

Reproductive biology and early life-history of
Rio Grande silvery minnow, *Hybognathus amarus*

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prepared for

U.S. Army Corps of Engineers
Albuquerque District
P.O. Box 1580
Albuquerque, NM 87103

under Purchase Order:
DACW47-94-P-0462

15 February 1995

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Executive Summary

Reproductively active Rio Grande silvery minnow, *Hybognathus amarus*, was successfully spawned in aquaria and their larvae reared to juvenile developmental stage. This species is a pelagic spawner that produces over 3,000 semi-buoyant, nonadhesive eggs during a spawning event. The eggs were about 1.6 mm diameter upon fertilization but quickly swelled to 3.0 mm and remained suspended in the water column during development. Egg hatching time was temperature dependent and appeared to occur in 24-48 hours. Recently hatched larval fish attempted to remain a part of the drift by swimming vertically (swim-up stage) in the water column. About three days after hatching, the gas bladder was developed, yolk-sac was almost completely absorbed, and protolarvae began feeding. These physiological developments corresponded with a shift in swimming behavior as the protolarvae ended their swim up period, moved horizontally, and appeared to actively seek low-velocity habitats.

Larvae were about 3.7 mm SL (standard length) upon hatching and grew about 0.15 mm per day during proto- and mesolarval stages. The first mesolarval Rio Grande silvery minnow was observed seven days after hatching but protolarvae numerically dominated the samples until 16 days after hatching. The first metalarvae was collected on day 22 and first juvenile 48 days after hatching. Growth for the first 41 days appeared to be allometric and seemed to shift to isometric between days 41-48.

The reproductive behavior, egg physiology, and larval fish behavior suggest that high flow events are important factors in this species life history. Eggs and larvae are presumed to remain a part of the drift for 3-5 days. Flows during the putative spawning period of Rio Grande silvery minnow could transport eggs and protolarvae 216-360 km (134-223 mi) downstream. Three diversion dams (Angostura, Isleta, and San Acacia) that currently divide the remaining range of Rio Grande silvery minnow prevent adults and larvae from returning to selected upstream reaches.

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Introduction

Rio Grande silvery minnow, *Hybognathus amarus*, formerly was relatively abundant and widespread in the Rio Grande, occurring from near Española to the Gulf of Mexico (Bestgen and Platania 1991). Recent studies in the Rio Grande basin documented the loss of this species from Rio Grande upstream of Cochiti Dam and downstream of Elephant Butte Reservoir. Rio Grande silvery minnow has been extirpated from its former range in the Pecos River and replaced by a congener, plains minnow, *Hybognathus placitus*. This 90-95% reduction in range was due, in part, to water resource development and resulted in the listing of this endemic cyprinid as a federal endangered species (U.S. Department of the Interior 1994).

Ongoing research on Middle Rio Grande fishes was designed to study the distribution and status of the ichthyofauna, habitat associations of selected species, and life history of Rio Grande silvery minnow. Studies of aspects of Rio Grande silvery minnow life history were designed to answer questions regarding mean and maximum age, age and time of reproduction, food habits, and growth rate of post-larval individuals. Information on early life history and reproductive ecology of Rio Grande silvery minnow, which could only be obtained in the laboratory, were deemed especially important in efforts to recover this species.

Although the reproductive biology and larval ecology of Rio Grande silvery minnow is virtually unknown, numerous authors have reported on life history aspects of plains minnow (Cross 1967, Miller and Robison 1973, Lehtinen and Layzer 1988). Most recently, Taylor and Miller (1990) reported the results of their two-year study of plains minnow in the Cimarron River, Oklahoma; however, field conditions prevented collection of eggs and direct observation of spawning and behavior of larval fishes. Problems associated with field collection of this information resulted in limited attempts at such studies thereby leaving important details of the reproductive biology of most North American mainstream cyprinids undescribed.

The present study was designed to address questions related to reproductive ecology of Rio Grande silvery minnow and early life-history of larval stages. Specific objectives were to learn the mode of reproduction, type and number of eggs produced during spawning, and the time between fertilization and hatching. The rearing of developing larvae through the metalarval stage allowed us to determine behavior and growth rate and produce a voucher series to be used as an aid in the identification of field collected specimens. Information from this study provided valuable insight, unobtainable from field studies, on the reproductive ecology and early life history of Rio Grande silvery minnow. The integration of laboratory and field life history information will assist in a better understanding of the relationships of flow and water management practices on this species.

Methods

This study was divided into two discrete phases. The first stage was concerned with Rio Grande silvery minnow egg and larval fish developmental rates and behavior while the second was designed to address specific aspects of their reproductive ecology. On 11 May 1994, 43 gravid female and 24 ripe male Rio Grande silvery minnow were collected at the confluence of the North Socorro Diversion Channel and the Rio Grande (Socorro County, NM). Specimens were transferred to plastic bags that contained river water and oxygen. The bags were then transported to the laboratory in ice chests that helped protect the specimens and maintain a relatively constant water temperature. Once in the laboratory, individuals were examined to determine their relative health, sex, and age.

Fish selected for the study were anesthetized using MS-222 and given an abdominal injection of about 0.1 cc (0.11 mg) of acetone-dried carp-pituitary extract (CPE). The fish were then placed in a 378.5-liter (100-gallon) aquarium where they were watched until they recovered from the anesthesia. Injection of Rio Grande silvery minnow collected for this phase of the study began at 23:00 (11 May 1994) and concluded by 23:55. Specimens remained in the 378.5-liter aquarium for about 36 hours to allow for the maximum number of spawning events and yield the maximum number of eggs and larvae for developmental studies. The approximate time between injection, spawning, and hatching was recorded. Diseased fish and those that did not recover from the anesthesia were preserved in 10% formalin.

Aquaria were kept in a greenhouse that received natural sunlight and maintained an ambient temperature of 27°C. Minimum and maximum water temperatures were recorded daily on submerged Taylor® minimum-maximum thermometers. A 25-cm tubular air-stone was placed in the lower corner of the 378.5-liter spawning aquarium to maintain the water's dissolved oxygen level and provide a current. Two aquarium nets (25 x 18 cm, fine-mesh) were suspended just below the water surface to collect eggs produced during spawning. The nets were installed about eight hours after the fish were injected with CPE and were checked every two hours. Eggs collected in the nets were transferred from the 378.5-liter aquarium to a 189.3-liter (50-gallon) aquarium for rearing. Compressed air and a tubular air-stone provided air and current in the rearing aquarium. A clear acrylic tray (37 x 15 x 8 cm) suspended 5 cm below the water surface provided low-velocity habitat for larval fish (Figure 1).

Samples of developing larvae were collected twice daily for the first 12 days following hatching, daily for the next 10 days, and then weekly until Rio Grande silvery minnow achieved the juvenile stage. Day 0 was designated as the day that larvae hatched. Fish were fed finely ground commercial fish food twice per day starting on Day 6. Their diet was also supplemented by algae that grew on the inside walls of the aquarium.

Larvae were examined for characteristics indicative of selected developmental stages as defined by Snyder (1983; Appendix I). As there were no discernible developmental differences between the two daily samples taken during days 1-12, only one sample per day was selected for study (Appendix II). A stereomicroscope fitted with an ocular micrometer was used to study and measure (standard length; SL, to the nearest 0.1 mm) developing eggs and fish. Terminology for reproductive behavior and egg type follow definitions provided by Breder and Rosen (1966).

On 26 May 1994, we collected about 20 Age II Rio Grande silvery minnow and, using the procedure described above, returned them to the laboratory. These specimens were used to determine reproductive behavior and the number of eggs produced by a single female during spawning. Three 56.8 liter (15-gallon) aquaria were prepared as described for the 189.3-liter and 378.5-liter aquaria. Three gravid females were selected, assigned a unique number, anesthetized,

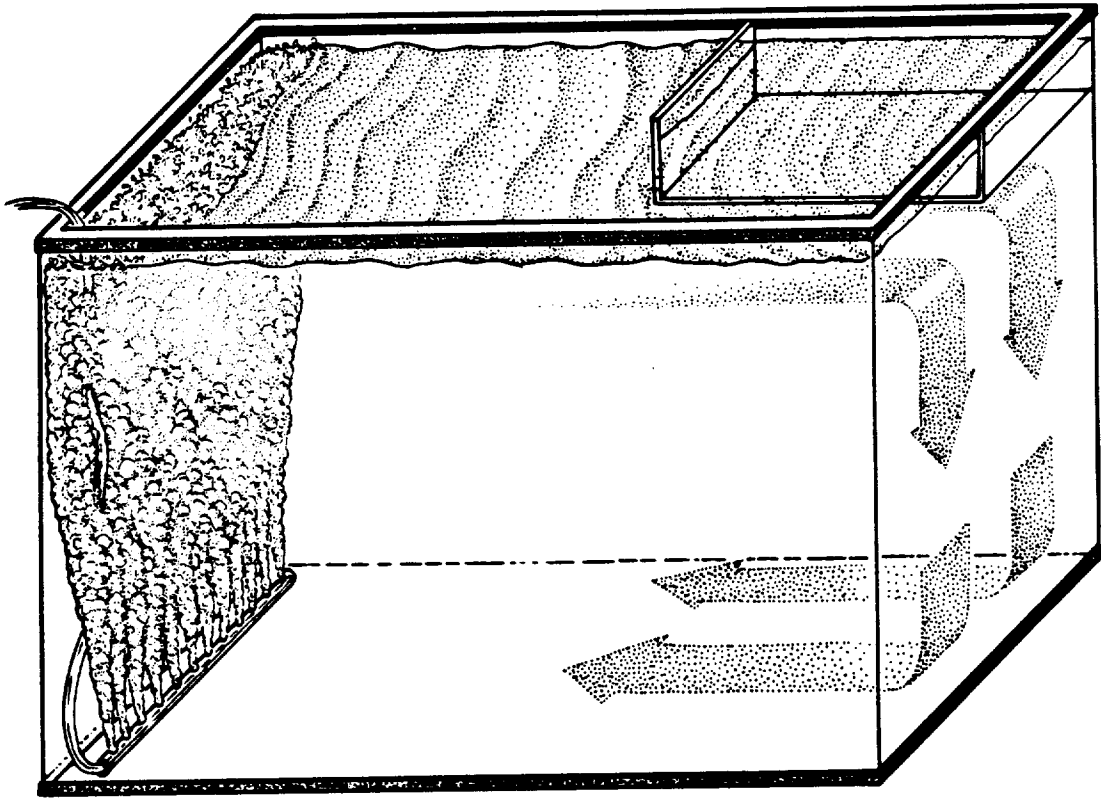


Figure 1. Spawning and rearing aquarium illustrating general water current (arrows) provided by air stone and artificial backwater (upper right corner).

weighed to the nearest 0.001 gm on a Sartorial scale, injected with CPE, and placed in one of the three aquaria. Injected males were subsequently introduced into each of the 56.8-liter aquaria.

Egg collecting nets were suspended in the aquaria and first checked about eight hours after fish were placed in aquaria. The nets were subsequently checked hourly. Fish were removed from the aquaria about 16 hours after injection and females were anesthetized, reweighed, and fixed in 10% formalin. All eggs were removed from the aquaria, fixed in 5% buffered formalin, counted, and assigned a museum number that corresponded with the female that produced the eggs. Determination of the level of spawning (high, medium, low) of females was based primarily on the decrease in her abdomen size. On 27 and 28 May 1994 we repeated the aforementioned sequence using six new individuals from the 26 May collection. Only one of the three fish injected on 28 May 1994 spawned. Specimens used for reproductive studies, and eggs, and larval fish produced as part of this work were deposited in the Fish Division of the Museum of Southwestern Biology and are available for future research.

Results

Spawning behavior and egg morphology

Rio Grande silvery minnow injected with CPE were moved throughout the aquarium but appeared to congregate in the middle of the water column. Pre-spawning events consisted of one to three males chasing a gravid female, nudging and nipping at her abdomen, and appearing to drive her toward the water surface. The chase usually lasted < 3 seconds and concluded when the male ended the chase. Although these chases began soon after the two sexes were placed in the aquaria, spawning did not occur until at least 11 hours after the fish were injected.

During the actual spawning event, the male appeared to align laterally on the side of the female in head-to-head orientation with his head and abdomen lower and tail slightly higher than the female (Figure 2). Both fish swam rapidly and in tandem. The male then wrapped his peduncle and caudal fin over the back of the female appearing to squeeze the female's midsection. Simultaneously, males and females released gametes. Both fish then reoriented head-to-head, facing downward, and drifted for several seconds. The duration of the actual spawning event (wrap-and-release) was less than one second. Spawning occurred at mid or upper water levels (=pelagic spawning) in water 20-24°C.

The semi-buoyant nonadhesive eggs dispersed throughout the aquarium and settled to the bottom where they quickly absorbed water and expanded (Table 1). Perivitelline space (distance between egg and embryo; Figure 3) was minimal upon fertilization but tripled within 10-30 minutes. As the chorion expanded and the perivitelline space filled with water, eggs became more neutrally buoyant, rose into the water current and remained in suspension. Eggs sank to the bottom if the water current was not maintained in the aquaria.

Upon fertilization, the embryo was not spherical but instead had sharp-angular edges. About 30 minutes later, the embryo was rounded and flattened (saucer-shaped) while the egg was spherical, the chorion smooth and ornamented with a single raised hexagon. Embryo development reached the morula stage at hour-15 and gastrula stage by hour-20. At 42 hours post-fertilization, some larvae had hatched but most were thrashing back and forth and attempting to tear the chorion to escape. All viable eggs had hatched within 50 hours of fertilization.

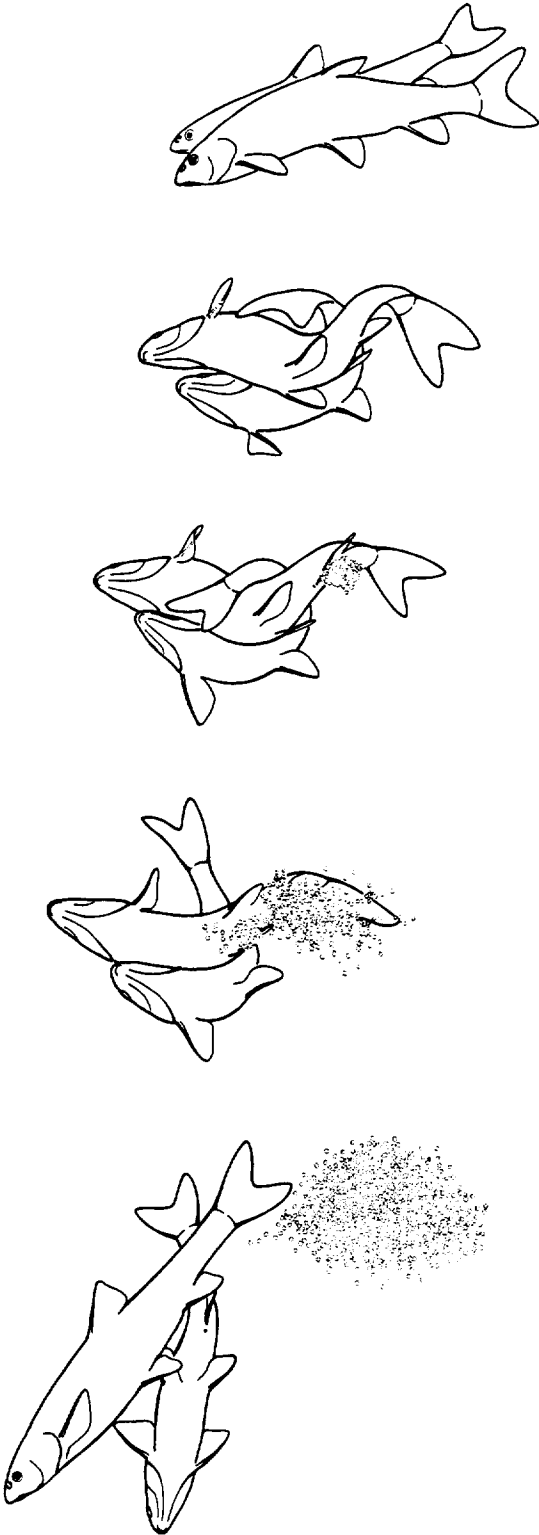


Figure 2. Spawning of Rio Grande silvery minnow.

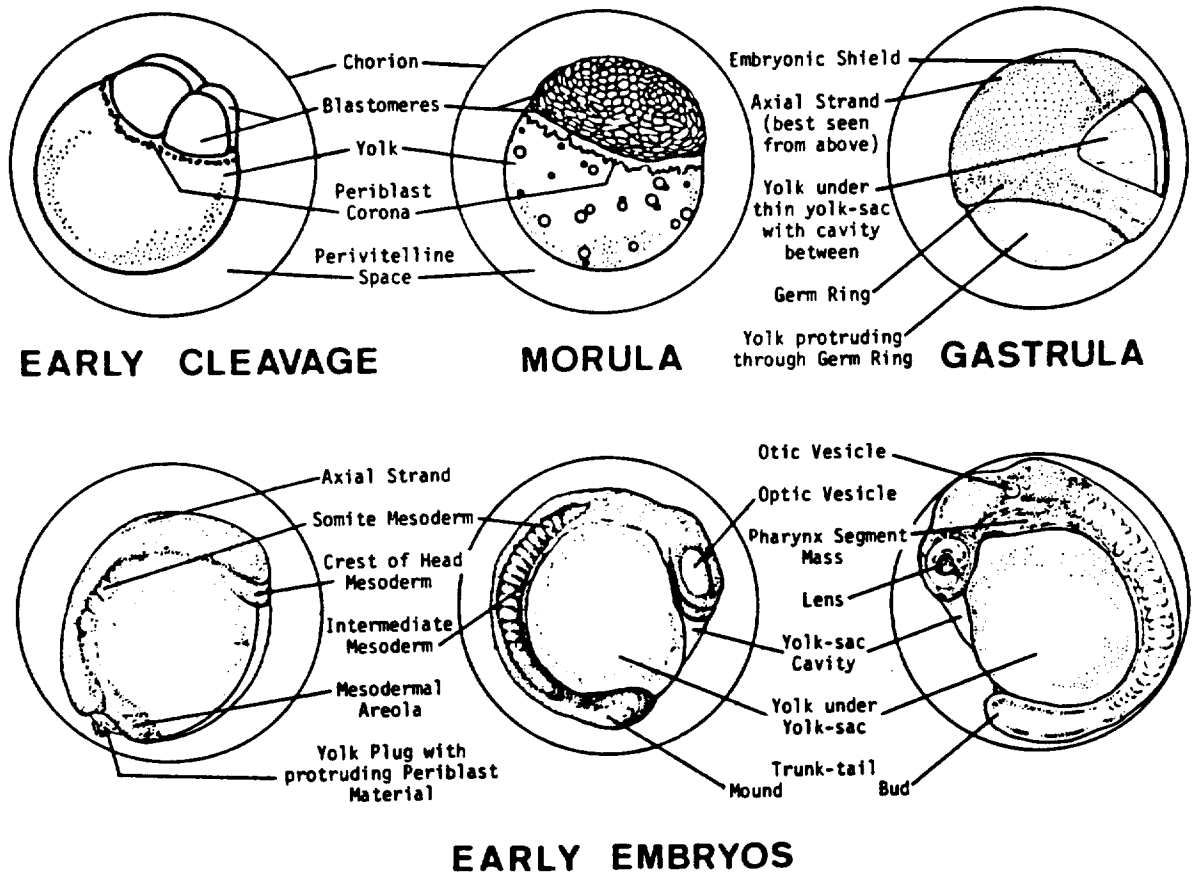


Figure 3. Selected anatomical features of cypriniform eggs and embryos (from Snyder 1981; based on drawings from Long and Ballard 1976).

Table 1. Rio Grande silvery minnow egg and embryo diameters.

TIME Post- spawning	Eggs Examined	EGG Mean Diameter (mm)	EGG Min-Max Diameter (mm)	EMBRYO Mean Diameter (mm)	EMBRYO Min-Max Diameter (mm)
0 minutes	50	1.6	1.4-1.9	1.0	0.8-1.1
10 minutes	50	2.9	1.5-3.6	1.1	0.9-1.4
30 minutes	50	2.9	2.0-3.6	1.1	0.9-1.4
15.5 hours	25	3.2	2.9-3.7	1.0	0.9-1.2

The numbers of eggs produced by spawning Rio Grande silvery minnow varied greatly (Table 2). The seven Age II individuals isolated and spawned in 56.8-liter aquaria collectively produced over 16,000 eggs. As these fish were not observed continuously, there was no means to determine if the number of eggs produced by a female represented single or multiple spawning events.

Table 2. Eggs produced during spawning by seven Age II female Rio Grande silvery minnow.

Specimen #	Length SL (mm)	Weight pre-spawn (gms)	Weight post-spawn (gms)	Egg weight (gms)	# of eggs	Spawning level
1	71.87	7.260	5.512	1.748	3,259	high
2	65.32	6.199	4.792	1.407	3,667	high
3	68.06	6.592	5.597	0.995	2,544	medium
4	65.66	6.150	5.380	0.770	1,480	medium
5	62.76	5.780	4.545	1.235	1,875	medium
6	60.88	5.743	4.354	1.389	3,643	high
7	65.26	5.327	5.050	0.277	773	low

Three of these fish were deemed to have released most of their eggs thereby achieving a high level of spawning or being very spent. The abdomens of these three individuals were extremely distended before spawning but subsequently were very concave. The abdomens of the three fish, whose spawning level was classified as medium, were distended before spawning but only slightly sunken after reproduction. Conversely, there was no noticeable change in the size of the abdomen of the one fish whose level of spawning was rated low.

There were considerable differences in the mean number of eggs for females with high levels of spawning ($\bar{x}=3,523$) compared to those with medium spawning levels ($\bar{x}=1,970$). Spawning production was not correlated with length as the smallest specimen (60.88 mm SL) released the second greatest number of eggs (Figure 4). There was a positive correlation between the difference in weight of pre-and post-spawning females and the number of eggs they laid (Figure 4); however, the limited sample size makes conclusions concerning various aspects of this phase of the study very speculative.

Larval fish development and behavior

Recently-emerged protolarvae (Day 0) were lacking pectoral fin buds, had clear eyes, no body pigment, slightly curved caudal fin, and a large yolk-sac that extended from the posterior portion of the operculum to the vent. Swimming behavior of recently-hatched larvae was characterized by vertical movement through the water column (=swim-up). Larvae spent 1-3 seconds swimming toward the surface, than stopped and drifted toward the bottom only to reinitiate the sequence after a few seconds. Larvae that contacted the aquarium bottom immediately resumed the sequence of vertical swimming.

On Day 1, there were 83 larvae in the artificial backwater (30 mm deep) and the behavior of specimens in this habitat was different compared to the larvae in the main aquarium. Larvae in the backwater did not attempt to maintain their position in the middle of the water column but instead lay on their sides infrequently swimming toward the surface.

By Day 3, the pectoral fins of larval Rio Grande silvery minnow were fully developed, their eyes well pigmented, the gas bladder apparent, and the yolk-sac considerably reduced in size (Figure 5). Of the 143 larvae in the backwater 50% were resting upright, 25% were laying on their side, and the remainder of the fish were tilted between those two positions. Fish in the main aquarium stayed in suspension in the current and continued their behavior of vertical movement in the water column.

Food was first observed in the gut of larvae on Day 4 and coincided with absorption of the yolk-sac. There were 122 fish in the artificial backwater with most of the specimens resting in an upright position. Many larvae in the main portion of the aquarium had abandoned their vertical swimming behavior and were instead swimming horizontally.

For the next several days there were few significant developmental changes as fish proceeded through the protolarval stage. Larvae grew relatively slowly as they absorbed their remaining yolk, started to feed, became deeper bodied, and grew caudal fin rays. Specimens were distributed throughout the aquarium moving freely between the main tank and backwater.

The first mesolarvae was taken on Day 8 but it was not until Day 16 that mesolarvae were >50% of the samples (Figure 6). Differentiation of the rays in the dorsal fin was first noted on Day 13 while anal fin ray differentiation was not observed until Day 17. As fish progressed slowly through the mesolarval stage, the flexion of the notochord completed, fish acquired additional pigmentation, and medial fin rays formed and segmented. Mesolarval were the most abundant developmental stage until Day 34.

The appearance of pelvic fin buds on a Day 23 specimen marked the first metalarval Rio Grande silvery minnow. On Day 27, the sample was 60% mesolarvae and 40% metalarvae. By Day 41, 63% of the fish were metalarvae and 37% were late-mesolarvae. The coiling of the gut became apparent and was associated only with metalarval specimens. Fish were feeding on commercial fish food and grazing on algae growing in the aquarium. Growth rate of metalarvae and development of the full complement of pelvic fin rays was slow.

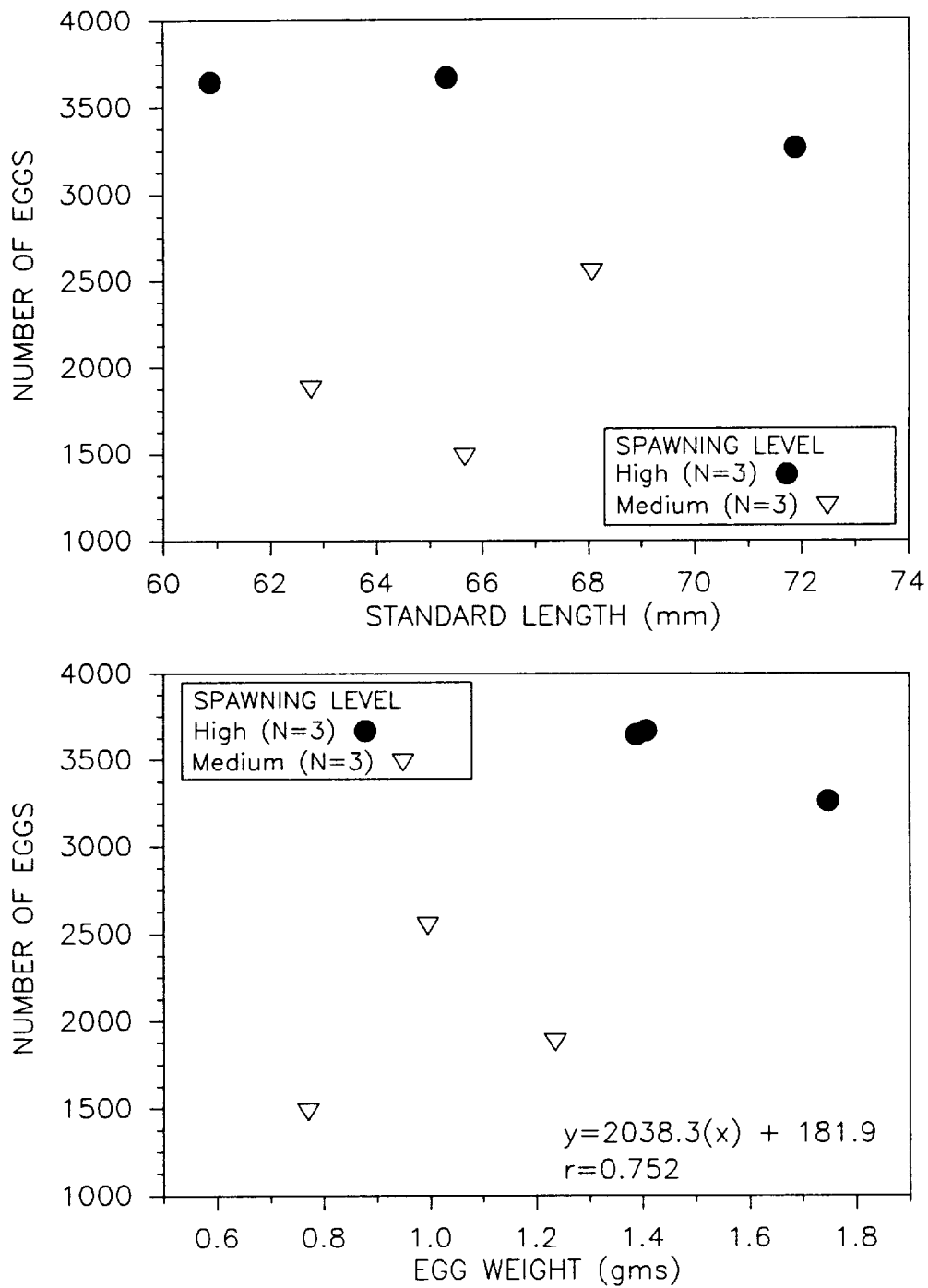


Figure 4. Comparison of number of eggs versus standard length and egg weight.

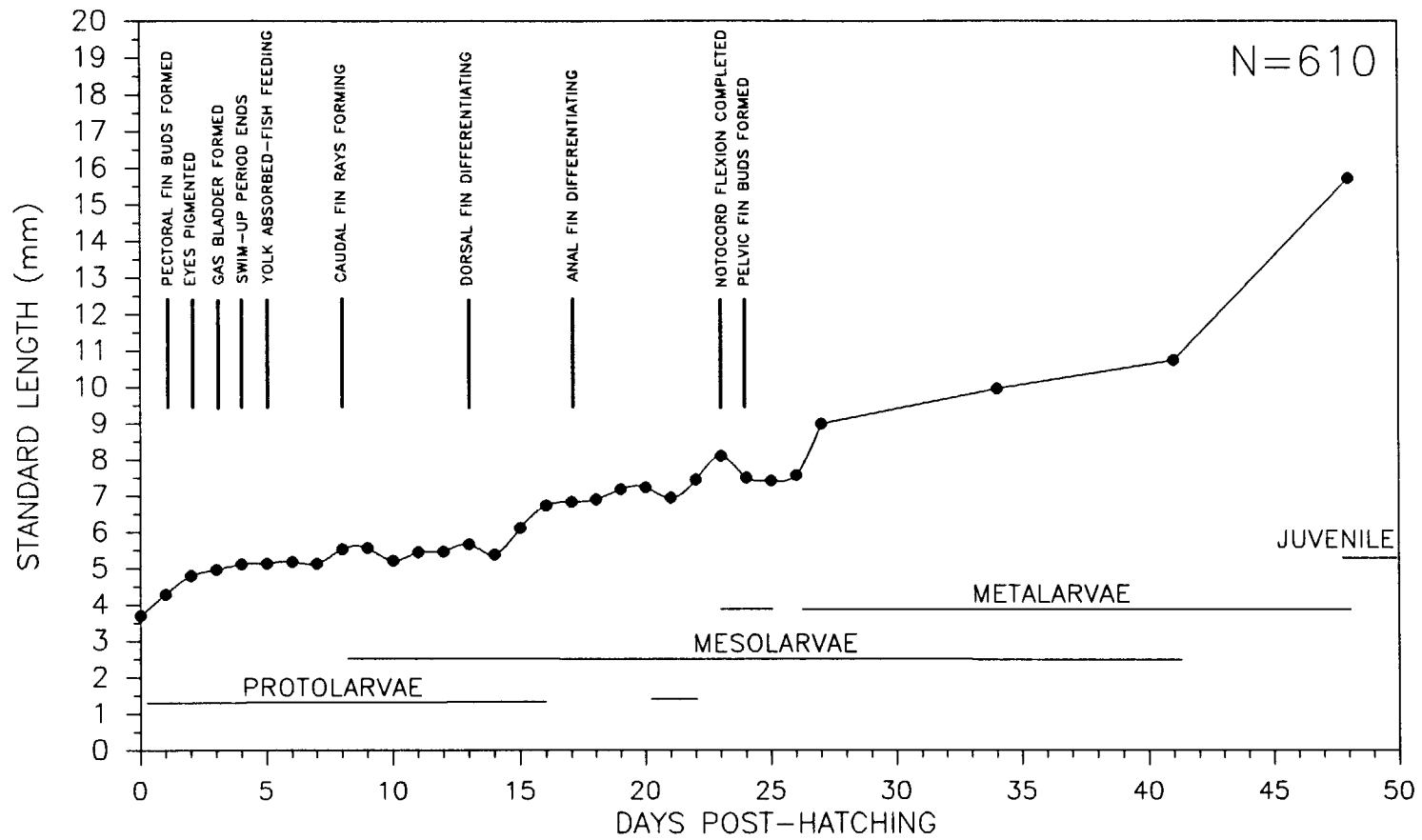


Figure 5. Summary of Rio Grande silvery minnow growth and development through larval stage. Dots represent mean daily length. Horizontal line represents range of ages over which developmental stage occurred.

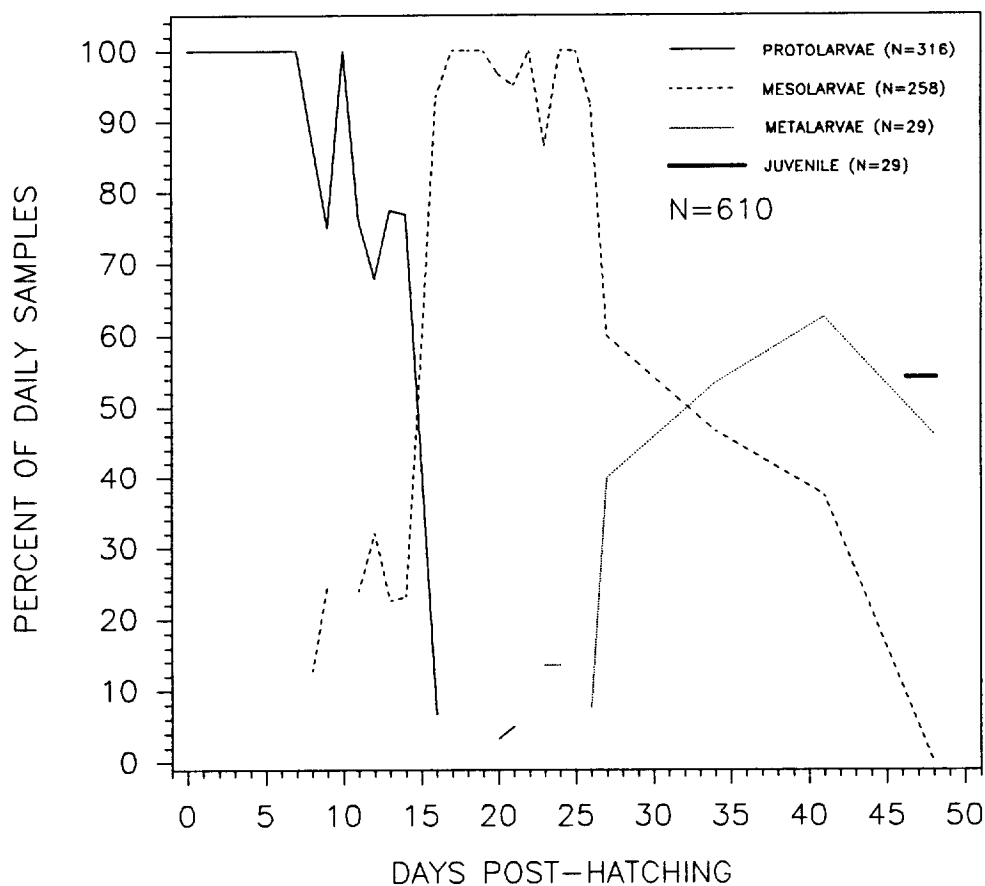


Figure 6. Relative abundance of developmental stages throughout the study.

On Day 48, over half (54%) of the sample was juvenile fish. These specimens had the full complement of fin rays, fully developed pelvic fins, beginning of a scale pattern, well segmented medial fin rays, and no remnants of fin folds.

Larval fish growth rate

A total of 610 laboratory-reared larval and juvenile specimens of Rio Grande silvery minnow was taken, examined, and measured during this study. The number of specimens preserved ranged between 8-66 per day and averaged 19.7. Most individuals (94.8%) were either protolarvae or mesolarvae (Figure 7).

Daily fish lengths were compiled and graphically presented as box plots (Figure 8). In this type of presentation, the box and its associated components represent all lengths, regardless of developmental stage, recorded during a single day. The box and caps illustrate the distribution of 10, 25, 50, 75, and 90% of the selected data. The caps represent the 10 and 90% marks while the lower and upper boundaries of the box depict the distribution of the middle 50% of the data (between 25 and 75%). Individual data points are not represented in the area between the caps.

The solid horizontal line in the box is the median measurement (50%) in the dataset; there are as many observations larger than the median as smaller. Conversely, the dotted line is the average or mean length of fish taken on that day. All data outside 10-90% of the daily lengths are represented as individual points.

During the beginning of the study (Days 0-7) fish had the smallest variance in size while specimens from the last three days of the work (Days 34, 41, and 48) had the greatest range and variance in standard length. Larval fish exhibited very little growth between Days 7-14 as they transformed from protolarvae to mesolarvae. There was also a decrease in mean length during their transition from mesolarvae to metalarvae (Days 23-26).

While growth of specimens between Days 0-41 seemed best described by two separate linear equations (Figure 9), the correlation coefficient (r) for growth rate during Days 0-26 ($r=0.955$) was not significantly different from that for Days 27-41 ($r=0.998$) or Days 0-48 ($r=0.947$). Mean daily length (SL) of specimens collected between Days 0-26 increased from 3.71 to 7.57 or 0.15 mm per day. From Days 27-41, mean growth rate of specimens was 0.13 mm per day. The greatest mean growth rate was between Days 41-48 as specimens transformed from metalarvae to juvenile fish and grew at a rate of 0.71 mm per day. However, this latter growth rate was based only on 21 individuals in two samples.

Discussion

Information gained from this laboratory investigation of the reproductive behavior and developmental biology of Rio Grande silvery minnow can be integrated with ongoing field studies to produce a comprehensive understanding of the life history of this species. Rio Grande silvery minnow is one of a group of fishes that evolved reproductive and early life history strategies for living in aquatic ecosystems in arid lands of the west-central United States. In New Mexico, both the Rio Grande and Pecos River are typical of plains lotic ecosystems characterized by flashy or unpredictable flow.

Observations of Rio Grande silvery minnow in aquaria indicate this is a schooling species with a reproductive behavior similar to that observed in other plains river fishes. Our laboratory and field studies suggest that numerous individuals congregate during spawning and that these

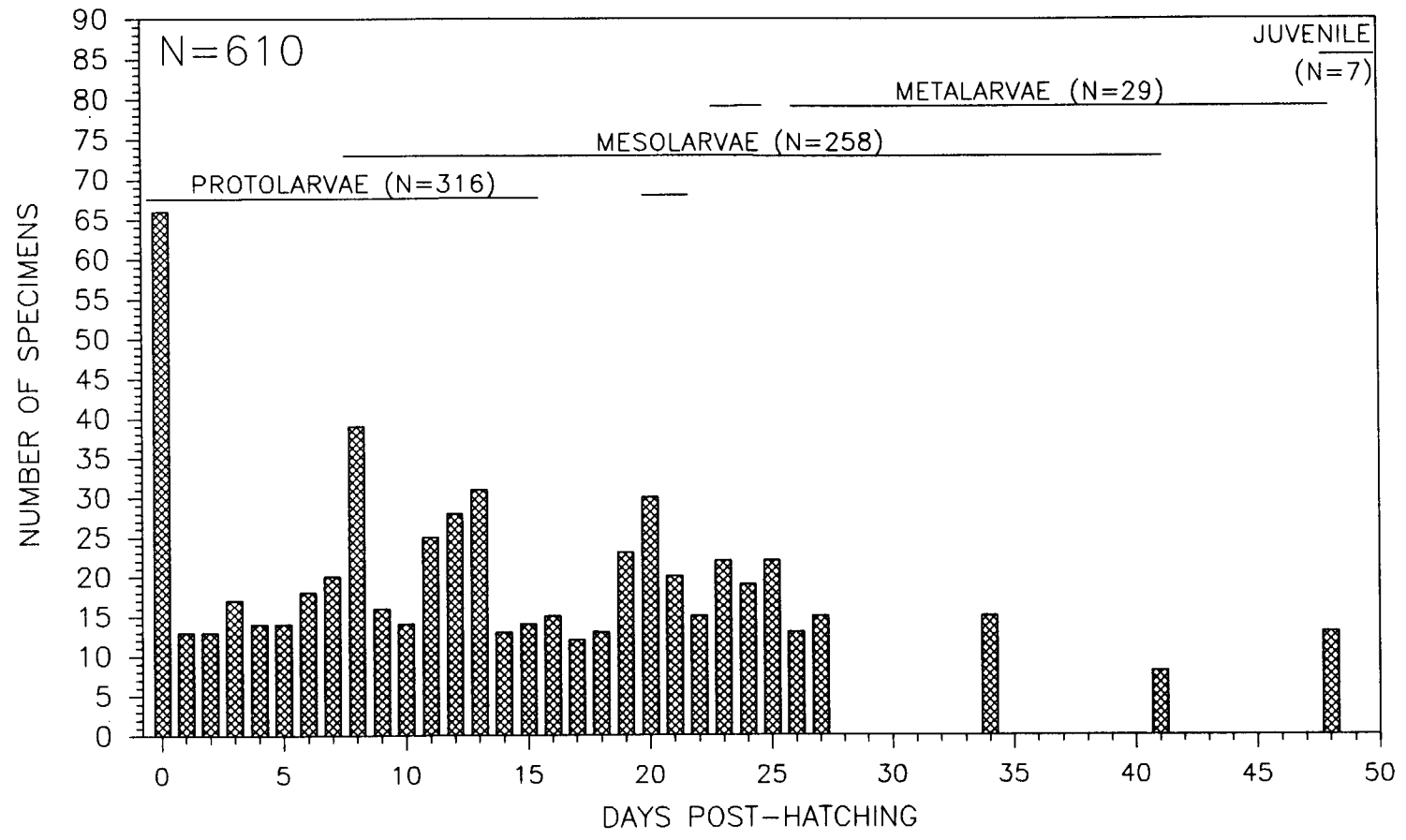


Figure 7. Number of larval fish specimens taken per day.

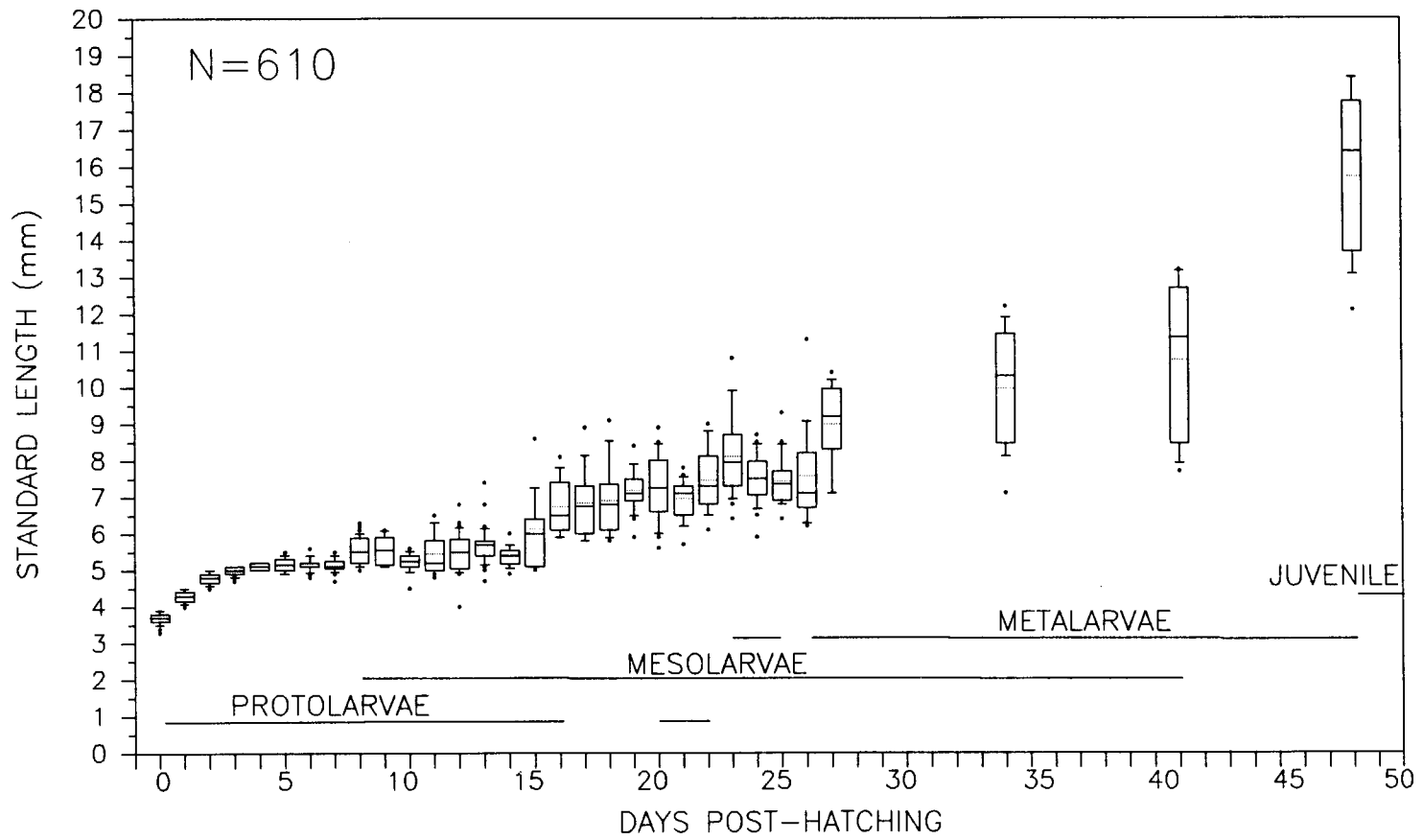


Figure 8. Box-plot distribution of daily growth of larval Rio Grande silvery minnow. See text for explanation of symbols.

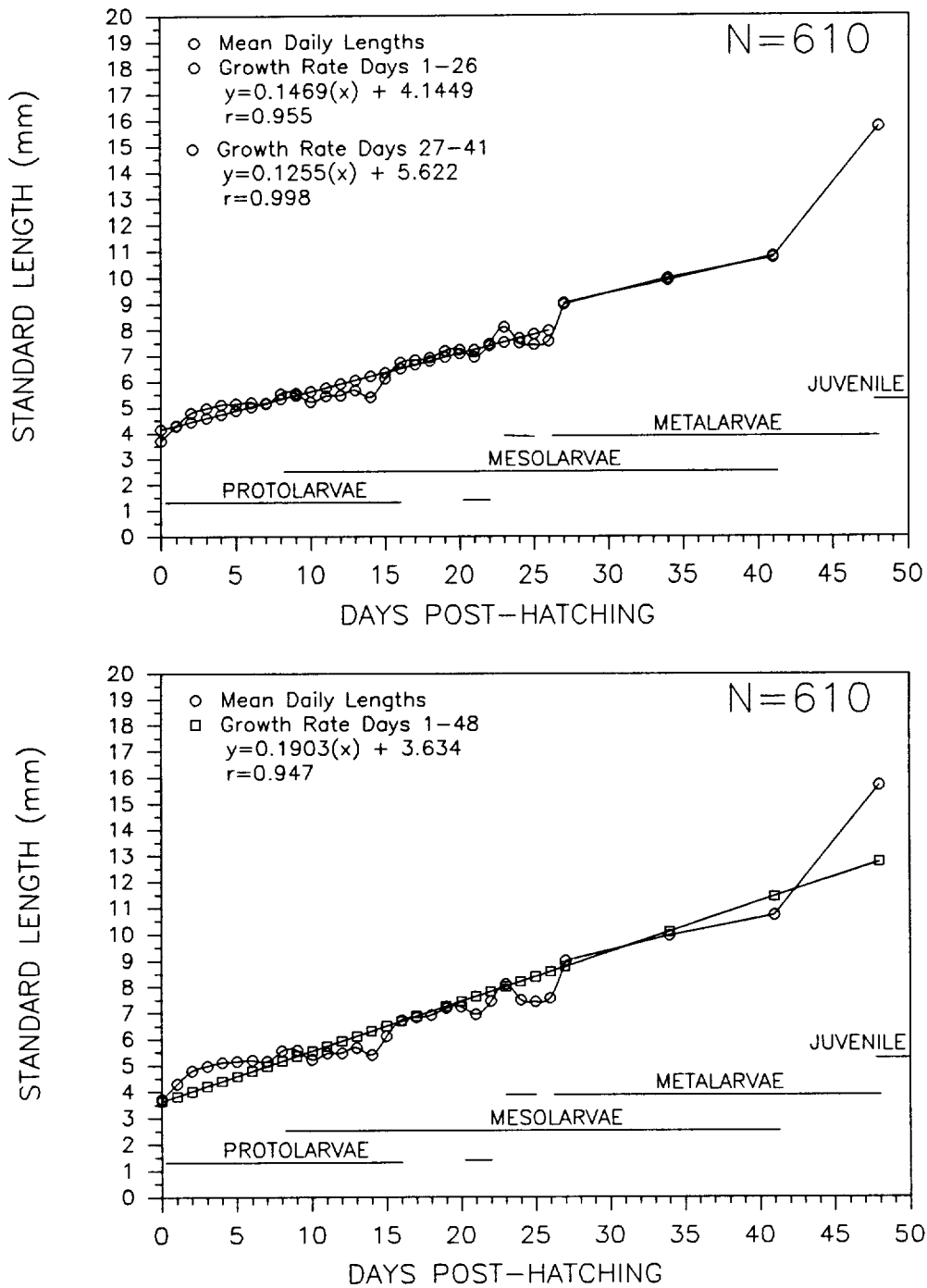


Figure 9. Growth rate of aquarium reared larval Rio Grande silvery minnow.

events may continue over several days or possibly weeks. We have not determined if spawning represents a relatively continuous event or is initiated and discontinued numerous times during the spawning season.

Examination of field-collected male Rio Grande silvery minnow indicate they are capable of spawning for a longer period than female silvery minnow. Males collected for the laboratory study freely expressed milt when slight pressure was applied to their abdomens. Conversely, none of the gravid females collected for the laboratory study released eggs before injection with CPE. Post mortem examination of ovaries of gravid but uninjected female Rio Grande silvery minnow revealed early-maturing, late-maturing, and mature eggs. None of the females examined contained ripe eggs necessary for viable spawning.

Observation of spawning behavior in aquaria demonstrated that males attempted to spawn almost immediately after being placed in the tank with females. The delay between injection of gravid females with CPE and spawning suggests that before the spawning can occur, eggs must transform from late maturing or mature to ripe developmental stage (Heins and Baker 1993). In the laboratory, the CPE injection probably stimulated egg development while in the field, an environmental factor or some combination of natural conditions is probably required for females to spawn. Our studies on the native fishes of the Pecos River suggested that the principal spawning stimulus was increased flow typical of spring runoff or summer rainstorms (Platania, personal observation). Moore (1944), in his work on early life history of Arkansas River shiner, *Notropis girardi*, was one of the first to suggest the correlation between spawning of plains fishes and flow spikes. Lehtinen and Layzer (1988) and Taylor and Miller (1990), in their studies on reproductive cycle of plains minnow reported, an apparent association between spawning and high flow.

Although we have not observed Rio Grande silvery minnow spawning in the field, characteristics of egg development gleaned from laboratory study suggest they spawn in current in the mid-or upper water column. Spawning in that habitat would allow the egg to remain in suspension during the 10-30 minute non-buoyant period that occurs immediately after fertilization but before the filling of the perivitelline space with water. Once filled with water, the egg became semi-buoyant and remained suspended in the water column as long as current was present. Without flow, eggs would sink to the bottom, become covered with silt, and die.

The scenario of spawning during periods of increased flow offers a possible explanation for the delay we observed between injection and spawning in laboratory specimens. Under natural conditions, females *Notropis* spp. cycle through three main stages of reproductive condition during spawning cycles (late maturing, mature, and ripe) with pre-spawning females carrying principally mature eggs (Heins and Rabito 1986). The onset of a flow spike may be the natural stimulus prompting cycling of eggs from the mature to ripe stage and allowing for spawning. Injection of CPE was an artificial stimulus that produced the same result. Such a stimulus would require several hours to effect a result. The laboratory experiment suggested this period ranged between 8-12 hours. The actual time for egg cycling may be considerably less (4-6 hours) and is probably most dependent on the developmental stage of the eggs and water temperature.

Under the aforementioned scenario, the delay between initiation of the stimulus (flow spike) and spawning would also be advantageous to zygote development. The delay would allow fertilized egg development to coincide with increasing flow and, under conditions of extended high flow, permit spawning to occur over several hours or days. This would provide eggs ample water and time to develop before hatching as they are carried in the wave of the flow event. In addition, deferred internal egg development would allow the opportunity to abort the spawn in case of an abbreviated flow spike.

While the number of eggs produced by an individual female during a single spawning event was not determined during this study, we discovered that Age II females could broadcast over 3,000 eggs during an eight-hour breeding session. The limited number of specimens available for this study (N=7) made it difficult to establish any correlation between length and clutch size. Additional fecundity and clutch studies will be designed to determine the relationship between length-weight and egg number, and the number of eggs laid by Age I and Age II female Rio Grande silvery minnow during a single spawn, over several hours of spawning, and throughout the spawning season.

The rapid development and hatching of eggs are perceived as a strategy necessary for survival of fish in plains or desert ecosystems. Development and hatching of Rio Grande silvery minnow eggs are correlated with water temperature. Eggs reared in 30°C water hatched in about 24 hours while eggs in 20-24°C water hatched within 50 hours. The swim-up period of larval fish was perceived as a behavioral mechanism that allowed them to remain suspended in the water column while their gas bladder developed, they absorbed their yolk-sac and started feeding. During egg and early protolarval stage, Rio Grande silvery minnow remained in the drift for about five days.

Once the gas bladder formed, larvae exhibited horizontal movement that allowed them to move from high to low velocity habitats. Low velocity habitats are characterized by high water temperatures, compared to mainstream habitats. In addition, low velocity habitats are typically shallow allowing substantial light penetration and high levels of primary productivity. This combination of warm water temperatures and abundant food would likely result in accelerated growth rate of larval fish. This type of habitat was replicated in the aquarium by the artificial backwater.

Examination of larval fish raised for the developmental series suggested slowing in growth of protolarvae as they switch from dependence on yolk to external feeding. Mid to late protolarvae Rio Grande silvery minnow that start feeding on algae soon transformed to mesolarvae. Apparently larval fish that consumed commercial food or algae had greater growth and developmental rates than those fish that relied on the yolk for nutrition. There was no obvious explanation for the many fish that had not started eating even after absorption of their yolk.

Blaxter (1976, 1984) warned of the problem of extrapolating growth criteria from tank experiments to natural populations noting that tank-reared fish are often shorter and fatter than their wild counterparts at the same developmental stage. Blaxter (1984) also suggested that fish activity was apparently enhanced by confinement in rearing tanks resulting in greater dietary needs. Despite these concerns, many aspects of growth information gained from this study have been corroborated by field observations.

Duration of the drift stage (egg and swim-up) of Rio Grande silvery minnow was most likely dependent on water temperature. However, the distance traveled by eggs and larvae during this phase of their life history is principally dependant on water velocity during the 3-5 day period immediately after spawning. Approximate water velocities in the Middle Rio Grande ranges from 8.6 km/hour (5.3 mi/hr) between Cochiti and San Felipe to 3.0 km/hr (1.9 mi/hr) between Albuquerque and San Acacia Diversion Dam (U.S. Army Corps of Engineers, pers. comm.). If Rio Grande silvery minnow spawn during the high flows that occur in May-June, the eggs and larvae could be transported downstream at 3 km per hour. Using the developmental scenarios determined in the laboratory and assuming a mean drift velocity of 3 km/hour, unimpeded eggs could be transported 72-150 km (45-93 mi). Developing protolarvae could be transported an additional 216 km (134 mi) during the swim-up stage. While there were no absolute means to determine the distance drifting Rio Grande silvery minnow eggs and larvae could be transported,

relatively high water velocity during the presumed spawning period would suggest those distances were substantial.

The downstream transport of eggs and larvae over long distances was, historically, likely beneficial to Rio Grande silvery minnow populations. This behavior may have promoted recolonization of reaches impacted during periods of natural drought. The tendency of fish and other aquatic organisms to move upstream toward more permanent sources of water potentially would concentrate reduced populations and allow for staging prior to annual runoff events. Increased temperature and flow would stimulate spawning resulting in redistribution of eggs and larvae throughout recently de-watered or impacted reaches. However, a crucial component of that scenario is the ability of fish to move upstream to reaches of sustained flow.

There are currently three structures between Cochiti Dam and Elephant Butte Reservoir that are barriers to upstream movement of fishes (Angostura Diversion Dam, Isleta Diversion Dam, and San Acacia Diversion Dam). These diversion dams effectively divide the Rio Grande into four discrete reaches: Cochiti to Angostura, 35.5 km (22.3 mi); Angostura to Isleta, 65.2 km (40.4 mi); Isleta to San Acacia, 85.6 km (53.1 mi); and San Acacia to Elephant Butte Reservoir, 92.3 km (57.2 mi). Fish (eggs and drifting protolarae) are likely transported downstream from one reach to another but are unable to return upstream past these barriers. Water velocity is at its maximum rate during the putative spawning period of Rio Grande silvery minnow. Given the reproductive ecology of Rio Grande silvery minnow (spawning behavior, egg type, and early life history traits), it is not surprising that this species is least common in the uppermost section and most common in the lowermost reach of its current range.

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Literature Cited

- Bestgen, K. R., and S. P. Platania. 1991. Status and conservation of the Rio Grande silvery minnow, *Hybognathus amarus*. *Southwestern Naturalist* 36(2):225-232.
- Blaxter, J. H. S. 1976. Reared and wild fish-how do they compare? Tenth European Symposium on Marine Biology, Volume 1:11-26, Ostend, Belgium.

- Blaxter, J. H. S. 1984. Ontogeny, systematics, and fisheries. pp. 1-6 in H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall, Jr., and S. L. Richardson, editors. *Ontogeny and systematics of fishes*. American Society of Ichthyologists and Herpetologists Special Publication 1. 760 pp.
- Breder, C. W. and D. E. Rosen. 1966. *Modes of reproduction in fishes*. T.F.H. Publication, Inc. Neptune City, New Jersey.
- Cross, F. B. 1967. *Handbook of fishes in Kansas*. Museum of Natural History, University of Kansas, Lawrence, Kansas. 375 pp.
- Heins, D. C. and J. A. Baker. 1993. Clutch production in the darter *Etheostoma lynceum* and its implications for life history study. *Journal of Fish Biology* 42:819-829.
- Heins, D. C. and F. G. Rabito, Jr. 1986. Spawning performance in North American minnows: direct evidence of the occurrence of multiple clutches in the genus *Notropis*. *Journal of Fish Biology* 28:343-357.
- Lehtinen, S. F. and J. B. Layzer. 1988. Reproductive cycle of the plains minnow, *Hybognathus placitus* (Cyprinidae) in the Cimarron River, Oklahoma. *Southwestern Naturalist* 33(1):27-33.
- Long, W. L. and W. W. Ballard. 1976. Normal and embryonic stages of the white sucker, *Catostomus commersoni*. *Copeia* 1976:342-351.
- Moore, G. A. 1944. Notes on the early life history of *Notropis girardi*. *Copeia* 1944:209-214.
- Miller, R. J. and H. W. Robison. 1973. *The fishes of Oklahoma*, Oklahoma State University Press, Stillwater, Oklahoma. 246 pp.
- Snyder, D. E. 1981. Contributions to a guide to the cypriniform fish larvae of the Upper Colorado River system in Colorado. U.S. Bureau of Land Management, Biological Sciences Series 3, Denver, Colorado. 81 pp.
- Snyder, D. E. 1983. Fish eggs and larvae. pp. 165-197 in L. A. Nielsen and D. L. Johnson, editors, *Fisheries Techniques*. American Fisheries Society, Bethesda, Maryland. 468 pp.
- Taylor, C. M. and R. J. Miller. 1990. Reproductive ecology and population structure of the plains minnow, *Hybognathus placitus* (Pisces: Cyprinidae), in central Oklahoma. *American Midland Naturalist* 123(1):32-39.
- U.S. Army Corps of Engineers. personal communication. Travel time of flood peaks, 12 August 1994.
- U.S. Department of the Interior. 1994. *Endangered and Threatened Wildlife and Plants; Final Rule to List the Rio Grande Silvery Minnow as an Endangered Species*. Federal Register 59(138):36988-36995.

APPENDIX I

Developmental stages of larval fish as defined by Snyder (1983)

- Protolarvae** Phase of larval development characterized by the absence of dorsal, anal, and caudal fin spines and rays. Transition to the mesolarval stage is based on the appearance of at least one distinct spine or ray in any of the median fins.
- Mesolarvae** Development stage of larval fish characterized by the presence of at least one dorsal, anal, or caudal fin spine or ray. Specimens in this stage lack the adult complement of principal fin rays in all median fins (=dorsal, anal, and caudal) and have not yet developed pelvic fin buds.
- Metalarvae** Larval fish developmental stage characterized by the presence of the adult complement of principal fin rays in all median fins and pelvic buds or fins.
- Juvenile** Transition to the juvenile requires that the finfold and atrophying fins must be absorbed beyond any recognition, the full adult complement of fin spines and rays must be formed in all fins, and segmentation must be evident in the rays of each of the fins.

APPENDIX II
Daily accounting of Rio Grande silvery minnow larval fish development

Rio Grande silvery minnow larval development

- Day 0 All larvae had hatched. Specimens had slightly curved caudal fins suggesting they had only recently broken free of their egg. The specimens had no body pigment and the eyes were also pigment-less. No air bladder present, all specimens had full yolk-sacs that extended posterior to the vent. Pectoral fin buds not apparent.
- Day 1 Body still pigmentless and eye (iris) still transparent. Yolk-sacs look a little smaller but are still a dominant morphological feature. Pectoral fin buds starting to form.
- Day 2 Eye pigmentation appears (brown), pectoral fin buds fuller, gas bladder formation started, and the yolk-sac was being absorbed. No body pigmentation was apparent.
- Day 3 Eye pigmentation changes to black. The developing gas bladder becomes apparent while the yolk-sac becomes obviously reduced in size. Some body pigment is evident.
- Day 4 Yolk-sac is almost completely absorbed and food is apparent in the gut of one of the larvae. There is pigmentation over the body with some pigmentation present on the tail. Pectoral fins are full and contain rays.
- Day 5 No major developmental changes. Additional specimens observed with food in their guts.
- Day 6 No significant developmental changes.
- Day 7 No significant developmental changes.
- Day 8 The first caudal fin rays started to appear indicating the transformation from protolarvae to mesolarvae. Specimens become deeper bodied as the yolk sac is absorbed and the gut continues developing.
- Day 9 Slow transition from protolarvae to mesolarvae.
- Day 10 Slow transition from protolarvae to mesolarvae.
- Day 11 No significant developmental changes.
- Day 12 No significant developmental changes.
- Day 13 No significant developmental changes.
- Day 14 Caudal fin rays still not well differentiated; majority of the sample comprised of late protolarvae, the few early mesolarvae in the samples also had developing dorsal fin rays.
- Day 15 No significant developmental changes.

- Day 16 Majority of sample comprised of mesolarvae with well formed caudal fin rays. No anal fin rays on any specimens.
- Day 17 First differentiation of anal fin rays.
- Day 18 Very few of the mesolarvae have both dorsal and anal fin ray differentiation. Many of the mesolarvae were barely past the protolarvae stage. Rate of development very slow compared to early protolarvae.
- Day 19 No significant developmental changes.
- Day 20 No significant developmental changes.
- Day 21 No significant developmental changes.
- Day 22 No significant developmental changes.
- Day 23 Notochord flexion completed and pelvic fin buds formed on few specimens. Majority of sample still mesolarvae. First metalarval fish taken.
- Day 24 Largest fish in this sample had a short gut coil near the vent and most of its principal anal fin rays. Most of the other specimens had some dorsal fin rays but no anal fin rays. Pigment patterns were strong.
- Day 25 All specimens were mesolarvae.
- Day 26 The lone metalarvae in this sample had full fin rays (including pelvic fins). The mesolarvae in the sample varied in developmental stage from not having any dorsal or anal fin rays to having a complete set of median fin rays.
- Day 27 Transition stage between mesolarvae and metalarvae.
- Day 34 About half of the sample were mesolarvae and half were metalarvae. Metalarvae had single coil gut that was not yet wrapped onto itself.
- Day 41 Gut coiling obvious in metalarvae but not apparent in the mesolarval specimens.
- Day 48 About half of the specimens were juvenile and half were late metalarvae. Juvenile had the full complement of fin rays, fully developed pelvic fins, beginning of a scale pattern, good segmentation of all medial fin rays, and lacked any remnants of the fin folds. Strong dorsal and lateral pigment.