MAY 2005

EFFECTS OF SALINITY AND SUSPENDED SEDIMENT ON PHYSICAL PROPERTIES OF THE EGG OF THE RIO GRANDE SILVERY MINNOW (Hybognathus amarus)

WRRI Technical Completion Report No. 334

David E. Cowley
Janelle Alleman
Ryan R. McShane
Patrick D. Shirey
Rossana Sallenave

NEW MEXICO WATER RESOURCES RESEARCH INSTITUTE
New Mexico State University
MSC 3167
Box 30001
Las Cruces, New Mexico 88003-8001
Telephone (505) 646-4337 FAX (505) 646-6418
email: nmwrri@wrri.nmsu.edu

EFFECTS OF SALINITY AND SUSPENDED SEDIMENT ON PHYSICAL PROPERTIES OF THE EGG OF THE RIO GRANDE SILVERY MINNOW (*Hybognathus amarus*)

By

David E. Cowley, Assistant Professor
Janelle Alleman, Technician
Ryan R. McShane, Graduate Assistant
Patrick D. Shirey, Graduate Assistant
and
Rossana Sallenave, Research Specialist
South**W**est **I**chthyological **R**esearch **L**aboratory

TECHNICAL COMPLETION REPORT
Account Number 01423990
May 2005

New Mexico Water Resources Research Institute
in cooperation with the
Department of Fishery & Wildlife Sciences
New Mexico State University

The research on which this report is based was financed in part by the U. S. Department of the Interior, Geological Survey, through the New Mexico Water Resources Research Institute.

DISCLAIMER

ABSTRACT

Specific gravity and diameter affect the potential and rate of downstream transport of semi-buoyant fish eggs. Salinity and suspended sediment were found to significantly alter both of these physical properties of the egg of the endangered Rio Grande silvery minnow (*Hybognathus amarus*). Under control conditions (no salts added to elevate salinity) egg specific gravity for *H. amarus* declined significantly in the first hour after fertilization as the perivitelline space of the egg filled with water. Egg specific gravity achieved a minimum value by 12 hours post-fertilization, which was approximately constant until hatching approximately 48 hours post-fertilization. Egg specific gravity was highly dynamic and it depended on the salinity of the water surrounding the egg. An egg transferred to water with a different salinity initiated immediate change in its specific gravity. Egg diameter and specific gravity of *H. amarus* eggs were reduced strongly by salinity but only modestly so by suspended sediment. In contrast, the length of newly hatched larvae was not affected by either salinity or suspended sediment. Assuming eggs were spherical, the reduction in egg diameter caused by increased salinity decreased egg volume by more than 20% and egg surface area by more than 15%. Our experiments showed that artificial eggs (Gellan beads) were a close approximation to the specific gravity of *H. amarus* eggs at salinities between 0.5 and 4 ppt. However, the specific gravity of artificial eggs was greater than that for *H. amarus* eggs at salinities near zero and was substantially less than the specific gravity of *H. amarus* eggs at 8 ppt salinity.

Keywords: Cyprinidae, development, egg drift, egg buoyancy, endangered species, fish eggs, fluvial transport, ontogeny, minnow, osmotic flux, pelagic, pelagophil

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

The Rio Grande silvery minnow (*Hybognathus amarus*), once found in its namesake river from the Gulf of Mexico (Girard 1856) upstream to northern New Mexico (Cope and Yarrow 1875), has persisted only in the Middle Rio Grande of New Mexico between Cochiti and Elephant Butte reservoirs (Sublette et al. 1990; Propst 1999). As a result of its extreme reduction in geographic range, the species was listed as endangered (U. S. Fish and Wildlife Service 1994).

Hybognathus amarus broadcast spawns nonadhesive, semi-buoyant eggs that can drift downstream as they develop (Platania and Altenbach 1998). This form of reproduction, pelagic spawning, is considered to be an ancestral, primitive mode of reproduction among teleost fishes (Balon 1984). It is often the mode of reproduction of species that undergo spawning migrations (Winemiller 1989). Downstream transport of eggs of the pelagic spawning fishes including *H. amarus* is facilitated by the perivitelline space of the egg, which swells with water shortly after fertilization and decreases the specific gravity of the egg while increasing its diameter. These features of the egg of *H. amarus* are likely an important factor in the species' once widespread distribution because they facilitate dispersal.

The passive movement of fish embryos and larvae is a common occurrence in many rivers (e.g., Gale and Mohr, Jr. 1978; Gallagher and Conner 1983; Schlosser 1985; Copp 1992; Scheidegger and Bain 1995). Long-term persistence of a fish species whose life history includes downstream transport requires at least one of two conditions to be true. Either a core population of spawners is resident in an upstream reach of the habitat or fish that drift downstream can pass back upstream to a spawning area. Under the "drift paradox" (Hershey et al. 1993), extinction is inevitable when downstream drift is the only transport process (Speirs and Gurney 2001). Thus, downstream drift of *H. amarus* eggs must be offset by upstream passage of adults to prevent eventual extinction of the species. The importance of this concern is highlighted by a call for upstream fish passage at irrigation diversion structures in the Middle Rio Grande (U. S. Fish & Wildlife Service 2003).

Because *H. amarus* eggs can drift downstream, it is important to understand how environmental conditions might affect the potential for egg transport to occur. Notable in our knowledge deficit are the effects that salinity and suspended sediment have on the rate and extent of drift of *H. amarus* eggs. Precise knowledge of egg size and specific gravity is necessary to better predict fluvial transport of *H. amarus* eggs. For example, the current velocity necessary to transport fish eggs would be expected to increase as specific gravity of the egg increases or as diameter of the egg decreases. Understanding the potential for eggs to drift provides insight for habitat restoration aimed at reducing egg drift and retaining them in shorter habitat reaches.

The objectives of this paper are two-fold. First, we evaluate the effects of salinity and suspended sediment on egg specific gravity, egg diameter, and the length of newly hatched larvae of *H. amarus*. Second, we evaluate how well Gellan beads mimic the buoyancy of *H. amarus* eggs. Our experiments were preceded by several predictions that led to testable hypotheses. First, we predicted that eggs increase in specific gravity as the *H. amarus* embryo develops and this would tend to reduce drift potential as embryonic development proceeds. Second, we predicted that salts and suspended sediment would increase specific gravity of *H. amarus* eggs and reduce their potential to be passively transported downstream.

METHODS

Egg Collection and Experimental Setup

Gravid adult *H. amarus* used in these experiments were obtained from the captive stock held at the A-Mountain facility on the New Mexico State University (NMSU) campus. Spawning aquaria were prepared with treated well water in Knox Hall on the NMSU campus (0.4 ppt salinity) or with well water at A-Mountain on the NMSU campus (~0 ppt salinity). A single gravid female *H. amarus* was introduced into a spawning aquarium along with one or two conspecific males. Early morning spawning in aquaria was initiated by intra-peritoneal injection of carp pituitary extract (CPE, 300 $\mu g \cdot \mu l^{-1}$, 0.1 cc per fish) approximately 12 hours earlier. Eggs were collected as closely as possible to fertilization. For spawning prior to sunrise, a small headlamp fitted with a red light was used to illuminate the aquarium and a small dip net was used to collect eggs that had not begun to swell. Unswollen eggs were transferred into a treatment aquarium and allowed to develop.

Specific gravity, egg diameter, and total length of newly hatched larvae were measured on random samples of eggs from the treatment aquaria. Larval *H. amarus* not used in the experimental measurements were collected from the bottom of the treatment aquarium with a flexible siphon tube and returned to the captive rearing facility at A-Mountain. At the conclusion of spawning, the adult fish were removed from the aquarium and returned to the captive stock.

Specific Gravity Measurements

Specific gravity of *H. amarus* eggs was measured using a density gradient column (Coombs 1981). The density gradient column used in our experiments (Techne Incorporated) included a filling device so that two solutions of different density could be introduced, filling the column from the bottom with progressively denser solution. Calibrated glass density beads [Techne (Cambridge) Ltd] were used as reference points of known specific gravity. Specific gravities of density beads were 1.0009, 1.0020, 1.0031, 1.0042, 1.0053, 1.0064, 1.0076, 1.0086, and 1.0097. Two salt solutions (Instant Ocean®, approximately 0-1 ppt and 30 ppt, respectively) were used to establish a linear density gradient in the column. The linear density gradient containing reference beads at known density was used for measurement of specific gravity of the eggs. The position of the calibrated density beads was recorded immediately before introducing each batch of eggs into the column.

Ten to twenty eggs were expelled into the bottom of the density column using a glass tube that contained eggs and water and that was attached to a thumb-wheeled pipetter.

Integrity of the density gradient was maintained by carefully inserting the glass tube along the inner surface of the density gradient column taking care not to disturb the calibrated glass floats. Using this technique, the eggs rose rapidly in the density column to their point of neutral density where they remained for several minutes, after which the eggs began to fall in the density gradient column. The position of neutral buoyancy of each egg in the column was recorded from reference marks on the glass column. These measurements were converted to specific gravity using the regression of specific gravity of the reference beads on their vertical position within the density gradient column. This procedure was repeated for every batch of eggs subjected to specific gravity measurement.

Up to 50 eggs could be introduced before the linearity of the density gradient was destroyed. After inserting each batch of eggs, the position of the calibrated density beads was recorded and evaluated visually for linearity. A new density gradient was established when departures from linearity in the density gradient were apparent, most obviously in the positions of the outer density beads (1.0009 and 1.0097) relative to those of intermediate specific gravity.

Experiment I – Does specific gravity increase with developmental age?

The effect of developmental age on the specific gravity of *H. amarus* eggs was studied using a fixed effects one-way analysis of variance design. The linear model for this experiment was

$$Y_{ij} = \mu + A_i + r_{ij} \, , \tag{1}$$

where μ is the overall mean, A_i is the *i*th developmental age, and r_{ij} is the residual for the *j*th individual in the sample. This experiment enabled tests of two null hypotheses: 1) H_0: egg specific gravity does not change between fertilization and hatching and 2) H_0: once the egg has swollen with water, its specific gravity remains unchanged during embryonic development.

Experiment I was conducted in a laboratory in Knox Hall and used treated well water available in the building. Salinity of the well water was approximately 0.4 ppt. Analysis of variance was conducted using the GLM procedure of SAS (SAS Institute, Inc. 1989b). Least squares means (Goodnight and Harvey 1978) were used for pair wise comparisons between treatments. Maximum experiment-wise error rate was controlled in the multiple comparisons of treatment means by adjusting the nominal significance level ($\alpha = 0.05$) by $\alpha' = 1 - (1-\alpha)^{1/c}$ (Sidak 1967). When data were unbalanced across treatments, Type III sums of squares (SAS Institute, Inc. 1989a) were used to assess significance of model terms.

Experiment II – Do salinity and suspended sediment affect egg specific gravity?

A factorial experimental design was used to learn if *H. amarus* eggs are affected by salinity and suspended sediment. We evaluated egg specific gravity and egg diameter at 24 hours post-fertilization and larval length at 48 hours. The linear model for this experiment was

$$Y_{ijk} = \mu + S_i + T_j + ST_{ij} + r_{ijk} \,, \tag{2}$$

where the symbols were defined: egg specific gravity, egg diameter, or larval length (Y_{ijk}), the overall mean (μ), an effect due to salinity (S_i), an effect due to suspended sediment (T_j), an interaction term to account for nonadditive effects from *S* and *T* (statistical interaction, ST_{ij}), and the deviation of the *k*th individual in the sample after accounting for the other model terms (r_{ijk}). Three hypotheses were tested for each of the three variables measured: 1) H_0: salinity does not affect egg specific gravity (or egg diameter or larval length), 2) H_0: suspended sediment does not affect egg specific gravity (or egg diameter or larval length), and 3) H_0: salinity and sediment do not interact to affect egg specific gravity (or egg diameter or larval length).

Treatment aquaria were of four types that form a 2 x 2 factorial analysis of variance design: (1) well water, (2) well water with Rio Grande sediment added, (3) well water with salt added, and (4) well water with salt and sediment added. Rio Grande sediment was collected at U. S. Highway 380 crossing, air dried, and pulverized to break up small clods of fine sediment. For aquaria with sediment treatments, 500 g of dried and clod-free river sediment was added to 15 l of well water. Sediment was retained in suspension with compressed air introduced through an air stone. Aquaria with salinity treatments were prepared by adding an amount of Instant Ocean (Aquarium Systems) to well water to yield a desired level of salinity. All aquaria were aerated and maintained at approximately 20º C.

Experiment II data were collected at the captive rearing facility at A-Mountain and used well water available at that facility. Developing eggs were transferred from the A-Mountain facility to the rearing laboratory approximately 12 hours post-fertilization. Analysis of variance was carried out using the GLM procedure of SAS (SAS Institute, Inc. 1989b). Additional details of the analysis were consistent with the description for Experiment I.

Egg diameter was measured on random samples of eggs taken approximately 24 hours post-fertilization and larval length was measured on random samples taken at approximately 48 hours after fertilization. These measurements were made at 20x magnification using a dissecting microscope fitted with an ocular micrometer. Measurements were recorded in ocular micrometer units and subsequently converted to millimeters.

Experiment III – How well do Gellan beads mimic fish eggs?

The specific gravity of Gellan beads (Technology, Flavors, and Fragrances, Inc.), a product used to mimic fish eggs (Davin et al. 1999; Will et al. 2001; Porter and Massong 2003; Kehmeier et al. 2004), was estimated with a one-way analysis of variance with salinity treatments of 0.4, 2, 4, 6, and 8 ppt. Gellan beads were prepared for analysis by soaking them in treatment water for 48 hours. Specific gravity of the beads was evaluated in the same manner as for *H. amarus* eggs. The relationship between salinity and specific gravity was estimated by linear regression. The specific gravity of artificial eggs was compared to that for *H. amarus* eggs at comparable salinities and a t-test (McPherson 1990) was used to compare regression slopes for artificial eggs versus *H. amarus* eggs across a range of salinities. Analysis of variance was conducted using the GLM procedure of SAS (SAS Institute, Inc. 1989b). Additional details of the analysis were consistent with the description for Experiment I.

RESULTS

Experiment I – Egg Specific Gravity by Developmental Age

The first experiment (Figure 1) provided estimates of egg specific gravity at a series of developmental ages including 10, 45, and 60 minutes post-fertilization and at intervals of approximately 12 hours until just prior to hatching, which occurred approximately 48 hours post-fertilization (Figure 1). The analysis of variance indicated significant variation between developmental ages (F = 84.92, P < 0.0001) and between replicate samples within a developmental age (F = 23.96, P < 0.0001).

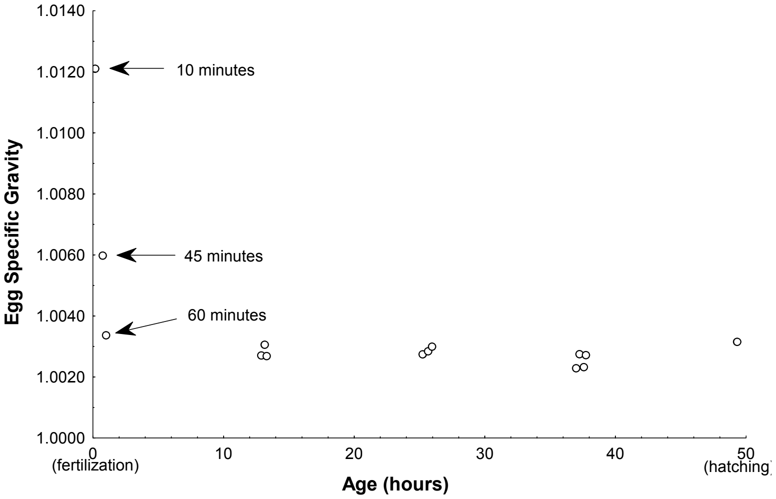

Figure 1. Specific gravity (relative density) of *H. amarus* eggs was estimated for well water having a salinity of 0.4 ppt. Circles denote least squares means from ANOVA for random samples of eggs. Standard errors of all means were less than 0.0002. All times are post-fertilization.

The specific gravity of newly fertilized eggs prior to swelling was estimated (Figure 1) because the unswollen eggs were well below the densest calibrated bead (1.0097). From 12 hours onward, specific gravity of *H. amarus* eggs was not significantly different except at 37 hours post-spawning when the eggs were significantly less dense (P <

0.005). Egg specific gravity declined significantly (P < 0.0001) between 45 minutes (N = 8, mean = 1.00598 ± 0.00018) and one hour (N = 37, mean = 1.00336 ± 0.00008) post-fertilization and between one and 12 hours post-fertilization (N = 53, mean = 1.00281 ± 0.00007).

Experiment II – Effects of Salinity and Sediment on H. amarus Eggs

Given the relatively constant specific gravity observed from approximately 12 hours post-fertilization, we chose to conduct measurements of specific gravity and egg diameter at 24 hours post-spawning. This enabled us to achieve better statistical power within the constraints of our endangered species permit. All replicates for Experiment II were conducted in 2004 at the NMSU A-Mountain facility.

Egg specific gravity was significantly altered by suspended sediment and salinity (Figure 2). The main effects of salinity and suspended sediment were highly significant in the analysis of variance (P < 0.0001, respectively). There was a significant interaction between salinity and suspended sediment, which indicated that the individual effects of sediment and salinity were not additive. This result occurred because the least squares mean for the combined salinity-sediment treatment was not significantly different from the least squares mean for the salinity treatment (P = 0.3420).

In general, salinity significantly increased egg specific gravity (Figure 2). Whereas the magnitude of change in specific gravity was large with changes in salinity, the increase in specific gravity with the addition of suspended sediment was significant (P < 0.0001) but numerically small (Figure 2).

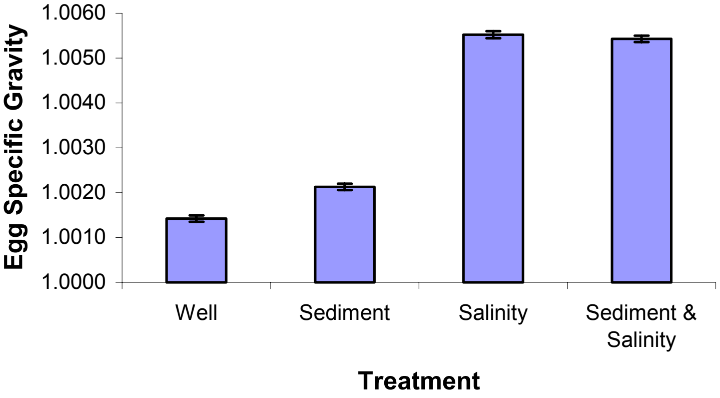

Figure 2. The effects of salinity and sediment on egg specific gravity were estimated from a 2 x 2 factorial experiment with two levels of sediment (0 g and 500 g per 15 l of well water) and two levels of salinity (0 or 4 ppt).

In the analysis of variance, egg diameter (Figure 3) was affected significantly by the main effect salinity (F = 332.32, P < 0.0001) but not by main effect sediment (F = 0.48, P = 0.4882). However, a significant interaction (F = 45.13, P < 0.0001) was observed between salinity and sediment. Eggs incubated in salinity plus suspended sediment had greater diameter than eggs incubated in salinity but smaller diameter than eggs incubated in suspended sediment (Figure 3). All of the pair wise comparisons of least squares mean egg diameter were significant (P < 0.0001). Salinity reduced egg diameter by approximately 22% relative to the well water control.

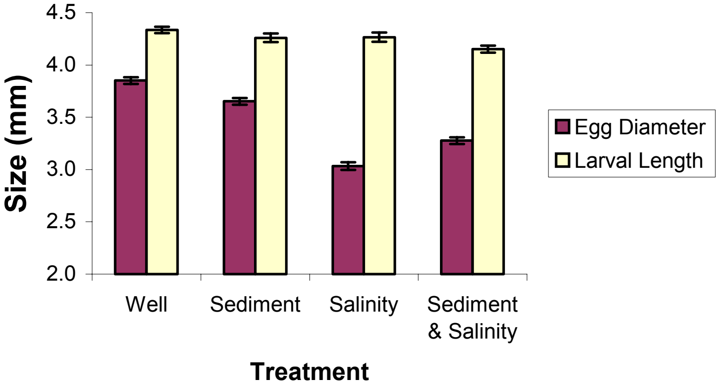

Figure 3. The effects of salinity and sediment on egg diameter and larval length for *H. amarus* were estimated from the 2 x 2 factorial experiment.

For larval length at 48 hours post-fertilization (Figure 3), analysis of variance showed significant (P < 0.05) effects due to the main effects salinity (F = 6.26) and suspended sediment (F = 5.58), but the interaction between them was nonsignificant (F = 0.26, P = 0.6076). The only significant pair wise comparison of least squares means (Figure 3) was between the well water control and the salinity plus suspended sediment treatment (P < 0.0001). Hatching appeared to occur a few hours earlier in the salinity treatments because more eggs had hatched by approximately 48 hours post-fertilization than in the other two treatments. However, we did not measure developmental time and water temperatures closely enough to assess statistical significance.

Experiment III – Specific Gravity of Artificial Eggs

The specific gravity of Gellan beads was estimated for NMSU well water with salinity of 0.4 ppt and at salinities of 2, 4, 6, and 8 ppt. Analysis of variance showed significant differences between treatments in specific gravity (F = 1323.53, P < 0.0001). Specific gravity of Gellan beads increased linearly with salinity. The regression of mean specific gravity on salinity was significantly different from zero (R^2 = 98.6%, P = 0.0007, see Figure 4. The regression intercept was significantly larger and the regression slope was significantly smaller than that for *H. amarus* eggs.

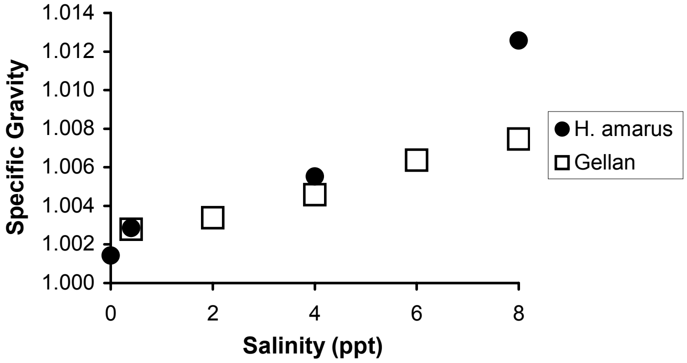

Figure 4. The specific gravity of Gellan beads is shown relative to the specific gravity of *H. amarus* eggs.

DISCUSSION

Physical Properties of H. amarus Eggs are Variable

Based on earlier literature, which described egg specific gravity as a static property (Hardy 1978; Hurley 1991; Davin et al. 1999; Platania and Altenbach 1998; Dudley and Platania 1999), we initially thought that the fish eggs could be placed on the top of the linear density gradient and that they would sink to a position of neutral buoyancy. However, eggs introduced in this manner fell continuously in the linear density gradient, reaching the bottom of the cylinder after approximately one hour, and there was no clear indication of the specific gravity of the egg. It was apparent visually that the eggs were much smaller in diameter by the time they reached the bottom of the density gradient and some eggs ruptured before reaching the bottom of the density gradient (> 25 ppt salinity). Thus, the buoyancy of the egg appeared to be highly dynamic and changed continually as the egg was exposed to increasingly higher salinity in the density gradient column.

Earlier authors did not note the highly dynamic nature of the egg that we observed. Whereas Dudley and Platania (1999) gave a single value of egg specific gravity that was intended to represent the entire guild of pelagic-spawning minnow species that includes *H. amarus*, our results clearly indicate that egg specific gravity is highly dynamic in response to the environmental conditions of incubation. Specific gravity and diameter of *H. amarus* eggs depend on the salinity of the incubation water. An increase in egg specific gravity is accompanied by a decrease in egg diameter, suggesting that the change in specific gravity of the egg is mediated through osmotic flux of water out of the perivitelline space into the incubation medium.

Platania and Altenbach (1998) underestimated the temporal duration of swelling of the perivitelline space of the egg. Whereas they reported that eggs were fully swollen by about 30-45 minutes post-fertilization, we found a highly significant decrease in specific gravity between one and 13 hours post-fertilization. As a result, their value of specific gravity was higher by an exponent of about 4 than what we observed as the minimal value attained after 12 hours post-spawning. Alderdice (1988) noted that permeability of the zona radiata reduced to and remained near a minimum during incubation, which occurs at different times in different species but is commonly 12 to 24 hours post-fertilization. Our results indicate that the *H. amarus* egg retains permeability of the zona radiata at approximately 24 hours post-fertilization, which allows osmotic flux to adjust its specific gravity.

Recovery and Habitat Management

Physical properties of fish eggs have been used in model simulations of pelagic egg drift in a river (Yeh and Yeh 1980). Our results show than egg specific gravity and egg diameter are significantly affected by salinity and suspended sediment. Because recovery of *H. amarus* provides a nexus for restoration of the Middle Rio Grande, it seems important to carefully study the biology of the species so that its ecological requirements can be better understood. Restoration plans will be improved by learning more about *H. amarus* biology, including factors affecting early life stages, food habits (Shirey 2004), life span (Cowley et al. *in review*), fecundity, sex determination, and spawning cues.

Laboratory studies have been used to speculate that *H. amarus* eggs and larvae could drift hundreds of kilometers downstream (Dudley and Platania, 1999). However, recent experimental data using Gellan beads to mimic drift of fish eggs suggest that passive drift of minnow eggs may be much shorter (Kehmeier et al., 2004). Our results will help comprehend the disparity between these two experimental outcomes by refining mathematical models of egg transport to accommodate variation induced in egg specific gravity and egg diameter by salinity or suspended sediment. With a good mathematical representation of egg drift it might be possible to compare the drift potential in river reaches that differ significantly in their salinity or suspended sediment load. Another use would be to compare alternatives for mitigation of elevated salinity or sediment with respect to their expected effects on egg drift.

The results here suggest several refinements to habitat restoration. First, while the specific gravity of *H. amarus* eggs increases with suspended sediment or salinity, the relative change in egg specific gravity is less than the change in the specific gravity of the river water transporting the eggs. Given the relative difference between densities of eggs and sediment-loaded water, we predict that most *H. amarus* eggs will drift nearer to the surface of the river when suspended sediment loads are high. As a consequence, suspended sediment should enhance egg transport. If suspended sediment indeed causes the eggs to drift high in the water column, extrapolation of number of eggs per unit volume of river discharge is probably less precise than extrapolating to river discharge in the surface layer.

Habitat conditions that provide "nursery" areas have been described for some species (e.g., Scott and Nielsen, 1989; Scheidegger and Bain, 1995) but for many others including *H. amarus*, it is unknown where juvenile fish successfully complete their larval development. Presently we lack an understanding of the full range of conditions that entrain drifting fish eggs, although for a given egg size and density one could calculate current velocities at which transport would be unlikely. It is also unknown which of the conditions that entrain drifting eggs also provide good nursery conditions for the larval stage. Field investigations will enable a fuller appreciation of channel features that limit egg drift.

Future studies should determine if larval *H. amarus* are pelagic and drifting freely in the water column, if they are benthic and associated with the river substrate, or if they exhibit diel changes between pelagic and benthic behaviors such as described (Gallagher and Conner, 1983) for freshwater drum (*Aplodinotus grunniens*) and shad (*Dorosoma* spp.). For each of these alternatives, it is important to understand how small differences in timing of reproduction can interact with flood-stage events to displace young-of-year fish downstream (Harvey, 1987). This is especially important when true flood stage events are rare and elevated river discharge is associated directly with flow regulation to deliver water to users. Thus, broader knowledge of the reproductive ecology of *H. amarus* is important for optimizing flow regulation to benefit recovery of the species.

ACKNOWLEDGMENTS

We are grateful to Jerry Landye for his knowledge, assistance, and encouragement throughout the project. Thanks also to William Knight and Cody Robertson for assistance with delivery and pick up of adult spawners. Colleen Caldwell kindly provided advice on rearing and animal husbandry techniques. We thank Victoria Pritchard for laboratory assistance with the ontogenetic study. We are grateful for three thoughtful critiques by Wiebke Boeing, Colleen Caldwell, and Michael Porter that significantly improved the presentation of results. The Middle Rio Grande Endangered Species Act Collaborative Program provided funding with a grant from the U. S. Bureau of Reclamation to New Mexico State University that was administered through the New Mexico Water Resources Research Institute. We thank Karl Wood, Director, and Cathy Ortega-Klett for their assistance in grant administration and report publication.

LITERATURE CITED

Alderdice, D. F. 1988. Osmotic and ionic regulation in teleost eggs and larvae. In: W. S. Hoar and D. J. Randall (eds.), Fish Physiology, Vol. XI, The Physiology of Developing Fish, Part A, Eggs and Larvae. Academic Press, Inc., London, U. K., p. 163-251.

Balon, E. K. 1984. Patterns in the evolution of reproductive styles in fishes. In: G. W. Potts and R. W. Wooten (eds.), Fish Reproduction: Strategies and Tactics. Academic Press, London, U. K., p. 35-53.

Coombs, S. H. 1981. A density-gradient column for determining the specific gravity of fish eggs, with particular reference to eggs of the mackerel *Scomber scombrus*. Marine Biology 63:101-106.

Cope, E. D. and H. C. Yarrow, 1875. Report upon the collections of fishes made in portions of Nevada, Utah, California, Colorado, New Mexico, and Arizona during the years 1871-1874. In: United States Army Engineers Department Report, in charge of George M. Wheeler, Geographer and Geologist of the Exploration and Surveys west of the 100th meridian, 5, Chapter 6, pp. 635-703.

Copp, G. H. 1992. Comparative microhabitat use of cyprinid larvae and juveniles in a lotic floodplain channel. Environmental Biology of Fishes 33:181-193.

Cowley, D. E., P. D. Shirey, and M. D. Hatch. *In Review*. Ecology of the Rio Grande silvery minnow (*Hybognathus amarus*) inferred from specimens collected in 1874. In: Cowley, D. E. and R. Sallenave (editors). Conservation and Management of Aquatic Resources in Arid Lands. Submitted to UNM Press, September, 2004.

Davin, W. T., C. Ethridge, C. Babb, and S. Hileman. 1999. Estimation of striped bass (Morone saxatilis) egg drift rate in the lower Savannah River. Final Report to the U. S. Army Corps of Engineers, Savannah District. December 1999. 68 p.

Dudley, R. K. and S. P. Platania. 1999. Imitating the physical properties of drifting semibuoyant fish (Cyprinidae) eggs with artificial eggs. Journal of Freshwater Ecology 14:423-430.

Gale, W. F. and H. W. Mohr, Jr. 1978. Larval fish drift in a large river with a comparison of sampling methods. Transactions of the American Fisheries Society 107:46-55.

Gallagher, R. P. and J. V. Conner. 1983. Comparison of two ichthyoplankton sampling gears with notes on microdistribution of fish larvae in a large river. Transactions of the American Fisheries Society 112:280-285.

Girard, C., 1856. Researches upon the cyprinoid fishes inhabiting the freshwaters of the United States of America, west of the Mississippi Valley, from specimens in the museum of the Smithsonian Institution. Proceedings of the National Academy of Natural Sciences, Philadelphia 8: 165-213.

Goodnight, J. H. and W. R. Harvey. 1978. Least squares means in the fixed effects general linear model. SAS Technical Report R-103, SAS Institute Inc., Cary, NC.

Hardy, J. D., Jr. 1978. Development of fishes of the mid-Atlantic Bight: An atlas of the egg, larval, and juvenile stages. Vol. 3, Aphredoderidae through Rachycentridae. U. S. Fish and Wildlife Service Program FWS/OBS-78/12, 394 pp.

Harvey, B. C. 1987. Susceptibility of young-of-the-year fishes to downstream displacement by flooding. Transactions of the American Fisheries Society 116:851-855.

Hershey, A. E., J. Pastor, B. J. Peterson, and G. W. Kling. 1993. Stable isotopes resolve the drift paradox for *Baetis* mayflies in an arctic river. Ecology 74:2315-2325.

Hurley, N. M. 1991. Transport simulation of striped bass eggs in the Congaree, Wateree, and Santee River, South Carolina. U. S. Geological Survey Water-Resources Investigations Report 91-4088.

Kehmeier, J., C. N. Medley, and O. B. Myers. 2004. Assessment of Pecos bluntnose shiner egg and larval drift potential in the Pecos River, New Mexico. New Mexico Interstate Stream Commission, Santa Fe, New Mexico, 43 pp.

McPherson, G. 1990. Statistics in Scientific Investigation: Its Basis, Application, and Interpretation. Springer-Verlag, New York, Inc.

Platania, S. P. and C. S. Altenbach. 1998. Reproductive strategies and egg types of seven Rio Grande Basin cyprinids. Copeia 1998(3):559-569.

Porter, M. D. and T. Massong. 2003. Progress report on Rio Grande silvery minnow egg habitat study – FY 2003. U. S. Bureau of Reclamation, Science and Technology Program, September 30, 2003.

Propst, D. L. 1999. Threatened and endangered fishes of New Mexico. New Mexico Department of Game and Fish, Technical Report 1, Santa Fe, 84 pp.

SAS Institute, Inc. 1989a. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 1. Cary, NC.

SAS Institute, Inc. 1989b. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 2. Cary, NC.

Scheidegger, K. J. and M. B. Bain. 1995. Larval fish distribution and microhabitat use in free-flowing and regulated rivers. Copeia 1995(1):125-135.

Schlosser, I. J. 1985. Flow regime, juvenile abundance, and the assemblage structure of stream fishes. Ecology 66(5):1484-1490.

Scott, M. T. and L. A. Nielsen. 1989. Young fish distribution in backwaters and main-channel borders of the Kanawha River, West Virginia. Journal of Fish Biology 35(Supplement A):21-27.

Shirey, P. D. 2004. Foraging habits and habitat utilization of Rio Grande silvery minnow (*Hybognathus amarus*) as inferred by diatom frustules. Master's Thesis, New Mexico State University, Las Cruces.

Sidak, Z. 1967. Rectangular confidence regions for the means of multivariate normal distributions. Journal of the American Statistical Association 62:626-633.

Speirs, D. C. and W. C. Gurney. 2001. Population persistence in rivers and estuaries. Ecology 82(5):1219-1237.

Sublette, J. E., M. D. Hatch, and M. Sublette. 1990. The Fishes of New Mexico. University of New Mexico Press, Albuquerque, NM.

U. S. Fish and Wildlife Service. 1994. Endangered and threatened wildlife and plants: final rule to list the Rio Grande silvery minnow as an endangered species. Federal Register 59: 36988-36995.

U. S. Fish and Wildlife Service. 2003. Biological and conference opinions on the effects of actions associated with the programmatic biological assessment of Bureau of Reclamation's water and river maintenance operations, Army Corps of Engineers' flood control operation, and related non-federal actions on the Middle Rio Grande, New Mexico. Cons. # 2-22-03-F-0129, March 17, 2003, Region 2, Albuquerque, NM (http://ifw2es.fws.gov/Documents/R2ES/FINAL_Middle_Rio_Grande_Water_Ops_BO_2003-2013.pdf).

Will, T. A., T. R. Reinert, and C. A. Jennings. 2001. Assessment of spawning sites and reproductive status of striped bass, Morone saxatilis, in the Savannah River Estuary. Final Report for Project 10-21-RR251-144, 1 January 2000 – 31 January 2001. Prepared for The Georgia Ports Authority, Savannah, GA. 42 p.

Winemiller, K. O. 1989. Patterns of variation in life history among South American fishes in seasonal environments. Oecologia 81:225-241.

Yeh, G. T. and C. F. Yeh. 1980. Mathematical modeling of the distribution of fish eggs from spawning regions of a river. Ecological Modelling 8:97-107.