, a capture probability of 20 percent and annual recruitment between 5 - 57 percent. Although sample effort by GA DNR was not equal among the years (1994 - 1999), calculation of a catch per unit effort revealed no significant difference among years. However, there may be population estimate bias relative to several factors:

- the insensitivity of juvenile fish (< 416 mm) to electrofishing gear,
- the limited reach of river under study (i.e., the reach fish were collected),
- drought condition low flows (depth < 1 m), and
- water clarity (< 10 NTU).

In addition, there seemed to be some movement of robust redhorse during the study, perhaps related to drought conditions, but whether it was driven by seasons, habitat availability, or turbidity is unknown. Further, the model estimates do not take into account human impacts in the watershed, either restoration or loss and degradation of habitat.

The long-term fate simulations project population estimates based on population size, survival rate, capture rate, new recruits, data's stochastic variation, and averaged over 200 replications. The long-term (defined as 100 years) fate estimate of the Oconee River population reveals a dynamic number of individuals any given year with a mean of 278 individuals and a sustained base level of greater than 100 individuals (Attachment 14 Long-Term Fate Estimate for Robust Redhorse in the Oconee River).



Moderately high survival and recruitment is expected in the Oconee River population. Although relatively low, the population estimate will be dynamic over time and the population is expected to persist beyond several generations at a minimum. Environmental conditions, such as water depth and clarity, are expected to effect population at any point in time. Future population research should be updated based on annual broodfish collection data and be conducted under a variety of environmental conditions. Individuals smaller than 417 mm should be targeted for sampling and the sampling area expanded.

#### Preliminary Results of Otolith Microchemistry Analysis - Dave Coughlan, DPC

At the 1999 RRCC Annual Meeting, information on the investigation of the ratios of strontium to calcium (Sr/Ca) in cross-sections of otoliths from 3 Oconee River robust redhorse was presented for the first time. The ratio of Sr/Ca in otoliths has been shown by researchers to increase with use of environments with elevated Sr concentrations (e.g., estuarine or saltwater environments by anadromous and catadromous fishes). Coincidentally, several authors have indicated the ability of some catostomid species to use saline waters and the robust redhorse, specifically, to tolerate salinities up to 16 parts per thousand. The otolith microchemistry research was initiated to evaluate whether the current distribution of wild robust redhorse populations is associated with use of saline environments during some life-history phase.

Otolith cross sections were cut, aged, and photographed. Dr. Karin Limburg, SUNY College of Environmental Science & Forestry, analyzed the entire cross-sectional surface of the otolith for Ca and Sr concentrations by proton-induced x-ray emission coupled to nuclear microscopy. Sr/Ca ratios for each pixel were calculated, color-coded, and graphed for presentation. Higher Sr/Ca ratios are indicated by darker colors (Attachment 15 Oconee River Robust Redhorse Otolith Microchemistry Analysis).

The pattern of Sr/Ca was variable among the robust redhorse individuals but seemed to indicate movement to an area of higher Sr concentration at a time that coincided with the onset of maturity. Some individuals may have visited this high Sr locale later in life while others did not. While the results of the 3 individuals were interesting, they were far from conclusive.

In an interest to increase the sample size somewhat, otoliths from 2 additional robust redhorse were sectioned, aged, and analyzed in January 2000. The figures from these 2 individuals are also presented in attachment 17 and tend to show the same movement pattern as the first 3 fish. This latest information was presented at the *Conservation of Nongame Freshwater Fishes in the Southeast* Symposium at the Southern Division American Fisheries Society Mid-year Meeting, February 3 - 6, 2000, in Savannah, Georgia.

The results tend to reinforce the paucity of information currently available on the life history of the robust redhorse. No information is currently available on the migratory behavior of the fish and if the bands of high Sr/Ca in otoliths correspond with time spent in a saline environment or one of localized high Sr levels. Efforts to implant immature fish with radio/ultra-sonic tags and subsequently track fish movement through the time of maturation could be extremely beneficial



to answering this question. Tracking individuals could lead to an understanding of robust redhorse migratory movements and life history requirements that would be invaluable to the restoration of the species. Future research should include analysis of a statistically significant number of otoliths and comparisons to analysis of otoliths from other sources such as pond-reared fish (to provide a negative control group), fish recovered from Clarks Hill Reservoir and silver redhorse.



#### Oconee, Broad, Savannah and Altamaha River Surveys — Jimmy Evans, GA DNR

Status surveys have been undertaken since 1998 to determine the presence of wild robust redhorse throughout its historic range and to attempt to estimate population sizes when found. Surveys conducted in 2000 were compared with those undertaken in 1999 (Table 3) in terms of the number of stations sampled, hours of electrofishing pedal time and capture results. In addition, total records of robust redhorse captures from 1980 to 2000 were provided.

Table 5. Re	suits of 1777 and 2000	Surveys and Total Cap	tules (1900 - 2000)
	1999	2000	Total Records
Ocmulgee	12 stations		
	26.4 hours		
	2 adults		2 wild adults
	2 juveniles (stocked)		2 juvenile escapees (stocked)
Ogeechee	6 stations		
	10.1 hours		
	2 juveniles (stocked)		5 stocked juveniles
Oconee	broodfish collection	broodfish collection	
	14.1 hours	16.2 hours	
	81 adults	79 adults	~ 800 wild records
		4 juveniles (1 stocked)	1 stocked juvenile
Savannah	6 stations	6 stations	
	16 hours	19.3 hours	
	23 adults	8 adults	37 wild adults
Altamaha		3 stations	
		16.6 hours	
		0 captures	0 captures

Table 3. Results of	1999 and 2000 Surve	vs and Total Captures	(1980 - 2000)
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Surveys on the Oconee River were conducted during the spring broodfish collection activities. One of the 4 juveniles collected from the Oconee River in 2000 had been stocked in February 2000. The juveniles collected from the Ocmulgee River in 1999 had escaped from the PNWR during a flood event in 1998.

Thirty-nine robust redhorse have been collected in Clarks Hill Reservoir, which is on the main stem of the Savannah River upstream of Augusta. The Clarks Hill records are survivals of stockings of 1995 and 1997 year classes released in the Broad River of northeast Georgia in 1996 and 1997. A total of 4 year classes have been introduced into the Broad River. These stockings were made prior to any recent records of robust redhorse in the Savannah. It was anticipated that stocked fish would remain in the main stem of the Broad River and the subsequent collection of individuals in the reservoir was unexpected. However, it appears survival rates of the Broad River stocking were fairly high and may indicate tolerance of or even preference for a lake habitat during a portion of the life cycle. All individuals collected from the Clarks Hill Reservoir were immature juveniles; the largest was just over 400 mm.

Thirty-one of the total captures in the Savannah River were collected from shoal areas between the Diversion Dam to just downstream of the Lock and Dam. The 6 remaining Savannah captures were collected in the upper one-half of the section of river between the estuary and the



New Savannah River Bluff Lock and Dam. There were fewer robust redhorse collected from the Savannah in 2000 than in 1999, possibly due to the drought conditions. The low flows seem to have influenced spawning cycles in 2000 with the peak occurring earlier in the year, which meant that the fish were not concentrated over gravel bars. Although spawning in the Savannah typically extends 2 weeks later than the Oconee, most of the fish collected in 2000 were in post-spawning condition. No spawning activities were undertaken with the Savannah fish in 2000 due to limited pond rearing space.

In comparing the Savannah and Oconee conditions, the Savannah robust redhorse were significantly free of spawning injuries. It was noted that the Savannah contained larger silver redhorse than has been observed in any other river. The area below the Lock and Dam includes documented prime gravel spawning habitat, although there has not been any mapping studies of the habitat. Robust redhorse were observed spawning in these areas 1 week before sampling in 2000 but were not observed during sampling. This is additional evidence that the spawning peak was past. In addition, there seems to be some movement of debris in the Savannah that may have changed the habitat since 1999.

The upper half of the Altamaha River was sampled with 3 electrofishing boats from the confluence to above Jesup in August – September 2000. This section of the Altamaha River has numerous outside bend areas, which are typically associated with good robust redhorse habitat. However, no robust redhorse were collected or even seen during surveys. These results can be attributed to a lack of suitable gravel spawning substrate, although several bedrock, boulder, and cobble outcrops were noted. Silver redhorse and spotted suckers were moderately abundant to uncommon. Carpsuckers were common to very abundant, additionally, 6 Atlantic Sturgeon were shocked during surveys. The sucker population was much depressed compared with the Savannah, perhaps largely the result of the huge flathead catfish population.

It is unlikely a resident robust redhorse population exists in the upper Altamaha River, although it is possible fish could migrate from the Oconee or Ocmulgee and there may be a resident population below Jesup. Future surveys include sampling the lower Altamaha in 2001 for the presence of robust redhorse in an extensive meander section below Jesup downstream to the beginning of tidally influenced areas. That section of the river is more meandering, velocities are fairly high in some areas and there are reports of some small gravel areas below Jesup.

Three additional juveniles were collected from the Ogeechee River, 4 from Clarks Hill Reservoir and 1 more adult was caught in the Savannah River in September and October 2000, after the surveys were completed.

#### Spring 2000 Pee Dee River Survey — John Crutchfield, CP&L

The Pee Dee River in North and South Carolina was surveyed for the first time in 2000 to determine if robust redhorse and Carolina redhorse are present in the drainage (Attachment 16 Pee Dee River 2000 Survey Study Reach). The surveys also intended to evaluate other resident and migratory fish populations and to determine critical spawning habitat areas for future survey



and other research efforts. Like other surveys, the Pee Dee study was a cooperative effort organized by CP&L and SC DNR and involving multiple state and federal agencies.

One robust redhorse was collected on the first day of surveys in the 23-mile Area 1, which represents the second verified specimen collected in the Pee Dee River drainage since Cope's 1869 description (Attachment 17 Historical and Present Distribution of the Robust Redhorse in Atlantic Slope Rivers). It was collected 0.9 miles below the Highway 74 bridge, approximately 4 miles below the Blewett Hydroelectric Dam, which is a transitional area along the Fall Line. The pre-spawning mature female was collected on shoal habitat in water temperatures of 17.4°C. It measured 605-mm total length and weighed 3200 g; its estimated age, based on scale counts, is 6 years. In addition, a great variety of sucker species were observed, including silver and short head redhorse.

Survey needs for the Pee Dee River in 2001 include additional efforts during peak spawning in the reach where the 1 specimen was found to better ascertain population status. Also the existence of adult holding habitat and spawning habitat in the upper coastal plain reach should be determined. In addition, an intense survey is planned for the 17-mile reach between Tillery Dam and Blewett Falls Lake that is upstream of the 2000 survey site. The Pee Dee effort represents partial accomplishment of a long-standing objective of the RRCC (to conduct surveys within the entire historic range of the robust redhorse), therefore, the re-discovery of robust redhorse in the Pee Dee is notable.

#### Plans for Broad River, South Carolina Surveys - Ross Self, SC DNR

Plans to survey the Broad River in South Carolina were initiated in the fall of 2000 using 10 sample stations surveyed in the spring and fall to provide a comprehensive inventory of the fishery resources of the basin. Surveys of the Broad River are part of a 30-year, aquatic resource enhancement fund related to the relicensing of 7 hydroelectric power facilities in the drainage. The fall 2000 surveys represent the first of a 3-year fishery resource survey, the recommendations from which will be implemented over the subsequent 27 years.

The 100-mile reach of the Broad River that is the focus of surveys is fragmented by the 7 hydroelectric power facilities. The oldest hydroelectric dam dates from 1895; consequently some of the river sections have been cut off for 150 - 200 years. Therefore, there is a great deal of uncertainty related to profitable surveys for robust redhorse even though the last hydroelectric plant has shoal habitat below the dam.

#### Survey Needs and Coordination - Discussion

It is critical to the robust redhorse recovery effort to determine the presence of wild robust redhorse throughout the historic range and to estimate population sizes when individuals are found. Therefore, status surveys have increased in frequency and distribution over time and more are needed. Experience has shown that when robust redhorse are targeted in surveys conducted by staff familiar with its specialized habitat preferences, small numbers are often



found. Table 4 lists survey needs in Georgia, South Carolina and North Carolina as identified in the discussion of the RRCC. The first three survey sites in each state are listed in priority order.

Survey	Georgia	South Carolina	North Carolina
Needs			
	Lower Altamaha	Savannah (overlaps with	Pee Dee (23 mile reach of the
1		Georgia's)	2000 capture)
	Lower Oconee	Lower Pee Dee (below Cheraw)	Pee Dee (between Lake Tillery
2			and Blewett Falls)
	Lower Ocmulgee	Broad River, of South Carolina	Upper Yadkin (this may be
3			completed)
	Savannah (up river of Port	Conagree (below Columbia	Cape Fear
4	Wentworth)	including Bypass portion)	
	Oconee (between Barnett Shoals	Lynches	
5	and Lake Oconee)		
6	Broad River, of Georgia	Edisto	
7	Briar Creek	Santee-Wateree	

Table 4. Survey needs in Ocorgia, South Caronna and North Caronna
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Although survey priorities were established, actual surveys undertaken are dependent on environmental conditions and resource availability. Successful surveys are logistically complicated, typically requiring the cooperation and coordination of many entities. In addition, success can be attributed in part to adequate flows. Particularly in Georgia, 2001 surveys may depend on relief from drought conditions.

#### Oconee River Bank Stabilization Project - Louis Kaduk, NRCS

The NRCS is completing a bank stabilization project on the Oconee River. Its main purposes are to help sediment collection and to prevent localized bank erosion. This project is a test case for the long-term effects of restoration practices on large rivers. The project is a 75 percent cost share with the landowner, funded by NRCS' Environmental Quality Incentive Program (\$32,806) and EPA's Partners for Wildlife Program (\$10,000); it required a general USACOE 401 permit. Approximately 1100 feet of bank stabilization has been installed at a cost of \$39 per linear foot.

The project site is located 7 miles below Georgia Highway 22/24 near Milledgeville, about 6-7 miles above Avant Mines. The bank is vulnerable to erosion due to the fluctuation of flows from Sinclair Dam resulting from power generation. It is anticipated that the placement of large trees will control the effect of fluctuating flows by temporarily stabilizing the bank until new vegetative growth provides permanent bank stabilization.

Restoration practices involved cutting 700, 8 - 24 inch diameter trees from the landowner's property and placing them along the 1100-foot section. Starting at the downstream end, 5/16-inch cable was laid across the bank and into the river every 25 feet. The trees were placed on top of the cable parallel to the bank, branches facing downstream with the larger trees on the bottom. The trees were compacted with a track hoe to a height that was slightly above the bank. The



cables were then walked back to the bank, cinched down over the tree mass and anchored around a large log (dead man, 12 - 24 inches in diameter, 6 feet long) that was buried in a trench perpendicular to the bank 6 - 8 feet deep. The final restoration practices will include reforestation of a 100-foot buffer along the bank, which will be fenced to keep cattle out of the river.



#### Update on the Candidate Conservation Agreement with Assurances - Mike Nichols, GPC

A Conservation Agreement with Assurances based on US FWS policy is being implemented in fulfilling the Conservation Strategy adopted by the RRCC. The Strategy is a planning document providing an overview for the conservation of the robust redhorse including long- and short-term conservation goals and a summary of the status and threats to the species' survival. The Agreement describes specific conservation actions for a specific location, consequences of those actions to the species, and also provides assurances to the applicant taking those actions in the event the species is listed.

The robust redhorse CCAA is the first use of this new US FWS policy involving an aquatic species in the nation and, therefore, its development has been a long, collaborative process several years in the making. For example, 3 different draft Agreements were written in 2000. One addressed all known issues and activities in the project site and provided assurances for all stakeholders with the Strategy serving as a supporting document. The second focused on Georgia Power's commitments to the recovery effort and assurances it would be provided while referencing the Conservation Strategy for supporting details. The third was developed as a stand-alone document incorporating necessary elements of the Conservation Strategy.

Activities in the Agreement include monitoring and conservation actions in support of the following phased conservation goals:

- 1. initial establishment of a juvenile refugial population during years 2 through 6;
- 2. establishment of an adult refugial population, years 6 through 11; and
- 3. establishment of a self-sustaining population, years 10 through 15.

Specific conservation actions include:

- stocking the project site with approximately 4,000 hatchery-reared robust redhorse fingerlings each year;
- studying the movement of introduced juvenile robust redhorse;
- monitoring the abundance and distribution of introduced robust redhorse; and
- estimating survival and the size of the population established through stocking.

The monitoring regime and adaptive management practices will allow flexibility to address potential migratory issues. Minimum flow provisions for operating Lloyd Shoals Dam were addressed and incorporated into its FERC re-licensing process in 1992 - 1993. In addition, the Georgia Power Company has committed resources to accomplish the conservation actions using the phased sequence and time line to meet the goals (Table 5).

Tuble 5. Conservation rigiteement Resource Communication				
	Juvenile	Adult	Self-Sustaining	
Movement	150K			
Abundance	60K	40K	20K	
Population		100K	50K	

Table 5. Conservation Agreement Resource Commitment



The Conservation Agreement and application process has been completed. Next steps involve development of an enhancement of survival permit and assurances. The completed application and supporting documents will then be submitted to the southeast regional director of the US FWS. Once approved, the Agreement is listed in the Federal Register where it remains open to public comment for 30 days.

The response to the Agreement cannot be anticipated, however, a great deal of effort was invested during the development process to address potential concerns. It was noted, however, that when the draft Conservation Strategy/Agreement policy was open for comment, it received 280 letters from federal and state agencies, businesses and corporations, religious organizations, trade associations, private organizations, and individuals. Responses to those comments involved addressing 24 policy and two regulatory issues. It is probable that additional issues will have to be addressed for the proposed CCAA before it is finalized.

#### Fall 2000 - Spring 2001 Robust Redhorse Stocking Scenario — Jimmy Evans, GA DNR

As in previous years, the harvest, tagging and stocking of Phase I (2000-year class) and Phase II fingerlings will begin immediately after the Annual Meeting. Consequently, the anticipated harvest from each hatchery and locations and rates of stocking were presented and discussed.

The anticipated total harvest from all hatcheries is approximately 20,000 - 29,000 Phase I and 2,000 - 3,000 Phase II fingerlings. The 2000 schedule for harvest and tagging robust redhorse fingerlings at Georgia and South Carolina hatcheries is provided in table 6.

Hatchery	Harvest Date(s)	Expected Harvest (Phase 1)	Expected Harvest (Phase II)
Burton	-Harvest Oct. 17 -Tag Oct. 18	3,000 - 6,000	-
All SC hatcheries (Fingerlings transported to DWC for tagging)	-Harvest Oct. 31 -Tag Nov. 1 - 2	8,000 - 10,000	200 - 700
Walton	-Drain ponds Nov. 14 -Harvest/tag Nov. 15 - 16	6,000 - 8,000	1,500 - 2,000
Richmond Hill	-Drain ponds Dec. 4 -Harvest/tag Dec. 5	3,000 - 5,000	-
McDuffie (Savannah)	-	-	300 - 500
Totals		20,000-29,000	2,000-3,200

#### Table 6. Schedule of 2000 Harvest and Tagging

Potential stocking scenarios were presented based on assumptions of anticipated harvests and on whether the Ocmulgee River Conservation Agreement will be in effect. Table 7 provides three stocking scenarios of low, medium and high harvest levels, incorporating the best guess estimate of harvest numbers from each hatchery. It indicates the locations (river or hatchery) of possible stocking sites and lists rates for stocking each age class.



Number Hammed 1		Number Starlad	Number Steeled
number Harvested	Location	Number Stocked	INUMBER STOCKED
Computer 1 T 1		Ochiuigee UCAA in Ellect	Ochiuigee CCAA NOT in Effect
Scenario I – Low nary	/est levels	1500 DL H	
	Osmulass	1500 Phase II	
15 000 Dhasa I	Ocmuigee	300  Phase III + (CGBS), 1200.07.08 year aloss (WS)	
15,000 Phase 1		1200 97-98 year class (WS)	
1 500 Phase II		2000 Phase I	2000 Phase I
(Oconee)	Diadmont NWD	5000 Fliase I	5000 Fildse I 600.07/08 year class (WS)
(Oconee)	Pleditiont IN W.K		750 Phase II
300 Phase III+	Phase II Production	8000 Bhasa I	0000 Phase I
(CGBS)	Fliase II Floduction	1000 Phase Life and use do	1500 Phase I
(0000)	CCBS	Stock Ogeocheo if not ready	200 Phase III + (CGPS)
1.200 97/98 year	COBS	Stock Ogeechee II not ready	200 Flase III + (CODS) 750 Phase II
class (WS)			Divide among sites if not ready
( )		2000 Phase Lor 3000 Phase Lif	1500 Phase I
100 Phase II	Ogeechee	CGBS pond not ready	600.97/98 year class (WS)
(Savannah)	Oconee		$100 \text{ Phase III} \pm (\text{SCRS})$
	Fort Gordon	100 Phase II (Sayannah)	100  Phase II  (Sevannah)
Scenario 2 Intermed	inte hervest levels	100 T hase II (Savannan)	100 Thase II (Savainiai)
Scenario 2 – Interineu		2500 Phase II	
	Ocmulgee	$500$ Phase III $\pm$ (CGBS)	
25 000 Phase I	Gemäigee	1200.07-98 year class (WS)	
25,000 T hase T		1000 Phase I	
2500 Phase II		5000 Phase I	4000 Phase I
2500 T huse 11	Piedmont NWR	5000 1 hase 1	600.97/98 year class (WS)
500 Phase III+			1250 Phase II
(CGBS)	Phase II production	12.000 Phase I	15,000 Phase I
. ,		1500 Phase Lif pond ready	1500 Phase I
1,200 97/98 year	CGBS	Stock Ogeechee if not ready	300 Phase III + (CGBS)
class (WS)	0020	Stoon Ogeeenee in not ready	1250 Phase II
			Divide among sites if not ready
250 Phase II		5500 Phase I or 6500 Phase I if	4500 Phase I,
(Savannah)	Ogeechee	CGBS pond not ready	600 97/98 year class (WS)
	Oconee	0	200 Phase III + (CGBS)
	Fort Gordon	250 Phase II (Savannah)	250 Phase II (Savannah)
Scenario 3 – High har	vest levels		
		3000 Phase II	
	Ocmulgee	700 Phase III + (CGBS)	
35,000 Phase I	0	1200 97-98 year class (WS)	
		1000 Phase I	
4000 Phase II		6000 Phase I	5500 Phase I
	Piedmont NWR	500 Phase II	600 97/98 year class (WS)
800 Phase III+			2000 Phase II
(CGBS)	Phase II production	16,000 Phase I	15,000 Phase I
		2000 Phase I	1500 Phase I
1,200 97/98 year	CGBS	500 Phase II if pond ready	500 Phase III + (CGBS)
class (WS)		Stock Ogeechee if not ready	2000 Phase II
500 DI 11			Divide among sites if not ready
500 Phase II		10,000 Phase I or 12,000 Phase I &	13,000 Phase I
(Savannan)	Ogeechee	500 Phase II if CGBS not ready	600 97/98 year class
	Oconee	100 Phase III + (CGBS)	300 Phase III + (CGBS)
	Fort Gordon	500 Phase II (Savannah)	500 Phase II (Savannah)

#### Table 7. Stocking Scenarios for 2000 Harvest



The discussion of rate and location of stocking involved a number of uncertainties related to harvest totals, the potential to stock the Ocmulgee (fulfilling the RRCC's objective to establish a refugial population), population estimates in other rivers and genetics. Like stocking discussions in previous years, potential risk and uncertainty were debated. Specifically, prudent genetic management, threat of sudden species decline, and recovery objectives related to broodfish production effort were discussed. Comments included a caution against stocking the Oconee River at the higher rates based on a population estimate that in some years may fall as low as 300 individuals, as provided in the Population Dynamics presentation. Given this caution, preliminary approval of the stocking scenario was provided. Specific rates will be decided after harvest totals are known.



# Maintenance of Genetic Diversity in Supplemental Breeding Programs — Anthony Fiumera, UGA

Supplemental breeding, as defined for this study, encompasses capturing reproductive adults from the wild, spawning these adults under controlled conditions and returning them to the wild. The progeny are reared in captivity for a period of time and stocked back into the wild prior to reproduction. There are often 2 main goals, high production of juveniles and maintenance of genetic diversity. Maintaining genetic diversity is important for supplemental breeding programs to prevent inbreeding, to protect the adaptive potential of the population to cope with changing environmental conditions, and to limit genetic alterations to the existing natural populations imposed by stocking.

The effective population size,  $N_e$ , is the 'genetic size' of the population, which is often different from the actual census size of breeding adults. For example, if the number of breeding males is different than the number of breeding females, the effective population size will be less than the census size.  $N_e$  will also be less than the census size if there are large differences in the number of progeny contributed by different parents. Effective population size can be a useful measure of the effectiveness of a supplemental breeding program to maintain genetic diversity. Therefore, the goal of supplemental breeding programs should be to attempt to maximize  $N_e$  by using as many breeding adults as possible (in as equal of a sex ratio as possible) and by equalizing the contributions of each to total offspring production.

There are 4 different breeding designs that will meet the supplemental breeding goals to varying degrees. In the first, equalization of family size, the contribution of each male and female is equalized by producing more eggs from under-represented females or by disposing of some eggs of over-represented females. This design will produce a high effective population size but lower overall production (when eggs are disposed of) and therefore does not achieve both goals. The second breeding design is a female bias in which as many females as possible are spawned but the minimum number of males needed to fertilize eggs are used. It yields high production but low effective population size and also fails to meet both goals.

The third and fourth breeding designs use full-sib and half-sib progeny arrays, respectively (Attachment 18 Full-Sib and Half-Sib Supplemental Breeding Designs). A full-sib breeding design is generated when each female is mated only to a single male and each male is mated only to a single female. In a half-sib design, every male is mated to every female and every female to every male. Under the full-sib design, the entire genetic contribution of 2 adults, 1 female and 1 male, would be lost if there was a problem with the gametes of either of the breeders (Attachment 19 Relative Genetic Contributions Under Full-Sib and Half-Sib Breeding Designs). Under the half-sib design, the genetic contribution of 1 adult would be lost if there were not viable gametes but the genetic contribution of the other would remain. The half-sib design provides a higher effective population size and retains the larger amount of genetic diversity, thereby best meeting the supplemental breeding goals of the 4 designs.



In this study, computer simulations were employed to generate hypothetical supplemental breeding programs using the full-sib and half-sib designs. Under the conditions investigated, the half-sib designs had an average increase of 30 percent in the effective population size compared to the full-sib designs. Likewise, the effective population size of half-sib designs were always larger across all investigated number of parents, an approximate 24 percent increase with 5 parents of each sex to an approximate 33 percent increase with 25 parents of each sex. In addition, when the effects of sterility were investigated, the half-sib design again provided increases in the effective population size, even when only 1 percent of males and females are sterile. Finally, the half-sib design did not reduce production compared to the full-sib breeding design.

The half-sib breeding design is the recommended protocol for the robust redhorse supplemental breeding program to maintain genetic diversity and maximize production. Each cross in the half-sib design needs to be conducted separately, mixing sperm from several males should be avoided. It is important to recognize that a complete half-sib design may be an idealized situation. Therefore, each male and female should be mated to as many different members of the opposite sex as is possible and under-represented males should be emphasized.

Preliminary and cautious conclusions support the theoretical basis of this study: a half-sib breeding design is likely to reduce the detrimental effects of supplemental breeding on the wild population. However, in the investigator's opinion, the number of captive-bred fingerlings released into the wild population should be limited to approximately 100 individuals based on the small population estimates in the Oconee River. Further, a full quantitative analysis of the effects of continuing the captive-breeding program should be undertaken if there is a small reproducing population in the wild. Genetic diversity in current robust redhorse stocks is expected to be highest in the wild population, next highest in hatchery-reared refugial fish and lowest in those fish harvested from hatchery ponds. Genetic diversity can be measured from breeding to release.

#### Population Structure and Genetic Diversity in Robust Redhorse from the Oconee, Savannah, and Pee Dee Rivers — Isaac Wirgin, NYU

Research on the genetic characterization of robust redhorse from various river drainages has been underway since 1997. Objectives of the research are to determine if there is:

- significant genetic differences among robust redhorse sampled from various collection sites in the Oconee River,
- significant genetic differentiation among robust redhorse from different rivers, and
- similar genetic diversity in natural populations from the Oconee River and their hatchery produced offspring.

Research approaches include analysis of mitochondrial DNA (mtDNA), which is maternally inherited and sensitive to population bottlenecks and analysis of microsatellites, which are biparentally inherited and evolve extremely rapidly.



In the mtDNA analysis, robust redhorse-specific mtDNA PCR primers were developed, which amplify a 1,300 bp mtDNA fragment containing the control region. One primer was used to manually sequence an approximate 310-bp section of the mtDNA control region, at which the frequencies of mtDNA haplotypes in populations were determined. Genetic material has been gathered from robust redhorse at various collection sites in the Oconee River representing four spawning aggregations and at two different times (1997 – 1998 and 2000) from the Savannah River at two different times (1998 – 1999). In addition, genetic material from a small number of robust redhorse from the Ocmulgee River and a single specimen from the Pee Dee River has been analyzed.

Assessment of genetic differentiation (using mtDNA analysis) among robust redhorse from the different rivers (Table 8) shows that the Oconee and Ocmulgee fish are not genetically distinct but that all Savannah River fish are different from the Oconee River fish at 3 polymorphic nucleotide sites. The extent of mtDNA differentiation of the single Pee Dee River fish from the other two populations was substantially greater. Applying a rate of mtDNA control region nucleotide change of 2.0 percent per million years, suggests that divergence of the Oconee and Savannah river populations occurred approximately 500,000 years ago. Further, this rate of change suggests that the Pee Dee fish diverged from the Oconee and Savannah populations approximately 1.5 million years ago.

		MtD	NA H	laplotyp	bes		
<b>Rivers/Aggregation</b>	Number	А	В	С	D	D'	Е
Oconee							
1	9	5	4	0	0	0	0
2	10	5	5	0	0	0	0
3	3	3	0	0	0	0	0
4	11	3	8	0	0	0	0
Unknown	1	0	1	0	0	0	0
Ocmulgee	3	1	2	0	0	0	0
Savannah (98-99)	22	0	0	10	11	1	0
Savannah (2000)	5	0	0	3	2	0	0
Pee Dee	1	0	0	0	0	0	1

Table 8. Frequencies of mtDNA Control Region Haplotypes in Robust Redhorse from the Oconee, Ocmulgee, Savannah, and Pee Dee Rivers

In the nuclear DNA analysis, 9 robust redhorse microsatellite loci were isolated by standard cloning procedures and library screening; PCR primers were developed from flanking single copy sequences. The DNA from tissue samples was amplified at diagnostic microsatellite loci, characterized by polyacrylamide gel electrophoresis, and the allelic frequencies determined (Table 9).

Table 9. Robust Redhorse Microsatellite Loci

Locus	Repeat	Size (bp)	# Alleles
RR13	(AC) <sub>13</sub>	196	6
RR15	$(GAT)_{12}$	217	1
RR24	(GT) <sub>20</sub>	190	4



RR33	$(AAT)_4$	174	1
RR37	(TA) <sub>25</sub> (TG) <sub>14</sub>	177	15
RR38	(AC) <sub>18</sub>	210	6
RR44	(GGAT) <sub>5</sub> (AT) <sub>27</sub>	113	7
RR55	(TG) <sub>5</sub> (GT) <sub>48</sub>	191	12
RR61	(GT) <sub>48</sub>	152	6
RR61	$(1G)_{5}(G1)_{48}$ (GT) <sub>48</sub>	191	6

There is almost a fixed difference of allelic frequencies at the RR37 microsatellite locus between the Oconee and Savannah fish (Attachment 20 Allelic Frequencies at Microsatellite Loci). Locus RR35 also shows a large but not fixed difference between the Oconee and Savannah and no difference between the Oconee wild and Oconee hatchery-reared robust redhorse. At the RR38 locus, there is a significant difference between the Oconee and Savannah fish and a statistically significant difference between the Oconee wild and hatchery-reared fish.

Assessment of the importance of these genetic differences used evolutionary significant units (ESUs) criteria (Waples), which states that ESUs are reproductively isolated from other conspecific units and they represent an important component in the evolutionary legacy of the species. The genetic criteria to designate ESUs (Moritz) involve reciprocal monophyly for mtDNA haplotypes and significant allelic frequencies at nuclear DNA loci. Based on either set of criteria, robust redhorse from both the Oconee River and Savannah River populations would qualify as ESUs. The management implication of the strong genetic differences between river drainages advises against the transfer of hatchery-reared offspring among populations, specifically against mixing between Oconee and Savannah robust redhorse.

Based on direction provided at the 1999 RRCC Annual Meeting, investigation of the genetic characterization of silver redhorse has begun. The reason for this research focus is that the robust and silver redhorse are closely related and share some life history characteristics, although the silver has wider distribution and larger populations. Preliminary results of mtDNA analysis of 20 Oconee and 20 Savannah silver redhorse show potentially significant genetic differences between the rivers. In addition, there appears to be differences in allelic frequencies at microsatellite locus but not fixed differences.

Future directions of the genetic investigations could include:

- complete comparison of microsatellite diversity in hatchery-reared robust redhorse to that in broodstock from the natural population in the Oconee river,
- comparison of microsatellite diversity in silver redhorse from the Oconee, Savannah and Pee Dee rivers,
- characterization of overall genetic diversity and haplotype frequencies in robust redhorse from other newly "rediscovered" populations, and
- species identification of redhorse larvae from the Oconee.

# **RECOVERY MANAGEMENT**



Management of the robust redhorse recovery involves many factors such as the existence and estimate of populations in the various river drainages, life history requirements, propagation and rearing techniques, habitat availability and quality, and genetic characterization of wild and reared stocks. Through research, much has become known about each of these factors, although perhaps much remains unknown as well. The interdependence of the management factors and areas of uncertainty provide a rich dialogue on short-term and long-term robust redhorse recovery approaches. The RRCC annually explores this dialogue, which is renewed each year by recent discoveries and new research results.

#### **Genetics** — **Discussion**

Results of the genetic research have played a large role in the discussion of management options and the final management decisions. Major points of the genetics-specific management discussion include the following concepts.

- It is important to keep genetically distinct robust redhorse reproductively isolated.
- There is evidence that robust redhorse can be established in a river through stocking, but evidence of any level of recruitment in the wild should effect re-introduction decisions.
- Re-introduction of robust redhorse into a system requires a commitment to continue stocking to prevent genetic bottlenecks.
- There is a need for an empirical assessment of the genetic diversity of wild versus hatcheryreared fish.
- Status surveys must be conducted with more frequency and distribution to determine the presence of wild populations.
- Objective criteria to determine a confident level of surveying and/or amount of electrofishing hours required to detect wild populations do not exist, especially for different sizes of river systems.
- There is evidence that sediment and toxins effect recruitment, perhaps larger fish should be introduced that are beyond the recruitment bottleneck. Wholesale decline of the species may pre-date recent times based on erosion and habitat issues. Habitat restoration, other than providing low stable flows, might be an increasingly important management factor in addition to managing for the presence of nonnative species.
- For a long-lived species, the robust redhorse has been studied for only one-third of a generation (population booms and busts occur) and not long enough to determine recruitment bottlenecks. Perhaps robust redhorse do not need to recruit every year for species survival.

#### Hatchery Production and Refugial Pond Populations — Discussion

The hatchery production program viably meets the goals for which it was established: 1) establish a refugial source, 2) provide for re-introductions into rivers with no wild populations, and 3) augment the existing populations. In addition to 5 ponds totaling over 30 acres at PNWR, Walton Hatcheries has a 3/8-acre refugial pond that is holding 2 older year classes. Two additional refugial sites at Central Georgia Branch Station are under development. Fort Gordon

# **RECOVERY MANAGEMENT**



began a refugial pond for Savannah River fish in 1999 and 3 ponds at the Central Georgia Branch Station (2, 4, and 7 acres), once refurbished, will be available as refugial ponds.

The success of the hatchery program, evidence that hatchery-reared releases are surviving in the wild, genetics concerns and the uncertainty of existing wild populations create a dilemma. The refugial population experiences annual losses in the hatchery ponds, therefore is it worth keeping the refugial hatchery program to try to produce broodstock or should there be a rotation schedule to release the larger fish? The following concepts capture the major concerns and questions related to hatchery production and refugial populations.

- Annual losses out of each year class of the refugial population were anticipated. However, perhaps better data of the pond environment, growth rates and mortality could be compiled to manage the refugial ponds to optimize survival related to water quality (dissolved oxygen and temperature), feeding and other stresses.
- Older/larger refugial fish have been stocked into the Oconee River to manage for predation.
- There is a need to keep the refugial population for potential broodfish until it is certain that the wild population(s) is stable.

Overall, there is potential to grow healthy fish in hatchery and refugial ponds through management. The future of the hatchery production program, especially refugial stock and broodstock production, may depend on the results of population dynamics research, status surveys and genetics research.

#### Population versus Species Management — Discussion

Particularly critical to the discussion of management options and the final management decisions is the tension between managing for the survival of the species and managing for the preservation of individual robust redhorse populations. On the one hand are decisions that help ensure the long-term adaptability and survival of the species through management of small genetically isolated populations and on the other hand is the risk that any unforeseen event could lead to sudden decline of the entire species beyond a reproductively viable level. The following concepts describe some of the discussion points related to management approaches.

- Should the Oconee River be augmented, given it is the only source of wild broodfish?
- What numbers (ratio of wild to bred) can be released to augment the Oconee (and other rivers) without genetically swamping the populations?
- The average age of the Oconee population is approximately 20 years old (the oldest captured is estimated to be 26 years old), but the species' life span is unknown. In the unlikely event that there is no further recruitment in the Oconee, it may be another 5 to 10 years (perhaps 15 20) before the population is senescent.
- A management directive could be based on the number of individuals required to maintain a genetically diverse wild and viable population, if the concepts of viable and self-sustaining population could be determined.

### **RECOVERY MANAGEMENT**



• Production has been taken out of the Oconee River through the supplemental breeding program and those numbers need to be replaced with juveniles that are beyond the recruitment bottleneck.

The RRCC remains concerned with the genetic implications of its management actions and committed to the recovery of the robust redhorse. All genetic evidence suggests keeping populations separate, but there is limited numbers of facilities and resources to manage separate strains. In addition, when the management approach shifts from the luxury of managing individual strains of robust redhorse to managing for survival of the species may not be clear. Genetic concerns are extremely important, but must be tempered with practical constraints of managing an imperiled species with limited resources. Inevitably, there will be the need to occasionally make management decisions equipped with less than perfect knowledge of all issues involved.

### E D U C A T I O N



#### Draft Robust Redhorse Video — Discussion

A rough version of the robust redhorse video was reviewed. Overall response to the video was very positive and enthusiastic. Meeting participants commented on potential refinement to the video with an understanding of time and budgetary constraints of making widespread or technically difficult changes. Comments included:

- Mention the Pee Dee River robust redhorse find in North Carolina.
- If possible, delete references to robust redhorse no longer being a threatened and endangered.
- Check on the possibility of editing video to make a 12- to 15-minute version.
- Get it done.

The final video will include:

- a map of the historic range of the robust redhorse,
- reshooting the aquarium fish segment using robust redhorse that still have their color,
- mention of all of the MOU signatory agencies, and
- exchange of one shot with signage from the Georgia Wildlife Federation as a representative of an environmental organization involved in the effort.

The filmmakers at GA DNR are very pleased with the film and believe it is of the quality and interest to be shown on GPTV.

#### Possible Robust Redhorse Display at the South Carolina Aquarium - David Wilkins, SCA

The potential installation of a robust redhorse exhibit at the South Carolina Aquarium was explored including discussion of the merits of the Reservoir, Piedmont River and Brownwater Swamp exhibits for display. The Reservoir tank has the most space but does not represent the native habitat of a riverine fish and has potential predator issues. The Brownwater Swamp exhibit displays fish found in brackish waters, also not the proper habitat for robust redhorse. The Piedmont River tank displays fish that are traditionally found above the Fall L and although it is rather small, it is the best immediate option with space to hold up to 10 juvenile robust redhorse. In approximately 3 years, the Aquarium expects to open a Large River tank that has the best potential to display robust redhorse.

Based on the experience of the RRCC members, it is believed the robust redhorse could survive an aquarium setting over several years. The hatchery developed feeding regime, catfish pellets and a gel diet formula, could guide the potential aquarium feeding supplemented with wild food. The Dennis Wildlife Center could serve as a fingerling source and receive fish that become too big for the tank.

There do not seem to be any major obstacles to display robust redhorse at the South Carolina Aquarium. Further interest and approval will be explored with the Aquarium director. The TAG will proceed with the possibility of placing a small number of fish in the Piedmont tank with the

# E D U C A T I O N



option of a larger number going into the Large River tank when it comes on line. This cautionary approach will afford the Aquarium time to gain experience with the biological requirements of robust redhorse and to establish a working arrangement with the hatchery.

#### **RRCC Internet Site** — Discussion

At the 1999 Annual Meeting, the RRCC directed the TAG to explore development of a web site to use as an educational tool for the robust redhorse and as a central repository of the growing number of reports, research findings, publications and audio-video materials related to the recovery effort. To that end, a domain name (www.robustredhorse.com) was purchased and web site development has been undertaken through The University of Georgia. A draft RRCC web site was presented and the RRCC provided its approval to proceed with development of the web site based on the following guidance.

The web site should be developed for a variety of audiences. It should provide a balance of information on the robust redhorse and the RRCC, but remain primarily the web site of the Committee. The home page of the web site should open onto a page with the agency logos of the MOU signatories that are hot-linked to each agency. The web site can include a generalized map of the historic range with location of captures. Options for specific contact information include the RRCC chair, the Project Manager and perhaps one other contact in each state, which were not identified. The website should include a listing of research activities and links to actual reports and articles, if possible. It also should include a photo gallery with descriptive information and credits.

# PRIORITIES IN 2001



Each year the RRCC develops a list of work items that are a priority to accomplish in the forthcoming year and charges the TAG with the authority to complete the work items to the best of its ability. During this discussion, new and continuous research needs are reviewed as well but due to time constraints at the annual meeting, the RRCC also charged the TAG with the task of establishing research priorities from those efforts that had been identified. The following lists the research and management priorities for 2001 established at the 2000 RRCC Annual Meeting.

- Support the search for a USFWS trainee who can take over coordinating and conducting spring spawning operations 100 percent within 2 years.
- Develop protocol for prevention, control and management of diseases at hatcheries.
- Develop protocol for managing the refugial ponds for broodfish production and/or for conducting cycled stocking scenario(s).
- Complete and distribute the video.
- Convene the Nominating Committee to nominate a vice-chair.
- Conduct status surveys per established priorities.
- Bring the web page on-line.
- Explore establishment of a RRCC coordinator personnel position.
- Pursue a possible display of robust redhorse at the South Carolina Aquarium.
- Secure site and complete logistics for the 2001 Annual Meeting.
- Determine genetics research priorities:
  - Continue characterization of wild Oconee population and any newly "re-discovered" populations (specifically from the Pee Dee and Savannah below the Lock and Dam) and assess in-breeding;
  - Larval robust vs. silver redhorse genetic identification;
  - Conduct a bottleneck analysis of the Oconee River robust redhorse to determine the history of genetic diversity/the recent history of population size.
  - Characterize hatchery-reared individuals to design a captive breeding program; and
  - Characterize silver redhorse from the Pee Dee (~20 specimens) and compare to the Oconee and Savannah silver redhorse.
- Other research priorities:
  - Sensitivity analysis to determine the potential impact of stocking on wild populations to help tailor an introduction program.

# PRIORITIES IN 2001



 Conduct microchemistry analysis of a statistically significant number of otoliths and comparisons to otoliths from other sources including: pond-reared fish (~ 5 specimens), Clarks Hill Reservoir captures, and silver redhorse.



#### Attachment 1 Participants of the 2000 RRCC Annual Meeting

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		wanstield tra 30005					



Attachment 2 Summary of Robust Redhorse Collection Efforts in 2000



Attachment 3 Robust Redhorse Length Frequency 2000





Attachment 4 Robust Redhorse Length Frequency Comparisons 1994 vs. 2000



Attachment 5 Robust Redhorse Catch Rates 1993 – 2000



### ΑΤΤΑСΗΜΕΝΤ



Attachment 6 Mean and Median Lengths of Robust Redhorse 1992 – 2000



Attachment 7 Trends in the Mean and Median Lengths of Robust Redhorse 1992 – 2000





Attachment 8 Minimum and Maximum Lengths of Robust Redhorse 1992 – 2000





Oconee Males (Tag Number)	Date	Savannah Males (Tag Number)	Date
21972	5/22/97	1701/1702	5/26/99
497/498	5/22/97	1703/1704	5/26/99
546/548	5/22/97	1714/1715	5/26/99
514/515	5/22/97	1732/1733	5/26/99
543/546	5/23/97	1730/1731	5/26/99
497/498	5/23/97	1719/1720	5/26/99
17982/17983 ?	5/23/97	1373/1375	6/7/00
21972	5/23/97	Mortality	6/7/00
536	5/23/97		
21972	5/24/97		
280/399	5/24/97		
84/88	5/21/98		
224/225	5/21/98		
586	5/21/98		
413/414	5/26/98		
84/88	5/26/98		
224/225	5/26/98		
425/21906	5/17/99		
614/616	5/17/99		
603/604	5/17/99		
630/631	5/17/99		
258/602	5/17/99		
1665	4/29/00		
327/1655	4/29/00		
17548/1672	4/29/00		
1636	5/2/00		
449/680	5/2/00		
1632/1633	5/2/00		
17983/17984 ?	5/3/00		
1630/1631	5/3/00		
17942/17943	5/3/00		

#### Attachment 9 Cryopreserved Sperm on Hand

As of 10/2/00 1 or more 0.5 ml. straws per male 26 Oconee males 8 Savannah males

5/3/00

34/1611

### ΑΤΤΑСΗΜΕΝΤ



Attachment 10 Movement Patterns of Juvenile Robust Redhorse in the Oconee River



Attachment 11 Daily Mean Water Discharge 1950 – 1998 vs. 2000





Attachment 12 Larval Redhorse Collected in 1999



Total Length (mm)

Attachment 13 Length Frequency of Robust Redhorse 1999-2000





Attachment 14 Long-Term Fate Estimate for Robust Redhorse in the Oconee River





								River mile		
Date Collected	Тад	PP #	SI (mm)	TL (mm)	Weight	Sov	Status	where	A	Date Aged
Date Obliceted	Tag	КК #	02 ()	()	(Ng)	007	Otatus	concercu	лус	Date Ageu
12-May-97	00411-00412	36	534	658	4.4	м	adult	87	22	3/30/99
14-May-97	00491-00492	37	555	nm	3.65	м	adult	87	21	3/30/99
13-Mav-97	00445-00451	35	514	638	4.11	F	adult	107	16	3/30/99
20-May-97	00528-00542	38	527	627	3.86	м	adult	86	23	3/30/99
4-May-99	00625-00626		548			F	adult	91	19	1/21/00
26-Apr-94		42	537	644	4.1	М	adult	85-88.2	24	1/21/00

Attachment 15 Oconee River Robust Redhorse Otolith Microchemistry Analysis

#### **1999 Robust Redhorse Otolith Analysis**



#### 2000 Robust Redhorse Otolith Analysis





### ΑΤΤΑСΗΜΕΝΤ



Attachment 16 Pee Dee River 2000 Survey Study Reach



Attachment 17 Distribution of Robust Redhorse Records in the Pee Dee River (1864 – 2000)









**Attachment 19 Relative Genetic Contributions under Full-Sib and Half-Sib Breeding Designs** 

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#### Attachment 20 Allelic Frequencies at Microsatellite Loci

#### Allelic Frequencies at RR37

River	1	2	3	4	5	6	7	11	12	13	15	16	17	18	20	21
Savannah	0	0	0	0	0	0	0	2	5	4	8	10	10	1	3	2
Oconee	18	29	13	42	19	0	2	0	0	0	0	0	0	0	0	0

#### Allelic Frequencies at RR55

River	1	2	5	6	7	8	9	10	11	13	14	15
Savannah	0	6	5	9	0	11	3	3	2	4	0	4
Oconee (Wild)	47	8	0	14	3	7	0	11	10	0	38	0
Oconee (Hatch)	53	3	0	19	1	4	0	5	11	0	56	0

#### Allelic Frequencies at RR38

River	0	1	2	3	4	5	6	7
Savannah	3	25	13	0	1	2	1	1
Oconee (Wild)	0	49	0	40	44	10	19	0
Oconee (Hatch)	0	19	0	45	51	8	11	0