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# Culture Techniques and Ecological Studies of the Robust Redhorse *Moxostoma robustum*: assessment of reproductive and recruitment success and incubation temperatures and flows.

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## Introduction

Interest into the status and fate of robust redhorse *Moxostoma robustum* has been ongoing since the species was discovered in the Oconee River, GA during the summer of 1991. Detailed information about the discovery and subsequent activities aimed at recovering this imperilled fish are given in Jennings et al. (1996). This report contains the results of research that was sponsored by Georgia Power Company and was conducted or completed during the period between April 1, 1996 and March 31, 1997. Specifically, this research included the development of culture techniques for robust redhorse, and the assessment of reproductive success in the Oconee River. Research on the swimming performance of larval robust redhorse and the implications for larval recruitment in the Oconee are given in Ruetz and Jennings 1997a.

## Project 1. Reproductive and Recruitment Success of Robust Redhorse in the Oconee River, GA.

During late May 1995, robust redhorse were observed spawning on a shallow (< 1 m) gravel bar in the Oconee River adjacent to the Thiele Kaolin Mine, about 32 km downstream of Milledgeville, GA. Robust redhorse in spawning condition also were encountered at a second shallow gravel bar about 52 km downstream from Milledgeville, but none of these fish were observed spawning. Ichthyoplankton sampling on the Oconee River last spring and summer has documented the presence of larval robust redhorse in the system, but the densities were low.

We investigated the reproductive and recruitment success of robust redhorse in the Oconee River for a second year. Specific objectives for this study were to: 1) document continued spawning activity, 2) determine abundance of larvae in spawning sites, and 3) determine the abundance of larvae in the Oconee River, GA.

## Materials and Methods

This study was conducted from April 1, 1996 to March 31, 1997 in the same reach of river identified and described by Jennings et al. (1996). Initially, as water temperature in the study reach approached 17 °C, known and suspected robust redhorse spawning sites were monitored for indications that robust redhorse had begun spawning. Robust redhorse were observed spawning (see Jennings et al. 1996) on a shallow gravel bar about 10-20 m upstream of the boat ramp at Avant Mine (N 32° 56' 36", W 83° 03' 98"), Washington, County, GA.

Weekly field sampling of larval and juvenile fishes was begun on May 14, 1996 and continued until November 19, 1996 (Table 1). Between May 14 and 20, 1996, gravel substrates on the bar where robust redhorse were observed spawning were sampled with a 0.1 $m^2$ diameter vacuum benthos sampler according to Brown et al. (1987), to confirm that fertilized eggs were being deposited, and to quantify the abundance (i.e., number per $m^2$) off eggs and larvae in the gravel. Larval emergence traps were used to sample larvae as they emerge from gravel at the spawning sites. Ichthyoplankton were sampled in the river with a 0.5 $m^2$ diameter plankton net (505$\mu$ mesh) and with light traps from May 27 to August 27, 1996. The drift nets sampled the faster flowing main-channel areas, and the light traps sampled the slower flowing off-channel and backwater areas; both gear were used at night. From June 21 to November 19, 1996, seines were used to sample post-larval and juvenile fishes in near-shore areas adjacent to sandbars, oxbows, and mud flats. Environmental conditions (e.g., depth, flow, water temperature, turbidity, and dissolved

Table 1. Gear types used to sample larval and post-larval fishes in the Oconee River, Georgia between May 14, and November 19 1996.

| Gear (mesh $\mu$m) | Sampling freq. (No.\week) | Stations sampled | Replicates per station | Mean volume water sampled ($m^3$) |
|---|---|---|---|---|
| Emergent Trap[1] | - | - | - | - |
| Benthic Pump[2] | - | - | - | - |
| Push-net (505) | 2 | 2 | 3 | 100 |
| Light trap[3] (N/A) | 2 | 2 | 3 | N/A |
| Seine[4] (800) | 1 | 3 | 3 | N/A |

[1]Larval emergence traps were used only once toward the end of the spawning season; they were deployed at Avant's Landing on June 5 and retrieved on June 7, 1996.

[2]Benthic pumps were used at Avant's Landing on May 14, May 19 and May 20, 1996. The benthic pumps covered a $^{0.1\ m2}$ area of substrate, and there were 3-5 replicates of each samples.

[3] Light traps were deployed for an average of 3-5 hours in slack water areas.

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

oxygen) at each station were recorded at the time of each sample. Samples were preserved in 10% buffered formalin, returned to the laboratory, and stored until they were examined for the presence of robust redhorse eggs and larvae.

Fish eggs and larvae were extracted from the formalin-preserved samples and placed in vials for later identification. Twenty percent of the sample residues were re-examined to assess the efficiency of project personnel extracting larval fish. The number of larval robust redhorse in the driftnets samples were used to determine the abundance; those from light traps were to be used to identify possible nursery areas for the species.

**Results and Discussion**

Four hundred two samples containing 38,715 larval and juvenile fishes were taken from the study reach of the Oconee River (Table 2). Generally, the catch was similar but a bit lower (about 16%) to that taken from the same reach of river during 1995 (see Jennings et al. 1996). At least 11 families were represented among the catch; minnows (Cyprinidae) comprised about 90% of the catch (by number), and suckers (Catostomidae), mosquitofish (Poeciliidae), sunfish (Centrarchidae), shad (Clupeidae), and silversides (Antherinidae) comprised another 6.5% of the catch (by numbers; Table 2). The estimated efficiency of project personnel extracting larval fishes was greater than 99%; therefore the adjustments of the catch data were unnecessary.

Most of the fishes taken from the study reach were sampled with seines, and fishes were sampled with all gear except the larval emergence traps (Table 3). Swift water currents ($> 1.5\ m^{-s}$) in the areas where robust redhorse were observed spawning made deploying these traps difficult. The traps eventually were deployed in an area adjacent to where robust redhorse were spawning,

Table 2. Number of larval and post-larval fishes, by taxonomic family, collected in the Oconee River, Georgia from May 14 - November 19, 1996.

| Family | Number collected |
|---|---|
| Lepisosteidae | 3 |
| Clupeidae | 393 |
| Cyprinidae | 35,373 |
| Catastomidae | 705 |
| Ictaluridae | 92 |
| Belonidae | 6 |
| Poeciliidae | 688 |
| Antherinidae | 311 |
| Centrarchidae | 459 |
| Percidae | 151 |
| Soleidae | 2 |
| Unknown | 532 |
| Total number of fish collected | 38,715 |

Table 3. Total number of samples (n=402) and fishes (n=38,715) taken by gear, from the study reach of the Oconee River during the period May 14 - November 19, 1996.

| Gear | Number of Samples | Number of Fishes |
|---|---|---|
| Emergent traps | 3 | 0 |
| Benthic pumps | 15 | 5 |
| Push-net | 73 | 1,056 |
| Light traps | 136 | 4,344 |
| Seine | 175 | 33,310 |

but this area may not have been optimal habitat and may explain why larvae weren't caught with this gear (Table 3).

Robust redhorse eggs and larvae were sampled with the benthic pump, but only in low numbers (Table 3). As a result, a laboratory experiment was conducted to determine the efficacy of this gear to sample robust redhorse eggs from gravel substratum. The sampler was about 33% effective in sampling fertilized robust redhorse eggs in gravel substrate (Ruetz and Jennings 1997b), which may account for the low number of eggs and larvae in field samples taken at the Avant's Mine site.

Larval and juvenile suckers were relatively abundant in the 1996 samples (Table 4), much as they were in the 1995 samples (see Jennings et al 1996). However, the numbers of larval robust redhorse in this year's samples of ichthyoplankton drift (Table 5) were lower (1.5-3.5 per 1000 $m^3$ of water) than those reported for 1995 (0.0-13.4 per 1000 $m^3$ of water). Whether the lower estimates represent differences in actual abundance of larval robust redhorse or simply differences in when the estimates were obtained is unclear. The estimates for 1996 were obtained during late May and early June, whereas the 1995 estimates were obtained during early to mid May. Therefore, the lower estimates in 1996 may represent the tail end of the spawning season when larval densities would be lower. The densities of larval robust redhorse sampled from the gravel substrates at the Avant's Mine spawning site on two different occasions (Table 5) also seemed low compared to the number of adults observed spawning in the area. Whether these densities represent actual abundance in the substrate or some artifact of the sampling protocol is unclear. Questions about the depth at which robust redhorse bury their eggs may have some bearing on the ability of this sampler to sample eggs buried deep (> 5 cm) into gravel substrates. None-the-less, overall densities of larval robust redhorse in the Oconee River remain low.

Table 4 Number of larval and post-larval catostomids (n= 705) collected, by gear, from May 14 - November 19, 1996 from the Oconee River, Georgia.

| | Gear | | | |
|---|---|---|---|---|
| Species | Benthic pump | Push-net | Light trap | Seine |
| robust redhorse | 4 | 3 | 0 | 0 |
| silver redhorse | 0 | 0 | 0 | 36 |
| carpsucker[5] | 0 | 103 | 9 | 546 |
| creek chubsucker | 0 | 0 | 0 | 1 |
| Unknown | 0 | 1 | 2 | 0 |

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

Table 5. Average total length (mm) and estimated density of larval robust redhorse *Moxostoma robustum* at specific sites in the Oconee River between Milledgeville and Dublin, GA. 1996.

| Date (1996) | Location (Lat\Long)[6] | Time | Water temp (°C) | Number/mean length (mm) | Estimated Density (larvae/unit)[7] |
|---|---|---|---|---|---|
| May 19 | N 32°56.358' W 83°03.975' | 1655 | ND | 1/10.5 | $6/m^2$ |
| May 20 | N 32°56.358' W 83°03.975' | 1210 | 25.0 | 3/10.8 | $18/m^2$ |
| May 27 | N 32°56.358' W 83°03.975' | 2228 | ND | 2/13.0 | $3.4/1000m^3$ |
| June 12 | N 32°46.876' W 82°57.956' | 2316 | 27.1 | 1/12.9 | $1.5/1000m^3$ |

ND= no data

[6]Latitude and longitude coordinates were determined by a Trimble™ ScoutMaster Global Positioning System.

[7]Estimates for the benthic pump are adjusted (i.e., increased) to account for the 33% efficiency of the sample (per Ruetz and Jennings 1997b)

Juvenile robust redhorse were not among the many juvenile suckers sampled during 1996 (Table 4). This absence was especially apparent compared to the 36 silver redhorse *M. anisurum* that were sampled during this same time period (Table 4). Juvenile robust redhorse also were absent from the 1995 samples, and only four silvers were sampled that year. However, the apparent nine-fold increase in silvers during 1996 as compared to 1995 (36 vs 4), suggests that environmental conditions during 1996 were sufficient to allow a nine-fold increase in the recruitment of silver redhorse. The stark difference in the abundance of these two congeners in our samples is puzzling. Both are thought to occupy the same or similar habitats. If this assumption were true, then the apparent differences may reflect actual abundance of both species. However, if these two species occupied different habitats (e.g., littoral vs mainstem) or behaved differently to the approach of a seine (e.g., hatchery-reared robust redhorse are very wary and have been difficult to seine from hatchery ponds), then the observed differences in abundance may not be representative of actual abundances of these two species.

Differences in when the two species spawn also may explain some of the observed differences in the abundance of silver and robust redhorses. For example, silver redhorse spawn at cooler temperatures (i.e., earlier in the spring) than do robust redhorse. By the time robust redhorse start spawning in early May, operation of an upstream hydro power facility has begun, which results in daily alterations to the natural hydroperiod and increased sediment load in the river. Water-borne sediments may influence incubation conditions in the spawning substrates, and increases in sediment load affects the abundance of some fishes negatively (Shields et al. 1992). Therefore, the earlier spawning of the silver redhorse during a time of a more natural hydrograph may explain the apparent differences in the recruitment of these two species.

## Conclusions

Robust redhorse continue to spawn in the Oconee River, and fertilized eggs and newly-hatched larvae have been taken from the gravel substrate at the Avant's Mine spawning site. None-the-less, densities of larval robust redhorse in the river remain low. Further, juvenile robust redhorse have not been sampled in either of two field seasons. Their absence and the abundance of other juvenile suckers, especially the congener silver redhorse, is puzzling. The mechanisms responsible for the apparent low recruitment remain unknown; however, the results of recently-completed research does not support one hypothesis postulated to limit recruitment (i.e., poor swimming ability of newly-emerged fry; see Ruetz and Jennings 1997a). Other hypotheses are being tested systematically. Although specific answers to the recruitment problem are not available, on-going research has produced new information and new insights into this problem and has focused attention on promising avenues for future research. These avenues include the relationship between sediment load in spawning substrates and the hatching success of incubating eggs, the effects of gear efficiency on abundance estimates, and the life history and recruitment patterns of long-lived species.

## Project 2. Effects of Temperature and Water Flow on the Incubation and Survival of Robust Redhorse (*Moxostoma robustum*) eggs and larvae

Robust redhorse spawning has been observed at several sites along the Oconee River between Milledgeville and Dublin, GA, and fish in spawning condition are abundant in late spring. However; in spite of considerable effort, very few larval, and no juvenile robust redhorse have been collected. Although researchers have been successful in producing healthy robust redhorse juveniles under carefully-controlled laboratory conditions, the near absence of recruitment in the

Oconee river suggests that environmental conditions may not be suitable for incubation of eggs and fry to the point of emergence from substrate as healthy larvae.

Unfortunately, little is known about the early life history of redhorse, and attempts to culture this group of fishes have been few (Hackney et al. 1967). This study was designed to provide vital information regarding the environmental requirements of robust redhorse eggs and larvae in a controlled laboratory setting. The establishment of optimal incubation parameters will be invaluable in establishing the environmental conditions needed to insure survival of early life stages of this species in the Oconee River and in other river systems where reintroduction is being considered. This study is a collaborative effort involving Georgia Power Company, the University of Georgia School of Forest Resources, the Wildlife Resources Division of the Georgia Department of Natural Resources, the U.S. Geological Survey, Biological Resources Division, and the U.S. Fish and Wildlife Service Fish Technology Center at Warm Springs, GA.

There are many factors that can affect the successful development of robust redhorse from the fertilized egg (embryo) stage through the pro-larva (yolk sac) stage, and finally the post yolk-sac larva (swim-up) phase. Factors most critical to early life stages of fishes in the hatchery environment include flow rate and temperature of incubation water. Adequate water flow is needed to replenish dissolved oxygen and remove toxic metabolites. When eggs and larvae are incubated at water temperatures above or below optimal, survival is extremely low, and the incidence of deformities high. This information is vital to the success of recovery efforts, since survival from egg to fry stage under hatchery conditions has been very low (10% during 1995 and 30% during 1996). Survival rates from egg to fry stage in the wild are unknown, but fertilized eggs and yolk sac fry have been collected from spawning areas in the Oconee River (Jennings et al. 1996). In a riverine environment, sedimentation also is likely to affect survival of eggs and larvae. Since the construction of Sinclair Dam in 1952, Oconee River flow volumes have fluctuated dramatically in the study area during the spawning period for robust redhorse (Figure 1). This fluctuation has a direct influence on river water temperatures (Evans and England 1995). Culturally-accelerated sedimentation has been well documented within the historic range of this species (Trimble 1969).

Our objectives for this task were to: 1) examine incubation success through a range of flow rates and turbulence levels, and 2) examine the effects of water temperature on incubation success.

## Materials and Methods

Fertilized robust redhorse embryos were obtained for this study by hormone-induced ovulation methods developed during the 1995 and 1996 (Barrett 1997). Embryos from a single female/male mating were transported to the Warm Springs Fish Technology Center for use in this study.

An incubation system was constructed that facilitated careful control of water temperature and flow velocity for replicate groups of eggs (Figure 2). The incoming water supply for the system had a temperature of 16 °C. An electric chiller and four electric heaters with thermostats (sensitive to the nearest 0.5 °C) were positioned to maintain desired water temperatures in head tanks above each incubation tank. Gravity flow water entered each incubation tank and was distributed to hatching units resembling small McDonald hatching jars (Figure 3).

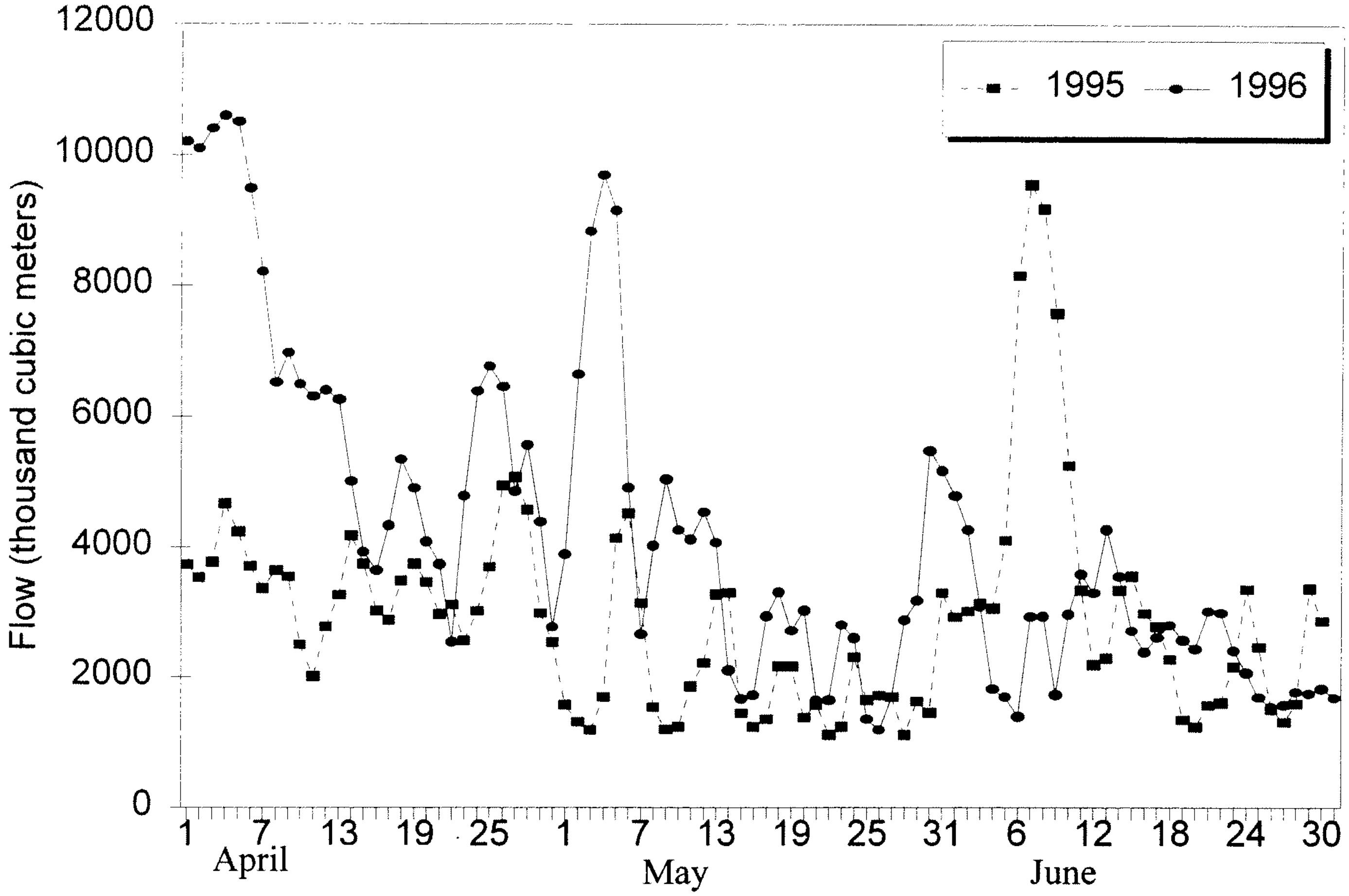


Figure 1. Oconee river flow volumes below Sinclair Dam at Milledgeville, GA during April-June, 1995-1996 spawning periods.

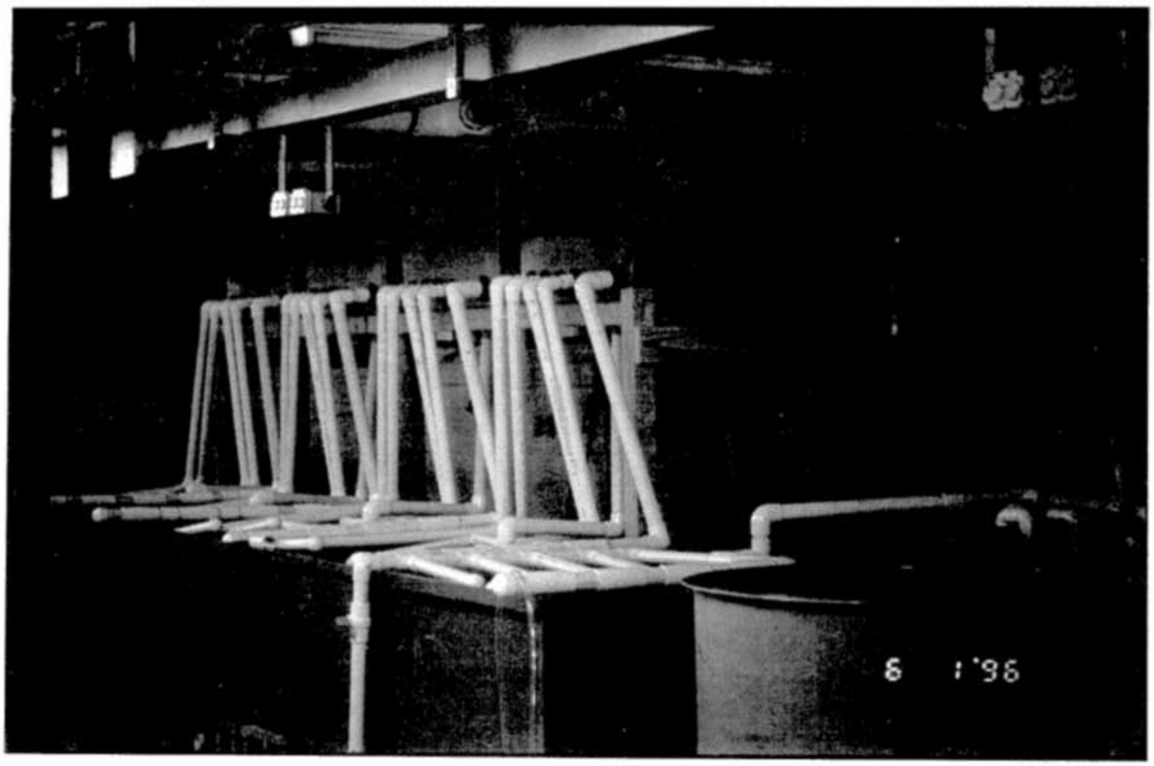


Figure 2. System used to regulate temperature and flows during incubation of robust redhorse eggs.

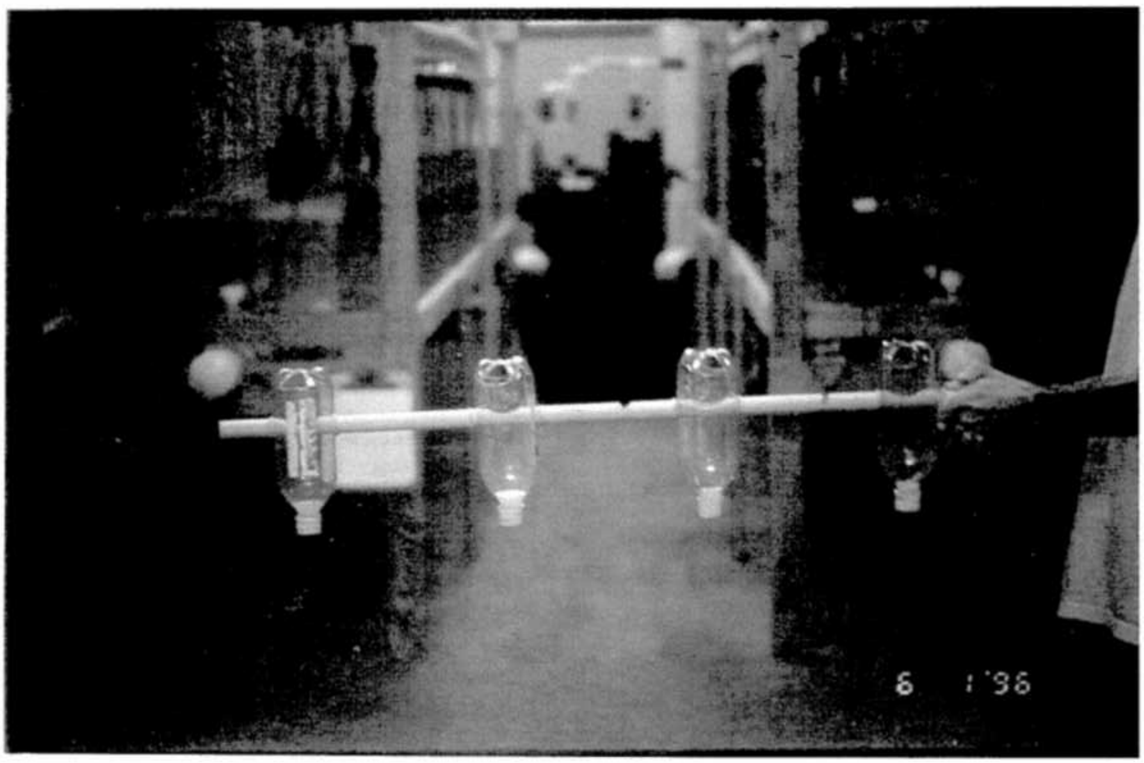


Figure 3. Hatching units used to hold incubating robust redhorse embryos.

A valve located above each jar allowed desired flow to be maintained. Each valve was calibrated with a graduated cylinder and stop watch prior to introducing eggs into hatching units, and then checked daily during the test. Replicate aliquots of 200 embryos by volume were incubated in each container. The incubation chambers were designed so that eggs could be introduced and removed while maintaining constant flow and temperature. Water flows ranged from a low rate, which produced no noticeable turbulence, to a high rate that resulted in highly-turbulent egg movement in chambers. Water exchange rate was the measurable criteria that related turbulence and flow conditions in each chamber. The four flow treatments were exchange rates of 30; 60; 120; and 240 seconds. The five temperature treatments were 15, 19, 23, 27 and 30 °C. There were six replicates of each flow rate at each temperature. Three replicates at each temperature and flow rate were kept in the hatching jars until swim-up/yolk sac absorption was completed (sustained flow group). The other three replicates at each temperature and flow rate were transferred to a low turbulence environment (Figure 4) as soon as hatching began (reduced flow group). Differences between the sustained flow group and the reduced flow group would thus reflect the relative effects of flow conditions on developing eggs versus developing larvae. Surviving larvae were enumerated and examined for gross deformities immediately after each trial was completed.

**Results and Discussion**

Immediately after embryos were placed in study chambers, they became positively buoyant. This was the first (and only) time that floating has been observed during incubation. A group of embryos from the same batch that had been placed in a standard McDonald hatching

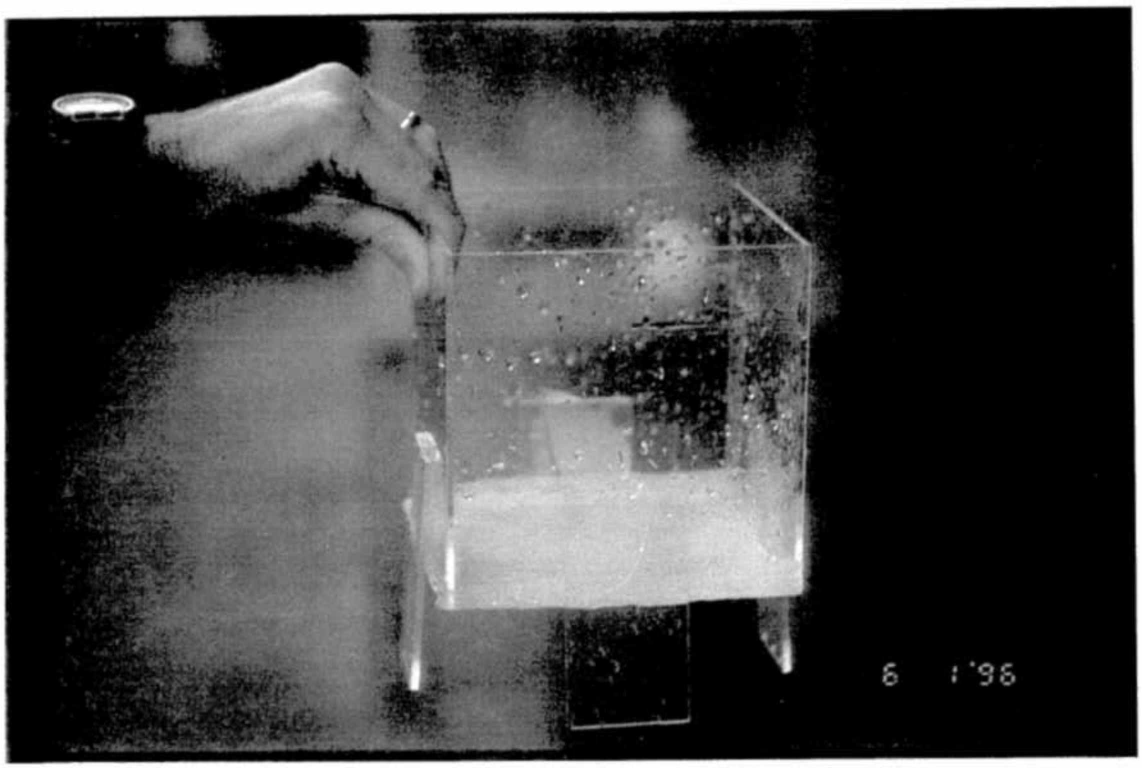

Figure 4. Flow chambers used to incubate robust redhorse in reduced-flow treatments.

jar and served as a control for the study also floated. Therefore, the phenomenon was not a function of the experimental system, but very likely a result of changes in internal gas pressure of embryos, due to temperature fluctuations during transport and/or tempering. Some (701 out of 3,668 surviving fry) of the floating embryos escaped from the study chamber during incubation. Information obtained on the effects of temperature during incubation are for all flow treatments combined. Information obtained on the effects of flow and turbulence on incubating embryos and fry will be presented, but inferences made based on this data **should not** be considered statistically defensible. These observations are included only in support of, and as a guide to future study of flow and temperature effects on incubation.

Of 4,800 embryos subjected to 30 °C water temperatures, none survived to hatch. Survival was highest at 23 °C and decreased as water temperature increased or decreased (Figure 5). Deformities were most prevalent at temperatures of 27 °C and 15 °C (Figure 6). The type of gross deformity observed most commonly was incomplete spinal development, which would almost certainly result in death. Figures 7 and 8 present survival rates (total number of individual alive at the time swim-up begins) for all temperature and flow treatments. Fry that escaped from hatching chambers were not included in the figures.

In the sustained flow treatments, where yolk-sac fry remained in the incubation chambers after hatching, survival was extremely low for the two high-flow, high-turbulence levels of 30 and 60 seconds per exchange (Figure 7). In the reduced flow treatments, where embryos were transferred to low turbulence chambers at the time hatching began, no strong relationship between survival and flow rate was observed (Figure 8).

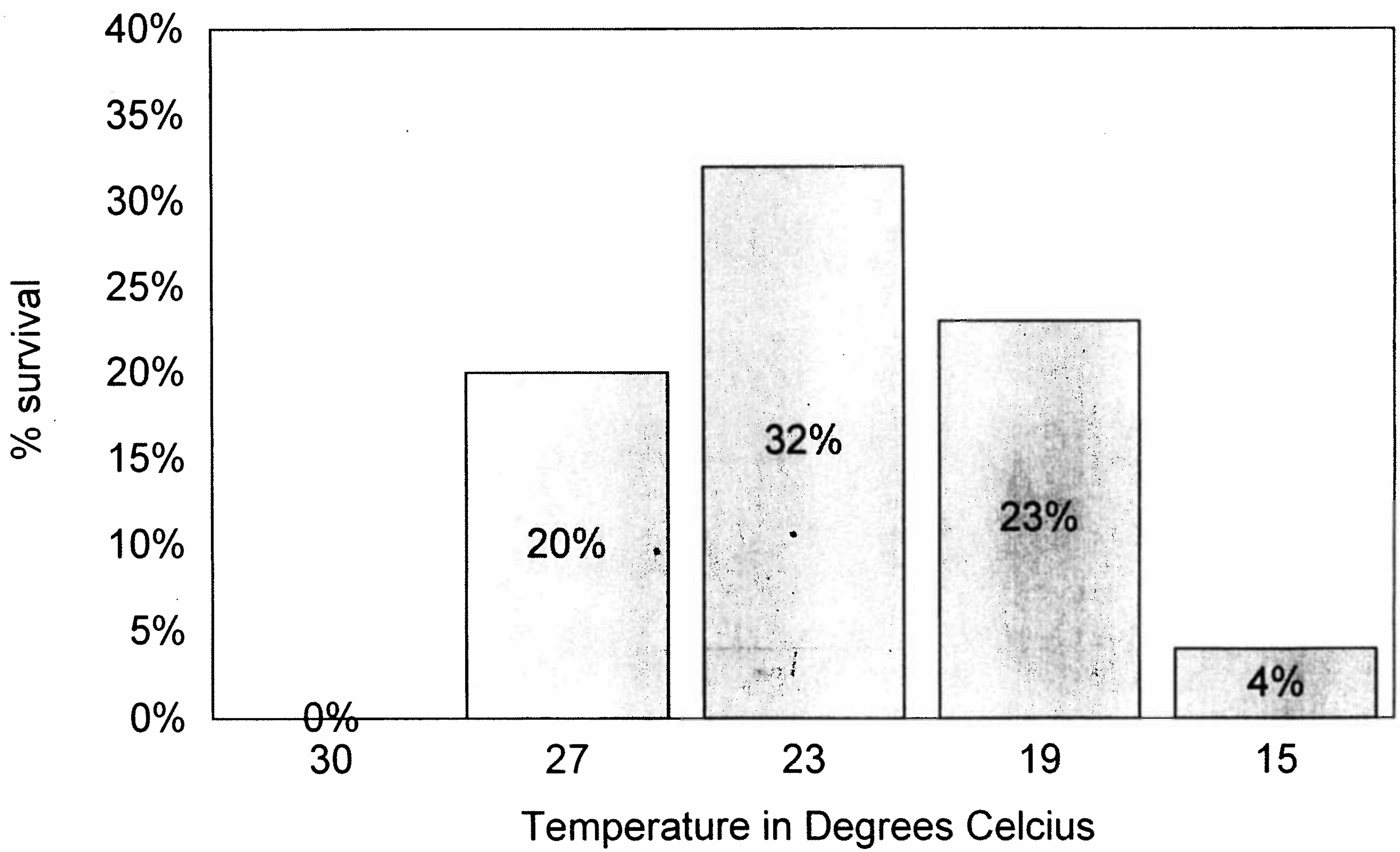


Figure 5. Survival of robust redhorse fry to swim-up at various incubation temperatures.

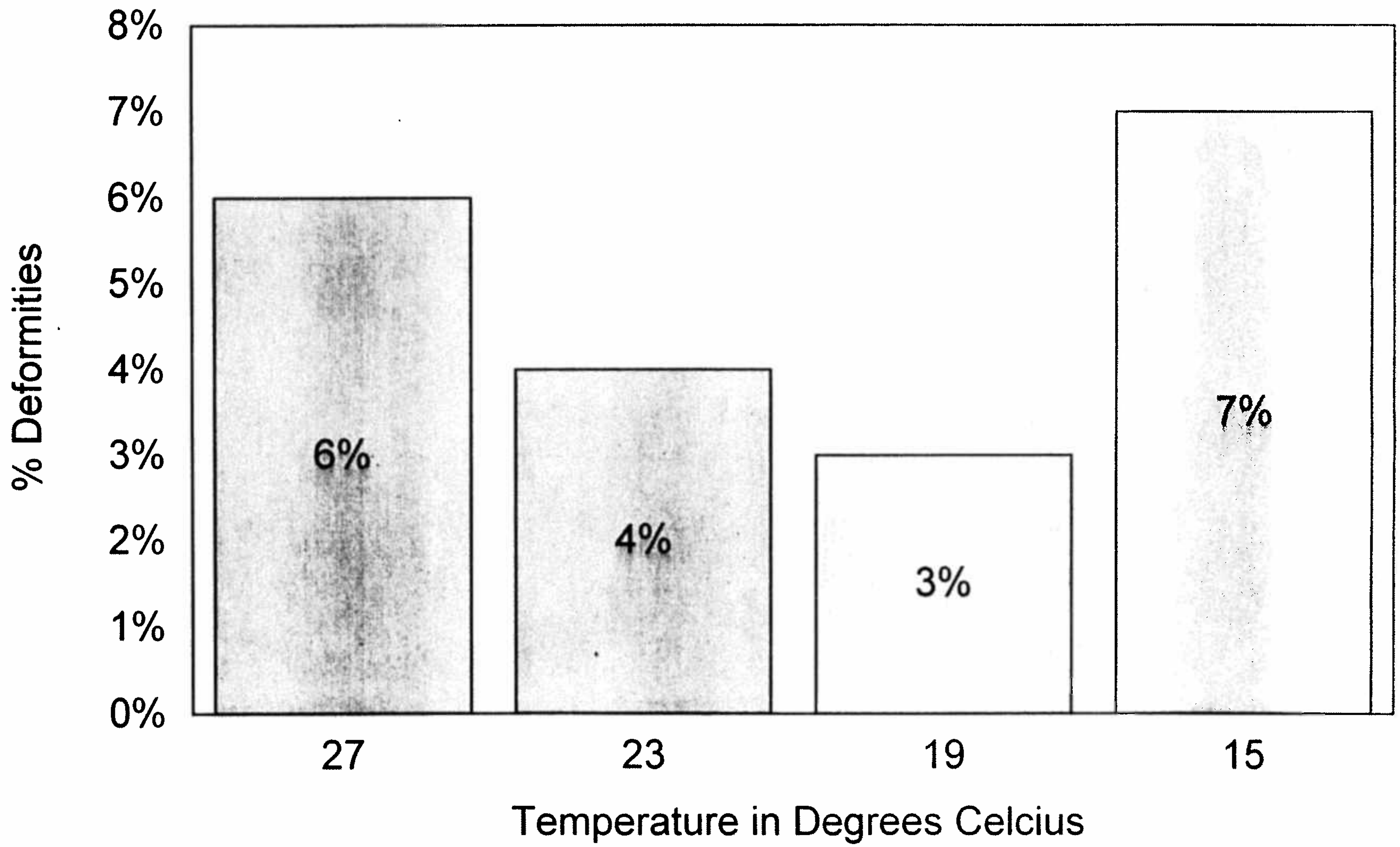


Figure 6. Incidence of gross deformities of robust redhorse fry at various incubation temperatures.

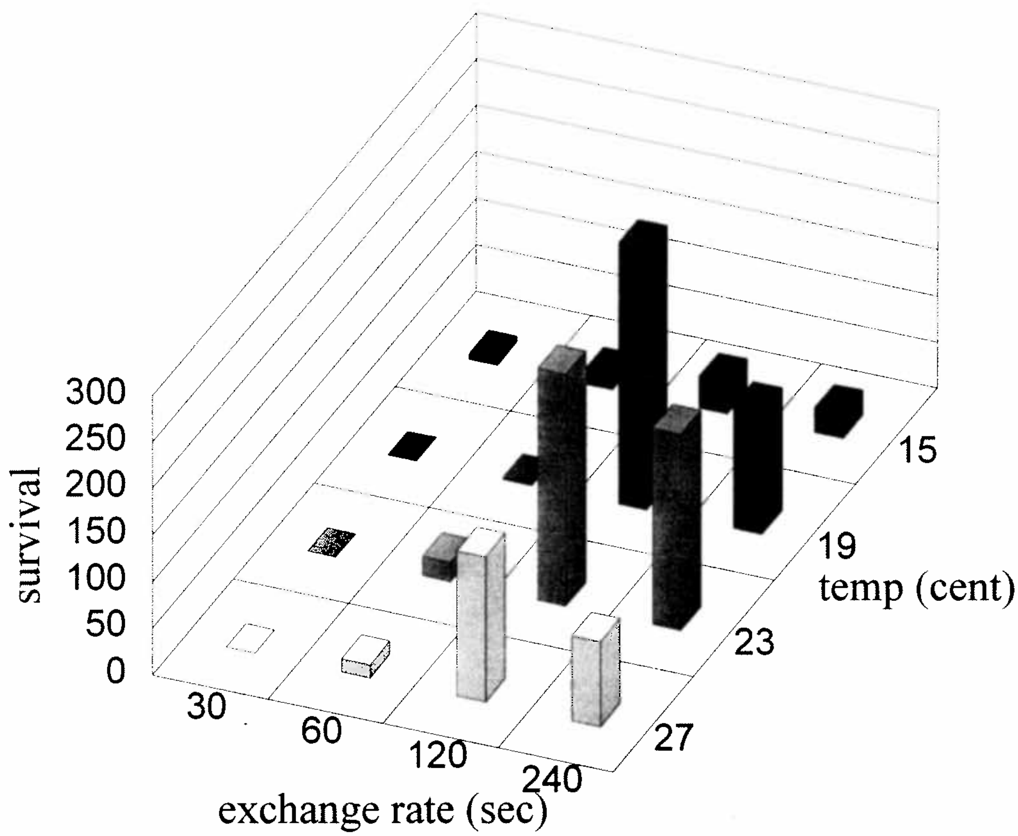


Figure 7. Survival of robust redhorse embryos under sustained flow conditions at various temperatures and exchange rates.

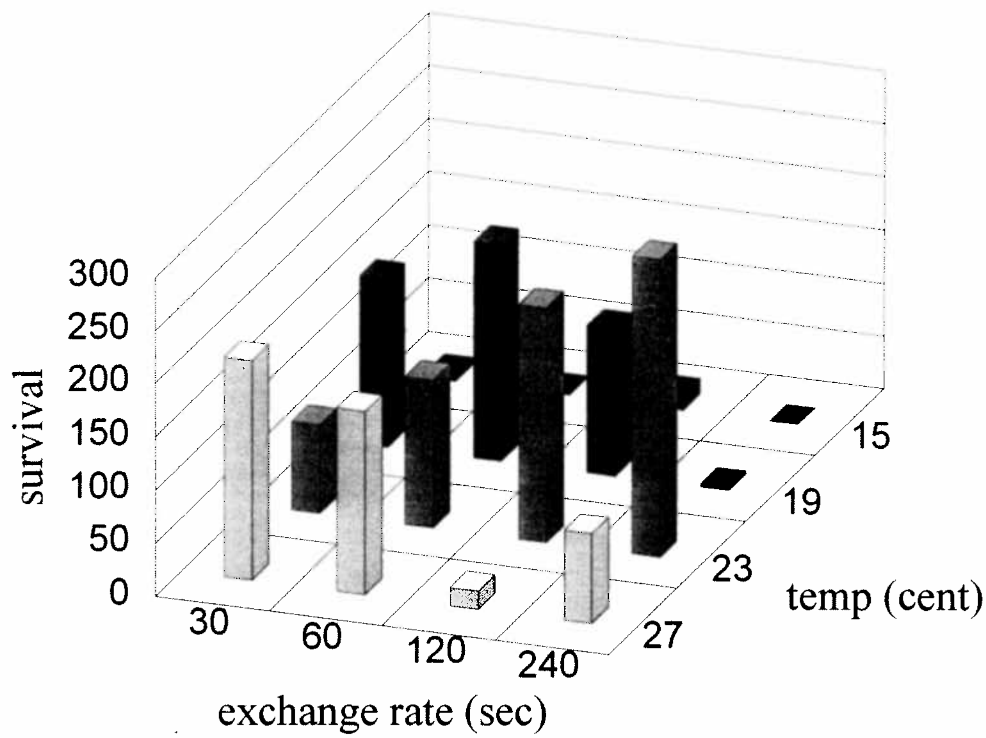


Figure 8. Survival of robust redhorse embryos under reduced flow conditions at various temperatures and flows.

The highest survival rates occurred at 23 °C and 19 °C (32% and 23%, respectively). Deformities were lowest at 19 °C and 23 °C (3% and 4%, respectively). These data suggest an incubation temperature optimum near 23 °C (Figures 5 and 6). Furthermore, temperatures above 27 °C or below 19 °C are likely to have a severe negative effect on incubating embryos and larvae. This upper temperature limit is quite significant since it is possible for wild-spawned fry, incubating in their natural habitat, to be exposed to such temperatures in late May. Water temperatures measured at Beaverdam WMA during 1996 when brood fish were being collected in spawning condition ranged from 17.3 to 26.1 °C (Figure 9).

Comparison of survival data for the sustained-flow versus reduced-flow treatments indicates a striking difference (Figure 7 and 8). Under sustained flow conditions, where yolk-sac fry were subjected to the same flow and turbulence as embryos, survival was extremely low at the two high exchange rates (30 and 60 seconds). Under reduced flow conditions, where yolk-sac fry were incubated in a low-flow, low-turbulence environment, survival does not appear to be affected by exchange rate. Total survival for sustained-flow and reduced-flow treatments were 1,237 and 1,730, respectively. These results suggests that robust redhorse larvae are more susceptible to adverse incubation conditions after hatching than before. Once larvae absorb their yolk-sac, swimming ability is quite strong, and they appear to be capable of tolerating turbulence and higher flows (Reutz and Jennings, 1997a). Therefore, the yolk-sac stage may be the most fragile if displaced from gravel too soon.

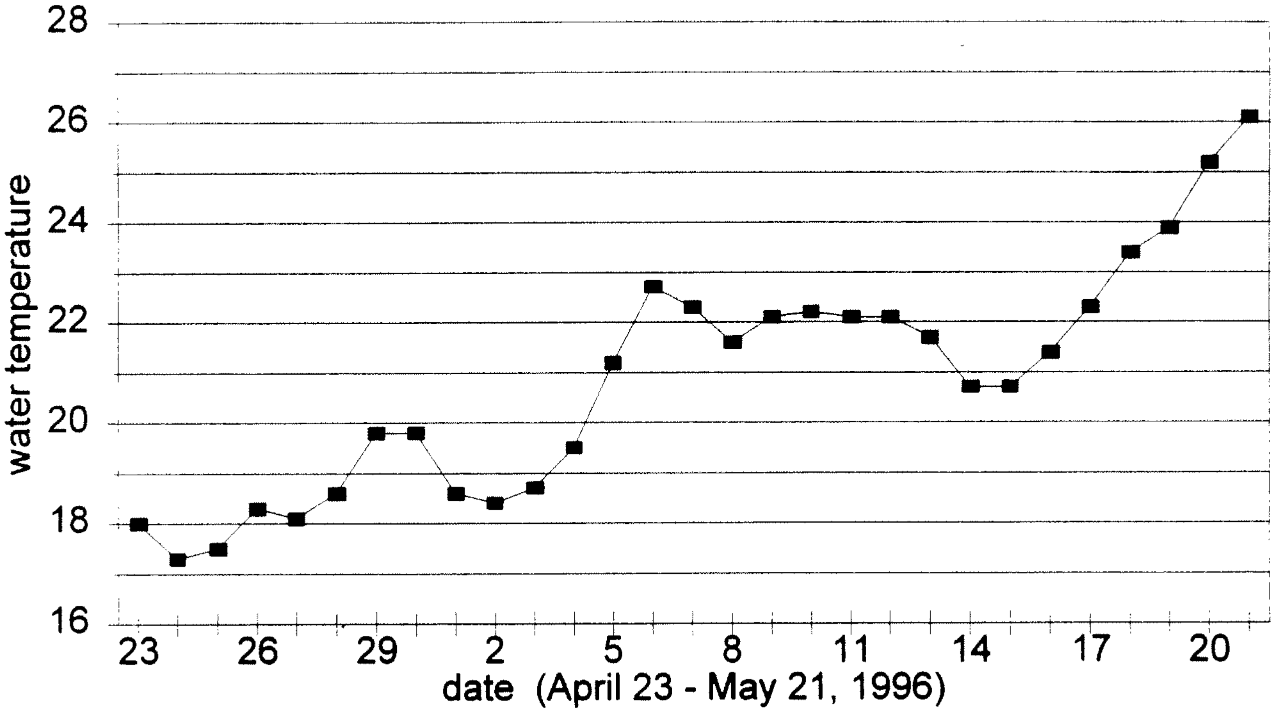


Figure 9. Water temperatures of the Oconee River, GA at Beaverdam WMA during the period of April 23 - May 21, 1996.

## Conclusions

Although some logistical problems have hampered this study, investigations into the importance of temperature, flow, and turbulence are of great value. The values for temperature and flow optima suggested for incubating embryos and fry in this report should be verified. This information has obvious value in our efforts to establish an effective reintroduction program. Furthermore, this information will assist in our systematic evaluation of the reasons for the near absence of robust redhorse juveniles in the Oconee River. This work suggests that yolk-sac fry are the most fragile stage in the early life history of this species. If this is true, then the conditions to which they are subjected during incubation are critical to recruitment. If physical or biological processes (such as scouring or superimposition) occur at spawning sites resulting in the displacement of these larvae, survival is unlikely.

## Literature Cited


Barrett, T. A. 1997. Hormone induced ovulation of robust redhorse (*Moxostoma robustum*). Masters Thesis, University of Georgia, Athens, GA.

Brown, A. V., M. D. Schram, and P. P. Brussock. 1987. A vacuum benthos sampler suitable for diverse habitats. Hydrobiologia 153:241-247.

Evans, J. W. and R. H. England. 1995. A Recommended Method to Protect Instream Flows in Georgia. Georgia Department of Natural Resources, Wildlife Resources Division.

Hackney, P. A., W. A. Tatum, and S. A. Spencer. 1968. Life history study of the river redhorse, *Moxostoma carinatum* (Cope), in the Cahaba River, Alabama, with notes on the management of the species as a sport fish. Proceedings of the Annual Conference of Southeastern Association of Game and Fish Commissioners 21(1967):324-332.

Jennings, C. A., J. L. Shelton, B. J. Freeman, and G. L. Looney. 1996. Culture Techniques and Ecological Studies of the Robust Redhorse *Moxostoma robustum*. Annual Report Prepared for Georgia Power Company, Environmental Affairs Division, Atlanta, GA.

Ruetz, C. R., III and C. A. Jennings. 1997a. Swimming performance of larval robust redhorse: implications for recruitment in the Oconee River. Annual Report Prepared for Georgia Power Company, Environmental Affairs Division, Atlanta, GA.

Ruetz, C. R., III and C. A. Jennings. 1997b. Efficacy of a vacuum benthos sampler for collecting demersal fish eggs from gravel substratum. Ecology of Freshwater Fish (6):241-244.

Shields, F. D., Jr., R. Milhous, J. R. Adams, and R. C. MacAuthor. 1992. Sediment and aquatic habitat in river systems. Journal of Hydraulic Engineering (ASCE) 118(5):669-687.

Trimble, S. W. 1969. Culturally Accelerated Sedimentation on the Middle Georgia Piedmont. Masters Thesis, University of Georgia, Athens, GA.