North American Journal of Fisheries Management 16:963–966, 1996

Field Propagation Techniques for the Endangered Razorback Sucker

GARY J. CARMICHAEL

U.S. Fish and Wildlife Service, Southwestern Fisheries Technology Center
Mora National Fish Hatchery and Technology Center
Post Office Box 689, Mora, New Mexico 87732, USA

J. HOLT WILLIAMSON

U.S. Fish and Wildlife Service, Southwestern Fisheries Technology Center
Dexter National Fish Hatchery and Technology Center
Post Office Box 219, Dexter, New Mexico 88230, USA

OWEN T. GORMAN

U.S. Fish and Wildlife Service, Arizona Fisheries Resources Office-Flagstaff
Post Office Box 338, Flagstaff, Arizona 86002-0338, USA

TERRENCE R. TIERSCH

School of Forestry, Wildlife, and Fisheries, Louisiana Agricultural Experiment Station
Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803, USA

Abstract.—We used plastic bags to incubate, transport, and hatch embryos for specific crosses of razorback suckers *Xyrauchen texanus* during two spawning seasons on the Colorado River (Nevada–Arizona). Fifty to 2,000 razorback sucker eggs were fertilized in plastic food storage bags for 26 matings in 102 trials. Experimental treatments prevented hatching in all bags, but hatching was observed when fresh milt and viable eggs were used (10–99% hatch). Observation of embryo development and treatment effects was facilitated by the transparency and portability of the bags. Water volume and exchange rates were chosen according to estimated metabolic rates, and the addition of oxygen to the bags was evaluated to increase holding times. Plastic bags were inexpensive and useful for gamete storage, controlled propagation, and transport of gametes, embryos, and fry under field conditions. These techniques are suitable for various applications in studies of reproductive biology, and they are especially suited to genetic management and research on endangered species that cannot be removed from the wild.

Endangered fish recovery plans often require controlled propagation that differs from the culture of traditional sport or commercial species. Additionally, there often is a need to keep specific crosses separate for genetic management and research purposes. Standard technology has been developed for propagation of commercially valuable fishes such as centrarchids, ictalurids, and salmonids (e.g., Dupree and Huner 1984a). However, techniques are needed to meet the unique breeding and rearing requirements for many rare, threatened, and endangered fishes. Incubation and hatching of fish eggs traditionally depends on methods that aerate and gently agitate fertilized eggs of captive broodfish populations (Piper et al. 1982). Although functional and efficient, traditional methodology has limited field applications. For example, techniques for controlled propagation of endangered fishes traditionally consisted of capturing and holding adult fishes for propagation. These methods may still be useful, but recent concerns about removing endangered fishes from native habitats, as well as small population sizes, and legal constraints make capturing and holding endangered fishes problematic.

Cryopreservation of sperm has been identified as a useful technology to manage the genetic diversity in broodfish populations (e.g., Cloud et al. 1990) and has received attention for commercially valuable fishes such as salmonids (Stoss 1983; Leung and Jamieson 1991) and ictalurids (Tiersch et al. 1994). Application of this technology to the endangered razorback sucker *Xyrauchen texanus* would aid conservation by improving controlled breeding programs, gamete storage, and development of hatchery broodfish populations.

We developed and tested techniques of storing gametes of razorback suckers for cryopreservation and controlled fertilization. Our purpose was to develop methods to isolate treatments and obtain offspring from individual crosses or matings of razorback suckers. Specific objectives included fertilization, incubation, and hatching of razorback sucker embryos obtained from Lake Mohave of

the Colorado River and transporting gametes, embryos, and fry to other locations. In testing methods for field use, we required large numbers of small, uniform, dependable, nontoxic, inert, and inexpensive containers. Various types of food storage bags were tested for use in fertilization, water-hardening, incubation, transport, and hatching of embryos of razorback suckers. Fish sperm and fry have previously been held and transported in polyethylene bags (Stoss 1983; Dupree and Huner 1984b), but fertilization and incubation of eggs through hatching has not been reported.

Methods

Ripe razorback suckers were collected in March of 1994 and 1995 by electrofishing the Colorado River between the Willow Beach National Fish Hatchery (WBNFH) and Hoover Dam. Experiments were conducted along the Colorado River and at WBNFH with eggs and milt from 52 fish to fertilize, incubate, observe development, or hatch eggs from 26 specific crosses in 102 trials.

Milt or eggs were stripped from towel-blotted fish directly into quart-sized (about 1-L) freezer or food storage bags. Some bags were supplemented with Hanks' balanced salt solution, prepared without calcium (added in equal volume to the eggs or milt), to store or transport gametes (Tiersch et al. 1994; in press). In the field and the hatchery, eggs were fertilized by adding milt following the "dry spawning" method of Piper et al. (1982). After gametes were mixed, about 50 mL of Colorado River water was poured into the bag to activate the gametes. After 30 s, the volume was increased to 250 mL to water-harden the eggs. Transportation trials included moving embryos in bags from the Colorado River to the WBNFH, to Las Vegas, Nevada, and to Albuquerque, New Mexico, and moving fry to the Dexter National Fish Hatchery and Technology Center, Dexter, New Mexico.

We evaluated three Ziplock® brand, Gripper® Zipper containers: (1) heavy duty freezer bags, (2) food storage bags, and (3) vegetable storage bags. Bag types 1 and 2 were evaluated for use in storage, fertilization, water hardening, incubation, and transport; bag type 3 was evaluated for use in incubation. These bags were inexpensive (US$0.06–$0.09 each in 1995) and were replaced easily when dirty or discolored. Individual bags were used only once. All bags were constructed of polyethylene, but the heavy-duty bags were thicker (2.7 mils; 1 mil = 25.4 μm) than the food storage and vegetable storage bags (1.75 mils). The food storage bags were permeable to gases, whereas the vegetable storage bags were porous due to manufactured perforations. The pin-hole-sized perforations were small enough to retain eggs but were large enough to allow the bag to drain water. All containers were labeled with permanent ink markers.

In most trials, 200–300 razorback sucker eggs with aggregate volumes of 2–3 mL were used per bag. In several tests for fertilization, however, up to 20 mL of eggs were placed in a bag. We estimated the metabolic rates of eggs to determine how often to change water in the freezer and food storage bags. A maximum metabolic rate of 150 mg oxygen/kg biomass per hour (Prosser 1973) was assumed, and about 250 mL of oxygen-saturated water (about 8.5 mg oxygen/L) was used in each bag. The bags were stored flat to increase water surface area and enhance gas exchange. The closed seal on the bags was slightly elevated as a precaution against accidental leakage due to incomplete sealing. Care was taken to enclose air, and in some tests, supplemental oxygen was added from bottles in place of air. Incubation temperatures were maintained at 21–23°C by thermostatically controlled air temperature.

Complete water exchange was used to maintain water quality in heavy-duty freezer bags and food storage bags. About 225 mL of water was replaced, twice daily, by opening the bags and decanting the water from the eggs. Intervals between water exchanges ranged from 10 to 16 h, and exchanges became more frequent as the embryos neared hatching. Water was obtained directly from the Colorado River (about 12°C), heated to 21–23°C, and aerated at the WBNFH prior to use. Vegetable storage bags containing fertilized eggs transferred from freezer bags were placed in aerated 23°C water in a 4,000-L recirculation system. To test if the perforations provided sufficient water exchange, no other water exchange or aeration was provided to the eggs in the vegetable bags.

Fungus was controlled by manual removal of hyphae-infected eggs with a transfer micropipette. A 50-mL watch glass placed on a grid was used to count and examine eggs and embryos sampled from the containers. A binocular microscope (70× magnification) was used to determine developmental stages of embryos. Hatching percentages were determined by dividing the number of hatched larvae by the total number of eggs.

Results and Discussion

Fertilization, water hardening, and hatching of razorback sucker eggs were obtained in both types of food storage bags and hatching was obtained in

TABLE 1.—Gastrulation and hatching of razorback sucker embryos in 102 trials with 50–2,000 (usually 200–300) eggs per plastic food bag. Three types of Gripper® Zipper food bags were compared. Embryos from some fish crosses were split into several lots such that the total number of crosses appears to be greater than 26.

Year	Number of:		Percentage of embryos reaching:	
	Crosses	Trials	Gastrulation	Hatching[a]
Heavy duty freezer bags				
1994	10	33	100	0–99
1995	16	39	100	0–96
Food storage bags				
1994	10	24	100	0–90
Vegetable storage bags				
1995	6	6	100	24–92

[a] Several experiments used unfertilized eggs or inactive milt such that gastrulation was the end point of development and hatching was not expected.

perforated vegetable bags (Table 1). In 72 trials of the heavy-duty freezer bags stocked with 50–2,000 eggs, we obtained up to 99% hatch. Twenty-four trials of the food storage bags yielded up to 90% hatch for between 100 and 900 stocked eggs. Six trials of the perforated vegetable storage bags yielded up to 92% hatch for 200–300 eggs. The trials with 2,000 eggs per bag were all above 99% hatch. Water hardening and incubation through the gastrula stage were observed in every trial. Experimental treatments prevented hatching in some trials, but hatching occurred in all bags that contained fresh milt and viable eggs. These procedures allowed isolation and replication of a large number of trials with multiple crosses. Our replicated cryopreservation and fertilization trials would have not been possible without development of these techniques.

The bags were portable and up to 15 at a time were transported from field sites to WBNFH for up to 4 h on the Colorado River in 19-L buckets or 45-L ice chests. On land, buckets, ice chests, or an attaché case were used to hold the bags for up to 7 h. Temperature was maintained by using ice in the buckets and ice chests. Some bags were flown to Albuquerque in the attaché case.

We preferred the heavy-duty freezer bags to the thinner storage bags because they resulted in high success and retained the atmosphere above the contained water better. Vegetable storage bags may prove useful for incubation and hatching by eliminating the need for water changes, but more experiments need to be conducted. The thin-walled food storage bags were apparently more gas permeable than the freezer bags and tended to deflate and lose all or most of the enclosed air. This deflation did not reduce development or hatching, but it might have a negative effect on hatching and embryo development if water exchange intervals were lengthened because of the loss of atmosphere above the water. However, water volume and exchange rates were adequate to prevent oxygen depletion and poor water quality with the numbers of embryos we studied. The addition of oxygen to the heavy-duty freezer bags may help to lengthen the time between water exchanges. The oxygen levels in the bags receiving bottled oxygen were measured with a meter and remained at saturation throughout the trials.

These three types of bags are manufactured with labeling areas that were useful for identifying individual treatments. Observation of egg and embryo development and treatment effects was facilitated by bag transparency. We used back illumination to evaluate water quality, presence of fungus, and embryo development.

The use of food storage bags should facilitate an expanded range of studies in field research of reproductive biology. This is especially important in research with endangered species, which typically cannot be removed from the wild. The bags can be used in large numbers in a variety of environments from wilderness to urban areas, and they are small, uniform, dependable, nontoxic, inert, and inexpensive. One hundred food storage bags weigh less than 1 kg, cost less than $10.00, and provide a complete, disposable microhatchery suitable for river bank research.

Acknowledgments

We thank M. Baker, K. Cobble, L. Gloshay, J. Hanson, B. Johnson, S. Leon, M. Lucas, J. Nathan, D. Parker, P. Plagge, J. Seals, and D. Stone for collecting fish and gametes. Razorback suckers were collected under the U. S. Fish and Wildlife Service, Southwest Regional Director's Endangered Species Permit PRT-67811; sub-permittee, Arizona Fisheries Resources Office. Housing was provided by J. Hanson and P. Carboni, Willow Beach National Fish Hatchery. Technical assistance was provided by J. Seals, W. Wayman, and W. Williamson. Mention of trade names or commercial products does not constitute endorsement or recommendation for use by Louisiana State University or the U.S. Fish and Wildlife Service.

References

Cloud, J. G., W. H. Miller, and M. J. Levanduski. 1990. Cryopreservation of sperm as a means to store salmonid germ plasm and to transfer genes from wild fish to hatchery populations. Progressive Fish-Culturist 52:51–53.

Dupree, H. K., and J. V. Huner, editors. 1984a. Third report to the fish farmers. U. S. Fish and Wildlife Service. Washington, D.C.

Dupree, H. K., and J. V. Huner. 1984b. Transportation of live fish. Pages 165–176 *in* H. K. Dupree and J. V. Huner, editors. Third report to the fish farmers. U. S. Fish and Wildlife Service. Washington, D.C.

Leung, L. K.-P., and B. G. M. Jamieson. 1991. Live preservation of fish gametes. Pages 245–269 *in* B. G. M. Jamieson, editor. Fish evolution and systematics: evidence from spermatozoa. Cambridge University Press, Cambridge, UK.

Piper, R. G., and five coauthors. 1982. Fish hatchery management. U. S. Fish and Wildlife Service, Washington, D.C.

Prosser, C. L. 1973. Oxygen: respiration and metabolism. Pages 165–211 *in* C. L. Prosser, editor. Comparative animal physiology. Saunders, Philadelphia.

Stoss, J. 1983. Fish gamete preservation and spermatozoan physiology. Pages 305–350 *in* W. S. Hoar, D. J. Randall, and E. M. Donaldson, editors. Fish physiology, volume 9, part B. Academic Press, Orlando, Florida.

Tiersch, T. R., C. A. Goudie, and G. J. Carmichael. 1994. Cryopreservation of channel catfish sperm: storage in cryoprotectants, fertilization trials, and growth of channel catfish produced with cryopreserved sperm. Transactions of the American Fisheries Society 123: 580–586.

Tiersch, T. R., W. R. Wayman, C. R. Figiel, O. T. Gorman, J. H. Williamson, and G. J. Carmichael. In press. Field collection, handling, and storage of sperm of the endangered razorback sucker. North American Journal of Fisheries Management 17.