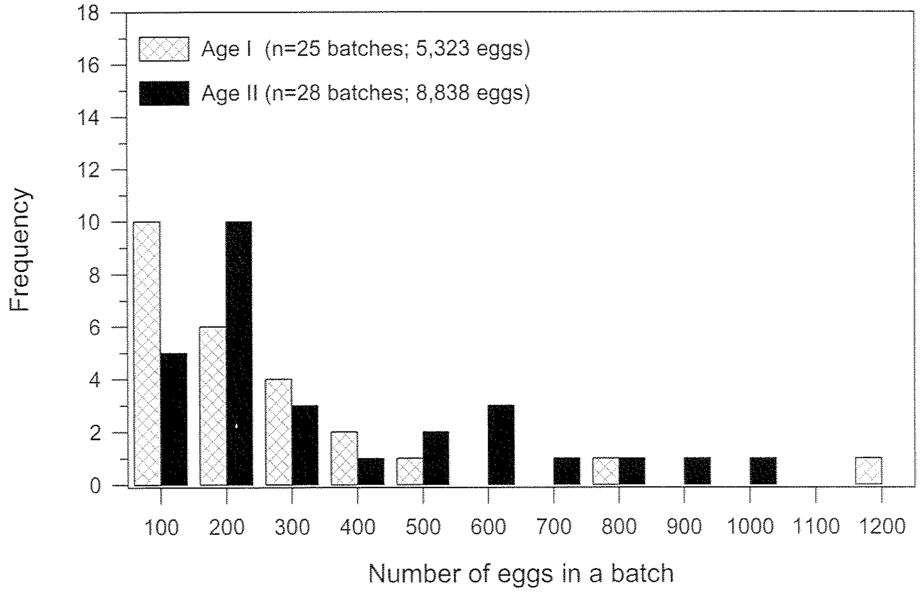
Reproductive Ecology of Rio Grande Silvery Minnow *Hybognathus amarus*: Clutch and Batch Production and Fecundity Estimates

Final Report



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Executive Summary

This phase of investigation on the reproductive ecology of Rio Grande silvery minnow, *Hybognathus amarus*, documented several additional aspects of this species' biology. Reproductive activities in a laboratory setting for this species was composed of a series of spawning events with 100 to 200 eggs expelled per event. This study also demonstrated significant differences in egg production between the two adult age classes of Rio Grande silvery minnow. Age II fish were engaged in more spawning events ($\bar{x}$=13), had greater modal number of eggs per batch (200), and higher total fecundity (up to 5,300 eggs).

The elevated level of spawning achieved by some individuals suggested that Rio Grande silvery minnow are capable of expelling all eggs in a relatively short time frame. This ability, in combination with this species apparent schooling behavior, suggests a high probability for synchronous spawning in populations in selected reaches of the Rio Grande. The lack of numerous larval silvery minnow cohorts in recent field collections may provide additional support for such spawning behavior.

Information gained from this study, when integrated with previous laboratory studies and ongoing field studies, produce a more comprehensive understanding of the life history of this species. This synthesis of laboratory and field life history information will provide insight of the relationships of Rio Grande silvery minnow population dynamics and water management practices in the Middle Rio Grande of New Mexico.

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Introduction

Determination of annual or lifetime fecundity in fish is dependant on clutch size and spawning frequency and is critical to studies of life history strategies. Fecundity estimates are typically obtained through enumeration of mature or maturing eggs in ovaries of preserved specimens. Several authors recently revealed the errors and spurious conclusions drawn from such data sets (Heins, 1990; Heins and Baker, 1988, 1993a, 1993b). Researchers have advocated the need to validate fecundity estimates from preserved specimen by obtaining comparable information on clutch size and frequency from live specimens observed under experimental conditions. Their studies concentrated on darters of the genus *Etheostoma* as many members of that genus lay multiple clutches of easily counted adhesive eggs (Weddle and Burr, 1991; Heins and Baker, 1993a, 1993b; Heins and Machado, 1993).

Although ubiquitous and generally more abundant than darters, there are little comparable information available for North American cyprinids and none for plains stream minnows. The lack of such information on plains stream fishes was due, in part, to the dearth of life history data regarding these forms. Our recent studies documented the reproductive behavior and egg physiology of many members of this guild of fishes inhabiting the Rio Grande and Pecos River (Platania, 1995). The discovery of broadcast spawning as the prevalent mode of reproduction in plains stream cyprinids of the American Southwest allowed us to undertake studies to determine clutch sizes, batch sizes, spawning frequencies and total fecundity from live specimens.

Previous reproductive biology studies on this endangered species resulted in the successful spawning of Rio Grande silvery minnow, *Hybognathus amarus*, in aquaria and rearing of their larvae to the juvenile developmental stage. That study (Platania, 1995) showed that this species is a pelagic spawner that may produce over 3,000 semi-buoyant, nonadhesive eggs during a spawning season. 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.

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. 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 clutch and fecundity studies were necessary to determine the relationship between length-mass 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. Preliminary investigations suggested significant differences in the relative abundances of Age I and Age II reproducing females and the number of eggs produced by each cohort.

This investigation had four principal objectives regarding fecundity estimates of Rio Grande silvery minnow: 1) determine relationship between the number of eggs spawned and female length and mass, 2) ascertain the relationship between the number of eggs spawned and female age class, 3) note the frequency of multiple spawning events, and 4) attempt to record the number of eggs laid per spawning event. Information gained from such a study, unobtainable from field investigations, can be integrated with ongoing field studies to produce a comprehensive understanding of the life history of this species. This synthesis of laboratory and field life history information will provide insight of the relationships of Rio Grande silvery minnow population dynamics and water management practices on the Rio Grande.

Methods

Rio Grande silvery minnow used in this investigation were collected on seven occasions between 5 May 1995 and 9 June 1995. Specimens were taken adjacent to the confluence of the Rio Grande and North Socorro Diversion Channel in Socorro County, NM. This locality was selected because of its ease of accessibility, extensive reaches of low-velocity habitat (despite the high mainstem spring runoff), and numerous gravid Rio Grande silvery minnow present there during the previous two years. In addition, fish from this locality appeared more reproductively active than similar size and age specimens from upstream localities (Rio Rancho). Fish were collected with small mesh seines and placed in 18.9-liter (5-gallon) holding buckets. Water temperatures during the study period and at the time of collecting were 15-19°C.

The reproductive status of retained fish were assessed in the field. Females that appeared most gravid (as determined by an increased body mass:length ratio) were selected and transferred to plastic bags of river water and oxygen. An attempt was made to procure specimens representing a wide range of lengths in both age classes. Fish were transported from the field to the laboratory in ice chests which helped maintain relatively constant water temperature and provided additional protection of the specimens. Once in the laboratory, individuals were re-examined to determine their relative health, gender, age, and reproductive condition.

All experiments were conducted in 12 sequentially numbered 56.8-liter (15-gallon) aquaria. Tanks were maintained in an experimental greenhouse in the Department of Biology at the University of New Mexico. The greenhouse received natural sunlight and maintained an ambient temperature of 27°C. Minimum and maximum water temperature in aquaria were recorded on submerged Taylor® minimum-maximum thermometers. A 25-cm tubular air-stone, placed in the lower corner of each aquaria, maintained the water's dissolved oxygen level and produced a water-current.

Rio Grande silvery minnow selected for this study were assigned a number (1-12) and anesthetized in a dilute solution of MS-222. Specimens were blotted to remove excess liquid, measured (0.1 mm SL), weighed to the nearest 0.001 gm on a Sartorial scale, and given an abdominal injection of about 0.1 cc (0.11 mg) of acetone-dried carp-pituitary extract (CPE) per gram body mass. Injected female Rio Grande silvery minnow were then placed in the appropriate corresponding numbered aquaria and observed until they recovered from the anesthesia. Fish were generally processed the day of their capture and injected with CPE between 1800-2400 hours. Our previous studies indicated female fish that were going to spawn would do so within 16 hours of injection.

Male Rio Grande silvery minnow were subjected to the same procedure except that males were kept separate from females for about eight hours, after which two males were introduced into each aquarium (containing one female). Attempts were made to select males and females of the same year class and similar lengths. Male Rio Grande silvery minnow were sometimes used for two spawning trials whereas females were never used for more than one. Diseased fish and those that did not recover from the anesthesia were preserved in 10% formalin.

Length-mass relationships of small fishes are usually based on eviscerated body mass of preserved specimens and can provide information on the reproductive state of individuals. One of our goals was to attempt to assess a gross level of fecundity in live females, therefore, we used uneviscerated body mass measures to determine length-mass ratios. These relationships were determined using regression analysis $y=mx+b$ where y was uneviscerated body mass, x was standard length and m and b were constants. Body mass measures were presented as logarithmic values to convert this ratio into a linear relationship.

Terminology used in this study, in reference to egg production, generally followed Heins and Rabito (1986). These terms are presented in a hierarchical progression of largest to smallest and are generally time dependent. **Fecundity** is a measure of the number of eggs spawned by a female without reference to temporal considerations. **Clutch** refers to a group of eggs that are spawned within a relatively short time frame and differs from **batch** in that the latter references a single complement of eggs produced during one spawning event.

While the aforementioned laboratory procedures were followed throughout this project, there were some phase-specific differences in the subsequent methodology. The four separate goals of this project necessitated slight differences in handling of specimens after they were placed in aquariums. Because of the potentially disruptive aspects associated with assessing the number of eggs per batch (objective # 4), that phase of the research was delayed until we had gathered sufficient information for the first three objectives of the study.

The principal focus of the study was to determine the number of eggs produced by Rio Grande silvery minnow and correlate those data with body mass. One egg collecting net (25 x 18 cm, fine-mesh) was suspended in each aquarium and first checked about 10 hours after fish were injected with CPE. Nets were subsequently checked and eggs removed hourly. We found this to be a sound technique collecting >99% of the eggs and minimizing disturbance of spawning behavior (Platania, 1995).

For the purposes of this study, a **spawning event** is defined as a male-female encounter that results in the discharge of eggs. Determination of the number of spawning events per female was accomplished by direct and indirect observation. We observed reproductive behavior in selected aquaria for trials 3, 5, 6, 8, and 9 and recorded the time of the event and relative location in the water column of the aquarium for each spawning event (surface, middle, bottom). Eggs produced during the spawning events were captured using the methodology described in the previous paragraph. Video cameras (8-mm) also recorded Rio Grande silvery minnow reproductive behavior and was later viewed to determine the number of batches produced. Fish were filmed in real-time mode with an internal digital clock image superimposed on the tape. Broadcast of eggs was easily seen when video tapes were viewed. All data recorded during direct observation were also available from video tape analysis. As with direct observations, there was a minimum of 16 hours between hormone injection and cessation of the trial.

Determining the number of eggs per batch was the most laborious portion of the research segment requiring continuous observation of individuals. In addition, it was also the most intrusive phase of the study as it necessitated removal of eggs immediately after a spawning event. We were aware, from previous laboratory reproductive studies, that spawning behavior consisted of a series of spawning events occurring over relatively short time frame (one-two hours). We were concerned that the activity required to ensure removal of all eggs immediately after a spawn might inhibit additional spawning and result in spurious data.

The number of eggs per batch were only determined from selected individuals in the next to last two trials (8 & 9). We were unable to obtain sufficient number of gravid female Rio Grande silvery minnow to continue this phase of the research. In trials 8 and 9, spawning fish were kept under direct observation. Eggs were netted soon after a spawning event had occurred and an attempt was made to keep disturbance of fish to a minimum. The eggs produced in this phase of the study were given a clutch number and number that corresponded with the parental female. These samples also provided data on number of batches and number of eggs-body mass ratio.

In all phases of this investigation, fish were removed from the aquaria about 16-20 hours after injection, anesthetized, reweighed, and fixed in 10% formalin. In the few cases where necessary, age determination was verified through examination of annuli on scales. Random subsets of eggs were taken from all tanks during each trial and allowed to develop to the gastrula stage thereby documenting egg viability. All eggs were fixed in 5% buffered formalin, counted, and assigned a museum number that corresponded with the female that produced the eggs. Terminology for reproductive behavior and egg type follow definitions provided by Breder and Rosen (1966) and Balon (1975). Specimens used for reproductive studies and eggs produced as part of this work were deposited in the Fish Division of the Museum of Southwestern Biology.

Results

This study resulted in spawning of 69 of 85 female Rio Grande silvery minnow and the production of 115,799 eggs. While all Rio Grande silvery minnow that spawned provided information on body mass-egg number relationships (n=69) a progressively smaller subset of those data were available to determine frequency of multiple spawning events (n=9), and number of eggs per batch (n=7). The 44 fish in the first two trials provided information exclusively on body mass-egg number relationships. Two of the four specimens in trial 3 that spawned were video-taped and resulted in data on the number of batches per clutch and six of the seven females that were used to determine the number of eggs per batch were in the last two trials (Table 1). The difference between the number of female Rio Grande silvery minnow weighted and measured and those that successfully spawned included individuals used as control (n=1), those that died after injection (n=5), females that got caught in the egg collecting net (n=3), and specimens that did not spawn (n=7).

Table 1. Accounting of trials and different phases of the clutch production-fecundity study.

DATE	TRIAL	Number of females	Number of spawning females	Length-Mass Ratio	Body-Mass Egg number Ratio	Number of spawning events	Eggs/batch
5 May	1	12	11	12	11	0	0
5 May	2	12	12	12	12	0	0
12 May	3	12	10	12	10	2	0
12 May	4	12	11	12	11	0	0
18 May	5	8	4	8	4	0	0
20 May	6	8	6	8	6	1	1
23 May	7	11	8	11	8	0	0
2 June	8	7	5	7	5	4	4
9 June	9	3	2	3	2	2	2
TOTAL		85	69	85	69	9	7

Length-mass relationships

Residual analysis of length-mass relationships obtained on 85 female and 133 male Rio Grande silvery minnow indicated a significant difference ($p<0.05$) between genders (Figure 1). Female silvery minnow consistently obtained greater body mass than males of equal length. The correlation coefficient between length and body mass was not only strong for both genders but was equal across sex.

The majority (72%) of female Rio Grande silvery minnow collected were Age I specimens that were 10-12 months old. Length and body mass of the 61 Age I females were 43.3-61.1 mm SL ($\bar{x}$=51.5 mm SL) and 1.85-4.42 gms ($\bar{x}$=3.03 gms), respectively. The largest Rio Grande silvery minnow collected during this investigation was an 76.6 mm SL, 9.72 gm Age II female. However, this individual was diseased and did not successfully complete spawning trials. Mean sizes of the 24 Age II female silvery minnow were 67.7 mm SL and 6.71 gms. There was a relatively clear separation in female length and mass between the two age classes (Figure 2).

About 80% of the male Rio Grande silvery minnow taken for this study were Age I fish. The size overlap between the two age classes was much greater in males than females. Age I male Rio Grande silvery minnow were 42.9-60.4 mm SL and 1.38-3.50 gms ($\bar{x}$=49.1 mm SL, 2.01 gms) while Age II specimens were 60.5-73.0 mm SL and 3.35-7.23 gms ($\bar{x}$=64.4 mm SL, 4.74 gms).

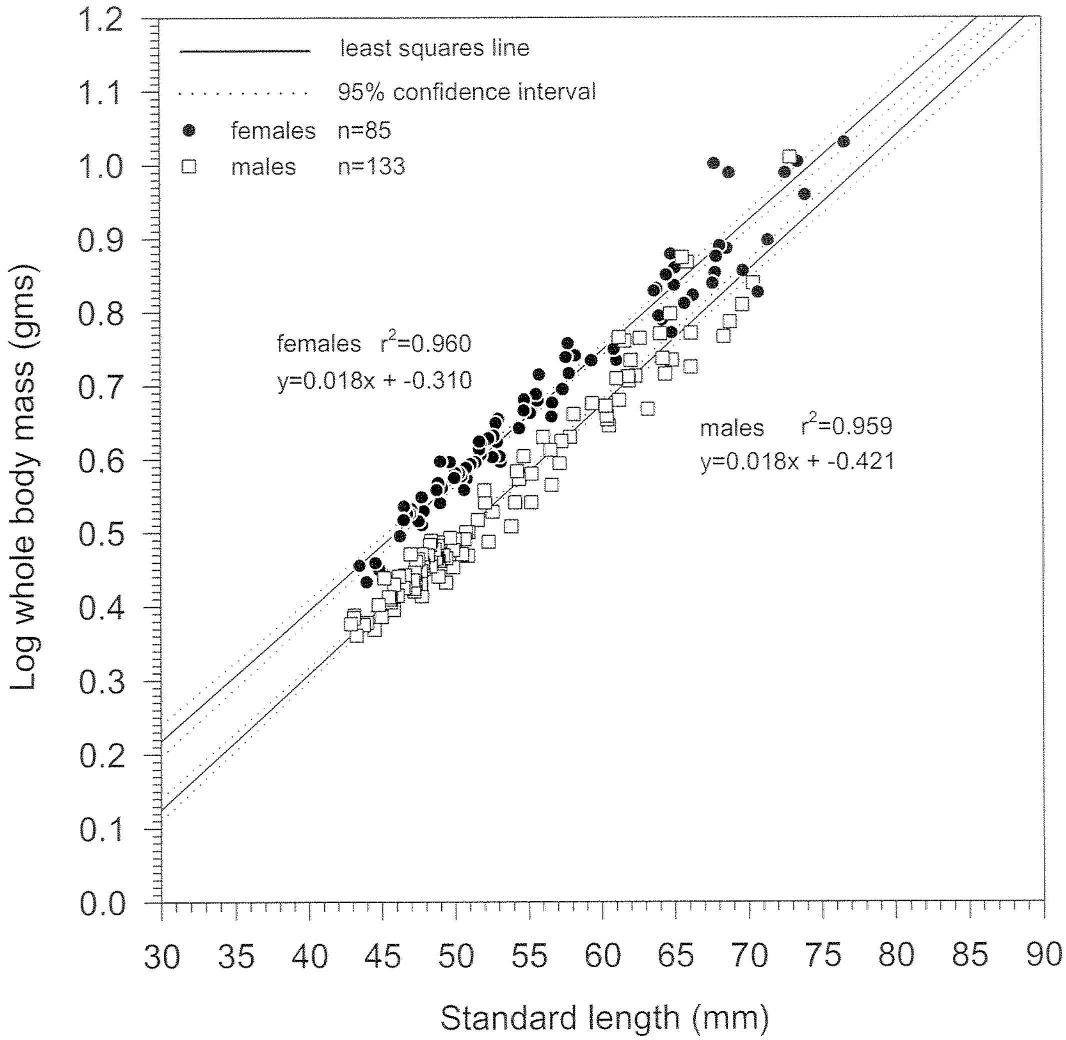
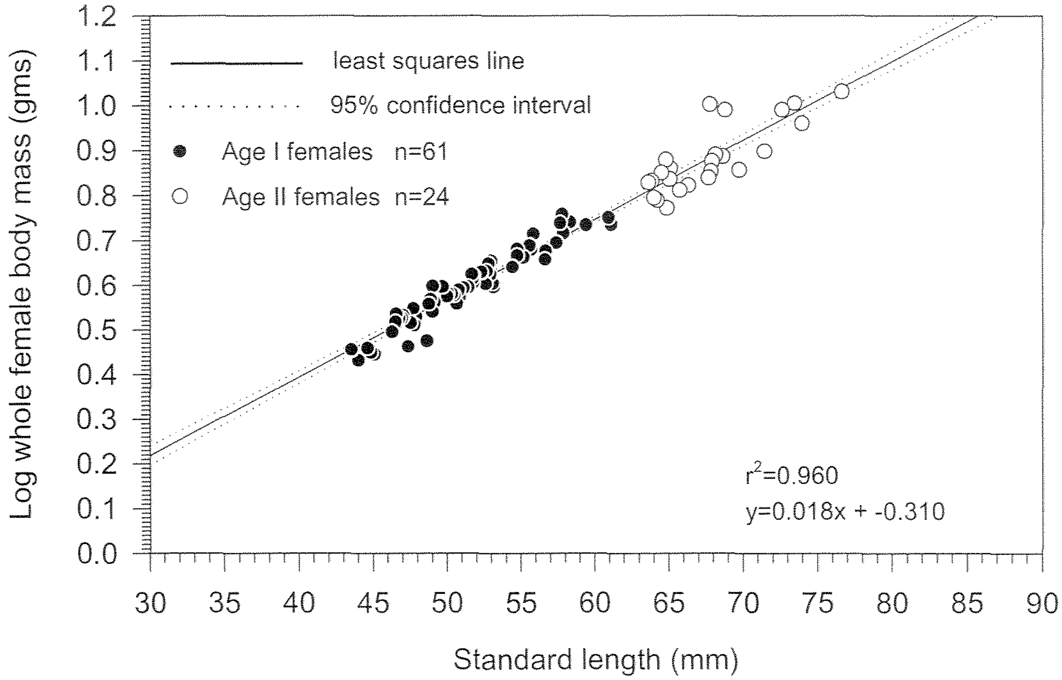


Figure 1. Rio Grande silvery minnow length-mass relationships.



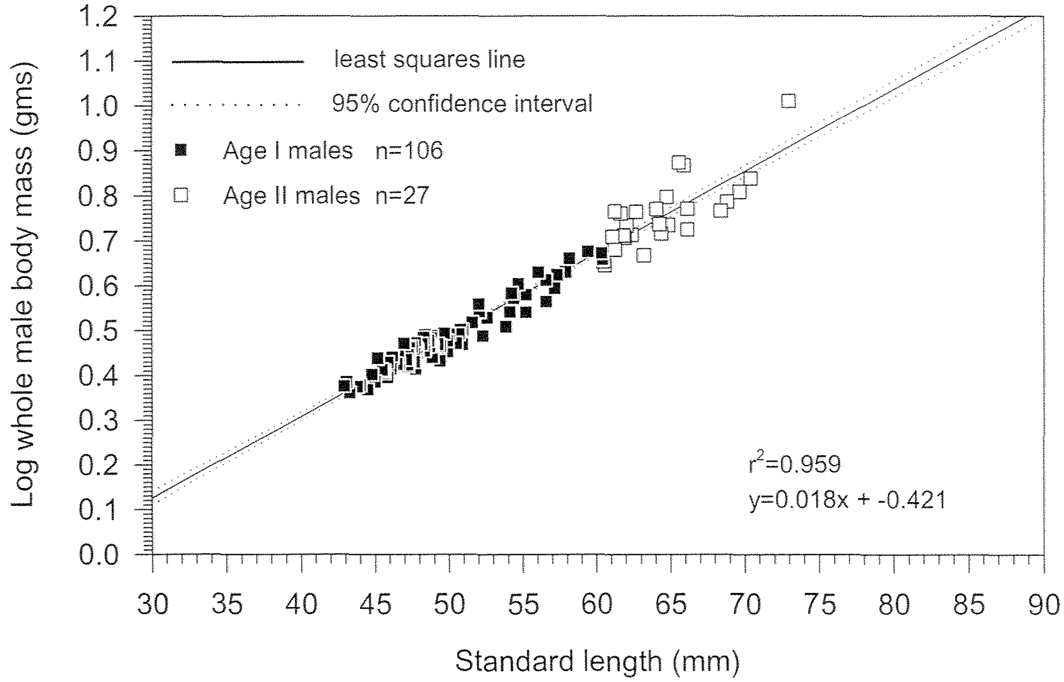


Figure 2. Length-mass relationships of Rio Grande silvery minnow based on age class and gender.

Body mass-egg number relationships

A total of 69 female Rio Grande silvery minnow and their clutches were examined for this portion of the study. Females that spawned during the study were 44.0-73.5 mm SL and 1.70-9.11 gms and comprised of 53 Age I and 16 Age II specimens. In general, larger females produced more eggs than smaller individuals. Regression analysis of the data indicate a positive correlation (r^2=0.596) between female length and the number of eggs produced (Figure 3). Close examination of the data revealed numerous outlying data points and considerable variation in the overall length-number of eggs dataset. These deviations resulted in lower fit of the line. This variation was most evident when the data were parsed and plotted by age class (Figure 4).

Correlation coefficient of Age I females (r^2=0.211) did not show a significant improvement when outliers at either end of the plot were removed. There was considerable variation throughout the Age I dataset in the number of eggs produced. With the exception of three data points representing spawns of about 3,000 eggs, the number of eggs produced by Age I females per length in mm SL remained relatively constant.

The small number of Age II Rio Grande silvery minnow available for study limited the level of analysis of putative trends. Age II silvery minnow were clustered into three separate length classes (ca. 64, 68, 73 mm SL) for additional study (Figure 4). Each of these groups exhibited significant variation in the number of eggs produced but still with some overall trend of increasing variance with increasing size. The largest number of eggs laid during this study (n=5,303) were from a 73.5 mm SL female in Trial 1. Conversely, a 74 mm female collected on the same date (run in trial 2) yielded only 1,294 eggs. A similar pattern, but of a lesser magnitude, was apparent for fish about 64 and 68 mm SL. This high level of variability was reflected in the very low coefficient of correlation score for Age II Rio Grande silvery minnow (Figure 4).

While female length was a relatively poor predictor of the number of eggs spawned, there was a strong positive correlation (r^2=0.70) in the relationship between change in female body mass and eggs laid (Figure 5). The 69 female Rio Grande silvery minnow used in that portion of the study indicated that about 1,700 eggs are spawned per 1 gram of loss in female body mass. Age II females produced 40% of the eggs while comprising only 24% of the sample. In addition, Age II fish exhibited greater change (reduction) in body mass than Age I females.

Even when examined by age class, there was a strong positive correlation between loss in female body mass and the number of eggs spawned (Figure 6). Only 10 of the Age I females lost more than 1 gram of body mass but within that group were the five largest spawns (2,169-3,007) by Age I Rio Grande silvery minnow. The majority (n=37; 71%) of Age I fish recorded a loss of between 0.4-0.9 gms of body mass and accounted for 63% (n=43,380) of the eggs produced by Age I fish. Regression analysis from these 53 female Rio Grande silvery minnow suggest about 1,750 eggs were produced per 1 gram of body mass lost (Figure 6).

Mean body mass loss in Age II silvery minnow (1.79 gms) was over twice that observed in Age I fish (0.73 gms). The two Age II fish with the smallest change in body mass (-0.81 gms) were still larger than the mean change in Age I silvery minnow. The three Age II fish with 2.9-3.2 gm decrease in body mass exhibited significant variation in the number of eggs laid (2,788-5,303). Similar high variation in body mass loss-egg production were observed throughout this age cohort. This phenomena was reflected by the relatively low r^2 value (0.367) for Age II silvery minnow. The regression model for Age II Rio Grande silvery minnow predicts about 2,400 eggs per gram of body mass lost.

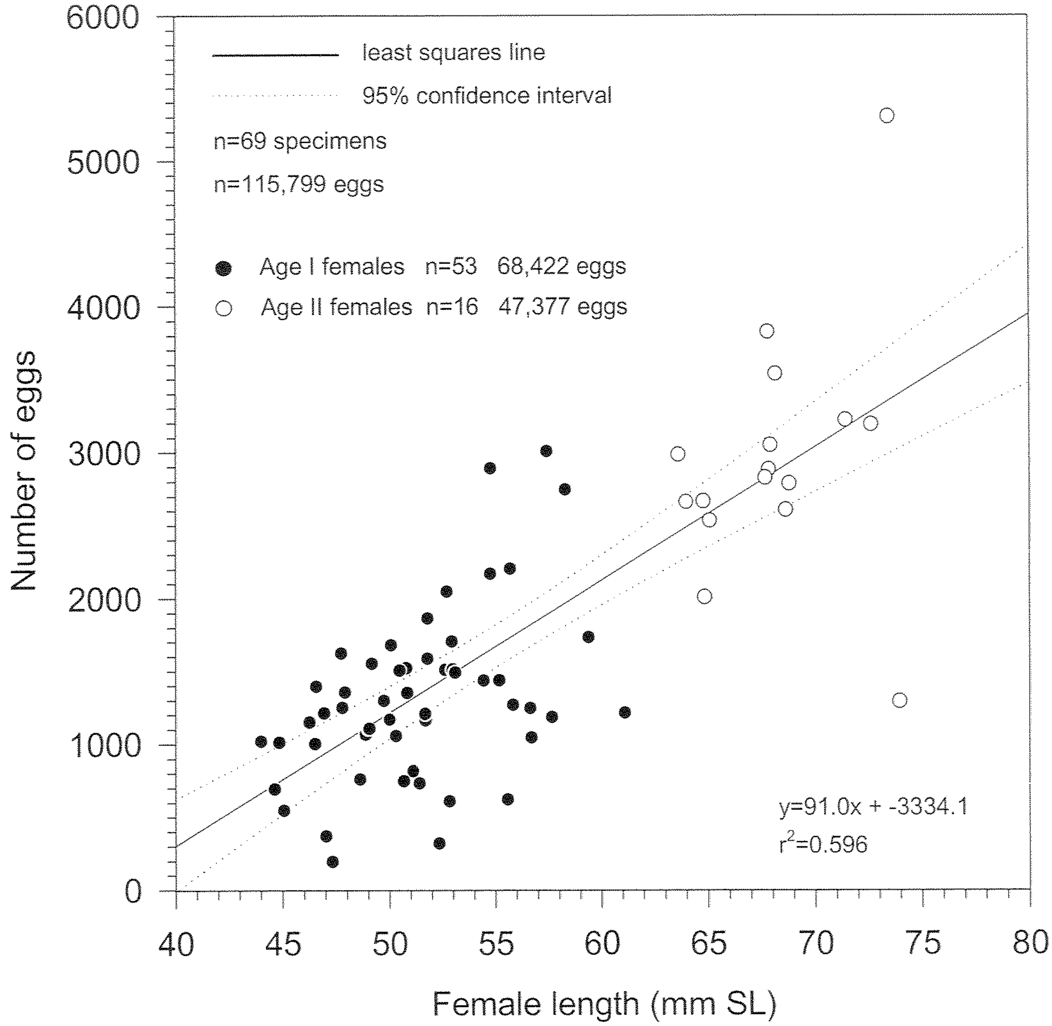


Figure 3. Correlations between female length and number of eggs spawned.

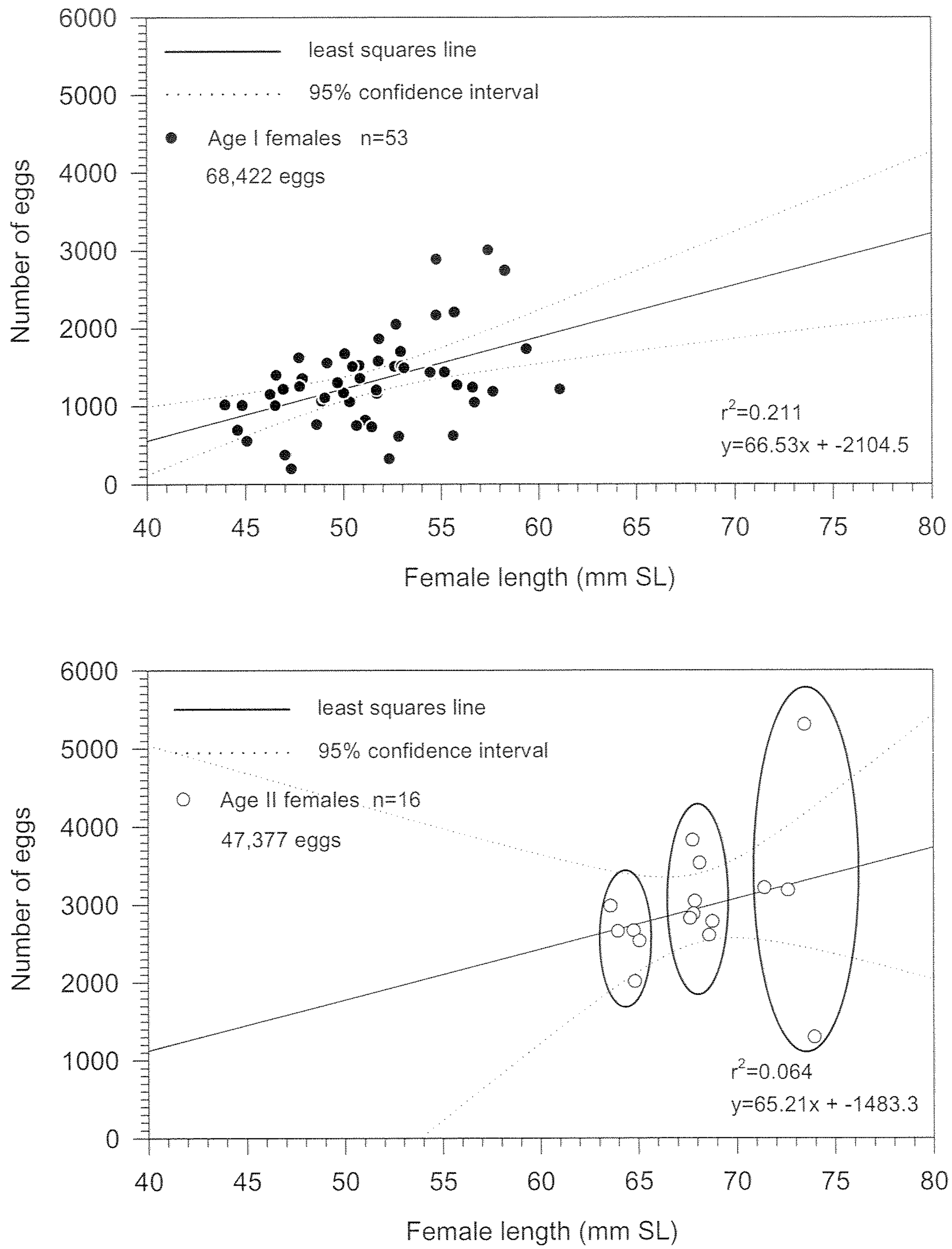


Figure 4. Comparison of female length versus number of eggs spawned by age class.

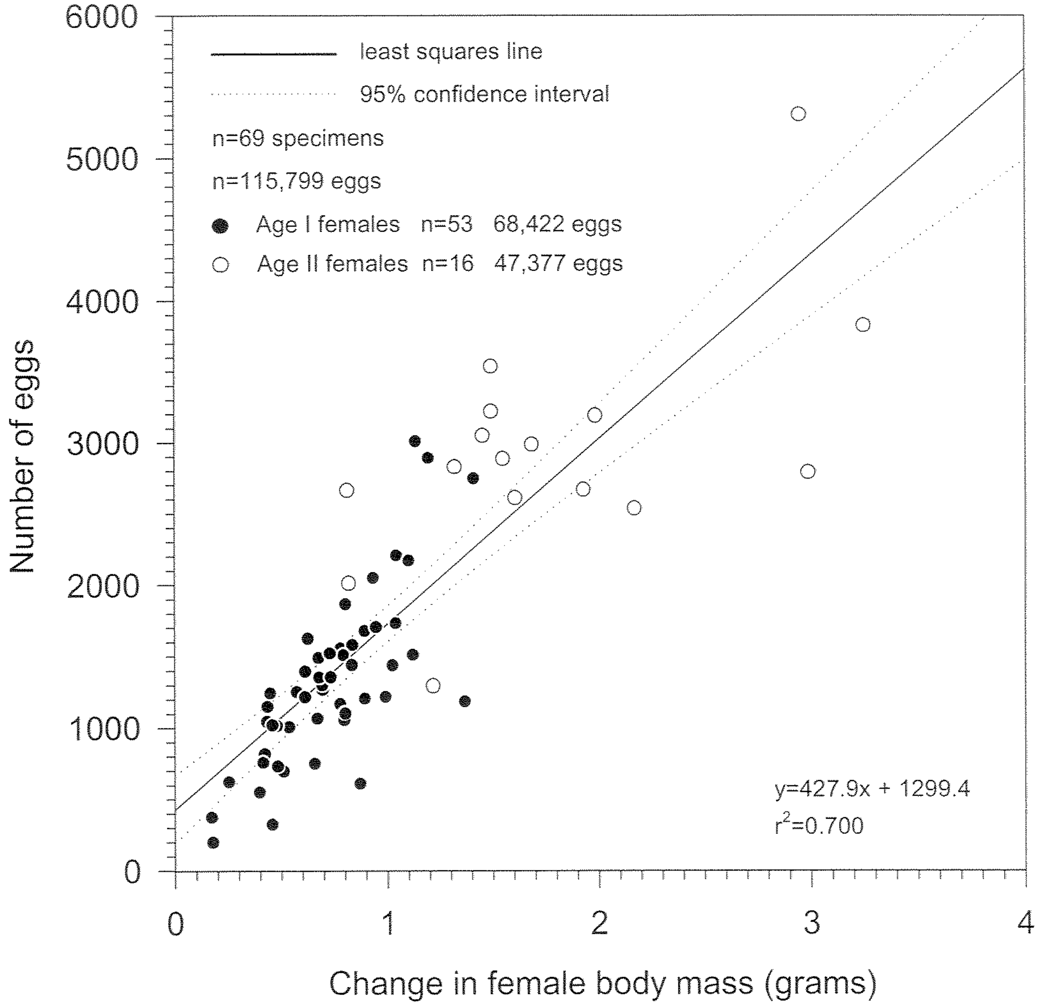
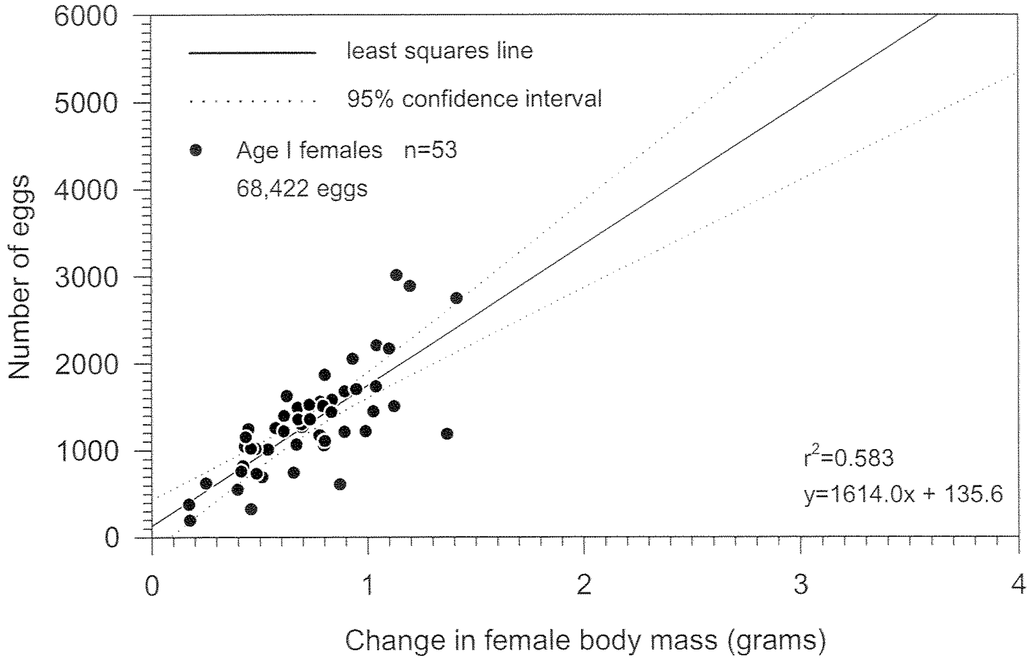


Figure 5. Relationship between change in female Rio Grande silvery minnow body mass and number of eggs spawned.



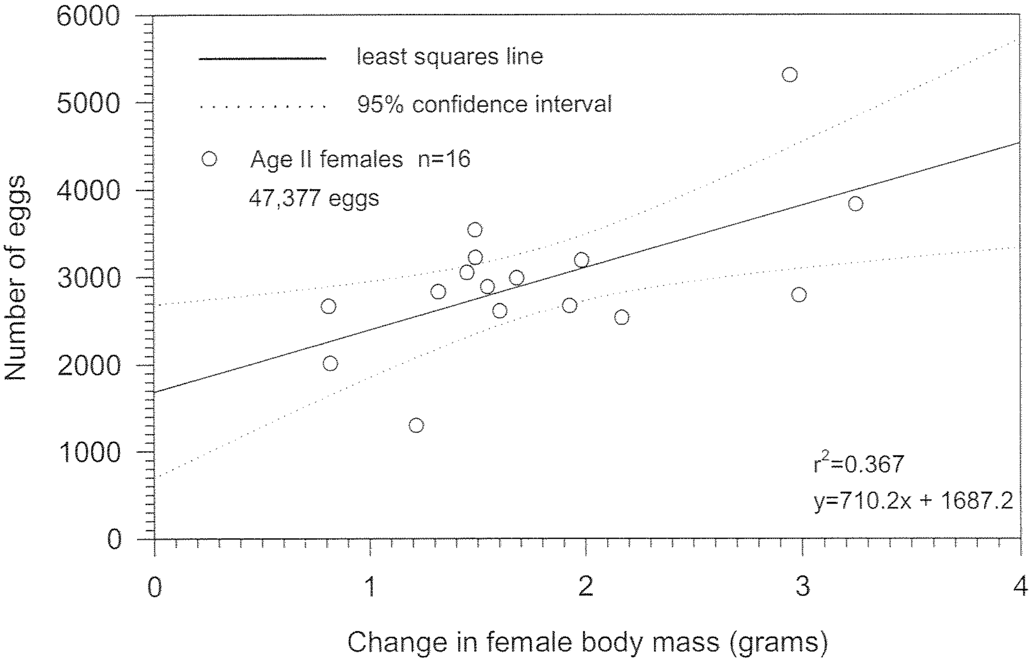


Figure 6. Comparison between change in female Rio Grande silvery minnow body mass and number of eggs spawned based on age class.

As with the differences between age classes, there were significant differences in the correlation between body mass loss and the number of eggs laid over the different trials (Figure 7). In general, the first four trial (=first two dates) had, individually and cumulatively, the lowest correlative scores. The general trend was for increasing strength of this correlation in later trials (and dates). The last two trials, while only comprised of seven females, produced 10,784 eggs and a correlation coefficient of 0.990. The overall correlation coefficient of the last five trials (n=25 clutches) was very strong (r^2=0.92) especially compared to that obtained (r^2=0.64) for the first four trials (n=43 clutches). Estimates of mean eggs per gram of body mass were about 25% higher in the final five runs as compared to the first four (Figure 8).

Multiple spawning events

We were able to enumerate the number of spawning events of nine females. We also attempted direct and indirect observation of spawning on four additional females but they did not spawn. Three Age II and six Age I female Rio Grande silvery minnow were used in this portion of the study. These individuals were 44.0-59.4 mm SL, 1.70-4.42 grams (Age I) and 67.7-74.0 mm SL, 5.90-9.11 gms (Age II). Two fish were observed in trial three, one in trial six, and the remainder in trials eight and nine (Table 1).

A total of 72 separate spawning events were observed. All nine of these individuals were observed in multiple spawning events with three being the fewest and 18 the maximum. Two of the three Age II females produced 10 clutches each with the largest female participating in 18 spawning events. The mean number of spawning events for Age II fish was 12.7.

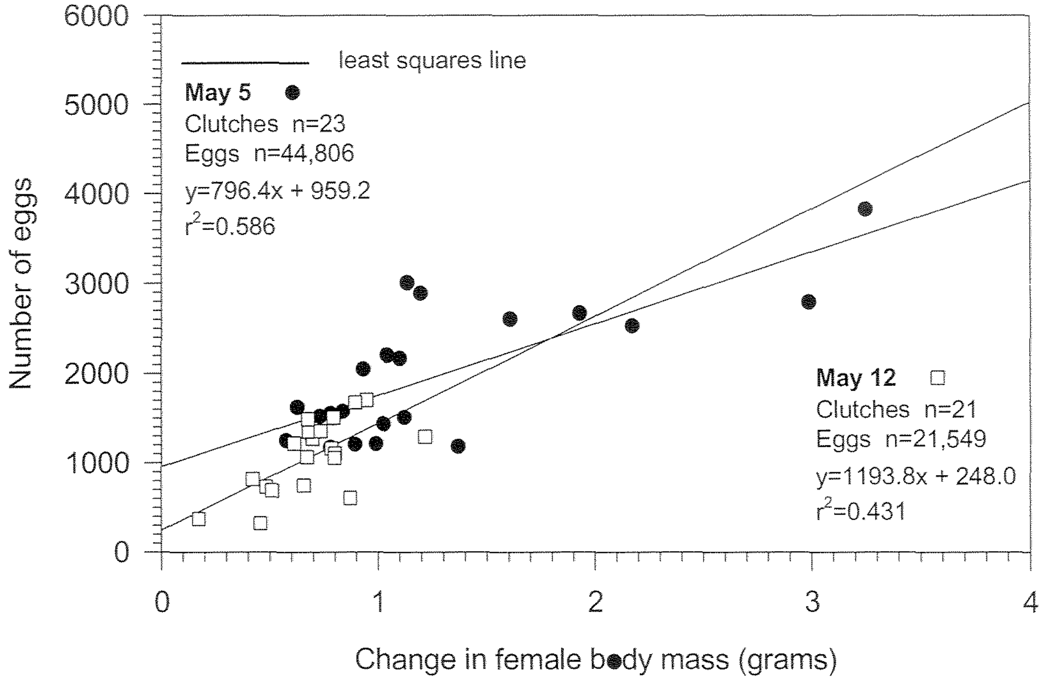
The six Age I female Rio Grande silvery minnow spawned a total of 34 times ($\bar{x}$=5.7) and ranged between 3-9. Three of the four Age I fish that were observed during trial 8 spawned five times with the fourth individual producing seven clutches. The two largest Age I females (55.8, 59.4 mm SL) were responsible for the minimum and maximum number of spawning events in this age class. Numerous false spawns (male envelops the female but no eggs are released) were also observed in members of both age classes. These false spawns occurred most frequently prior to the first actual spawning event.

There was considerable variation in the timing between spawning events. In cases where more than five clutches were produced, there was typically 15-30 minutes between spawning events. On one occasion, a single female spawned five times in 10 minutes. This same individual also recorded a maximum time of 90 minutes between clutches.

Number of eggs per batch

We were able to obtain 53 batches of eggs from seven female Rio Grande silvery minnow. These batches contained 14,161 eggs with the number per clutch ranging from 621-5,303. The two largest clutches were produced by the two Age II females and accounted for 62% of the eggs in this portion of the study. There were a cumulative 18 batches of eggs laid by these two individuals. The minimum number of eggs per batch for both Age II fish were 62 while 816 and 975 eggs were the maximum number produced by these two fish in a single batch.

There was considerable variation in the number of eggs per batch produced by Age I Rio Grande silvery minnow (Figure 9). Four of the five individuals had at least one batch of 44 or fewer eggs and all produced at least one batch with >260 eggs. The mean number of eggs per batch for Age I fish was 213 and ranged from 108-591 per specimen. The largest Age I female Rio Grande silvery minnow produced the largest clutch (n=1,773), batch (n=1,120), and highest average number of eggs per batch (n=591).



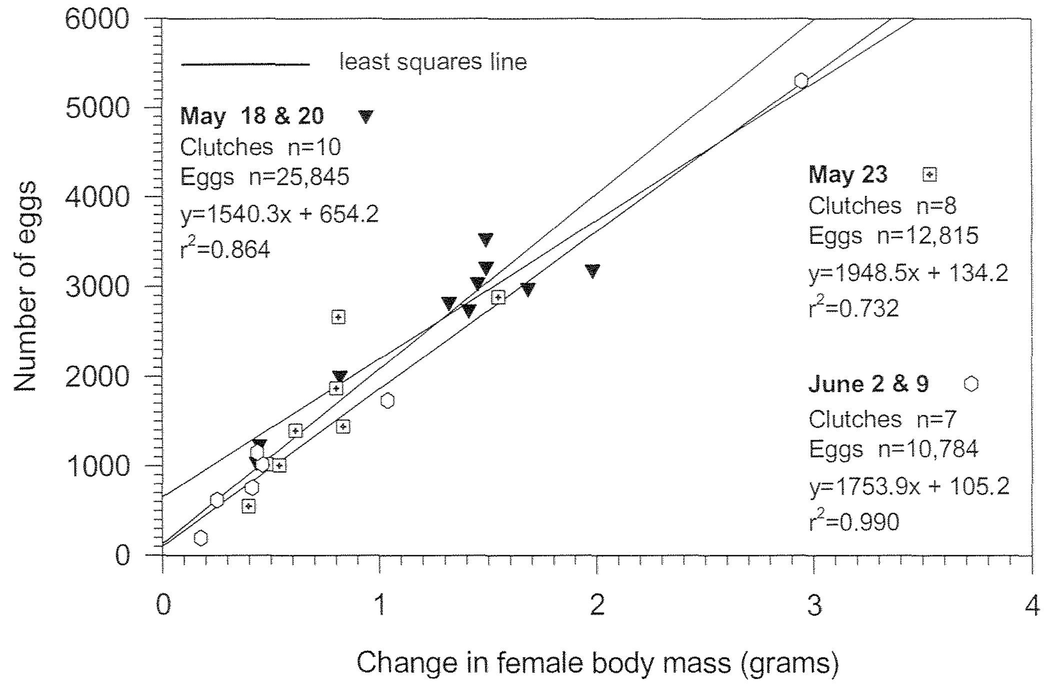
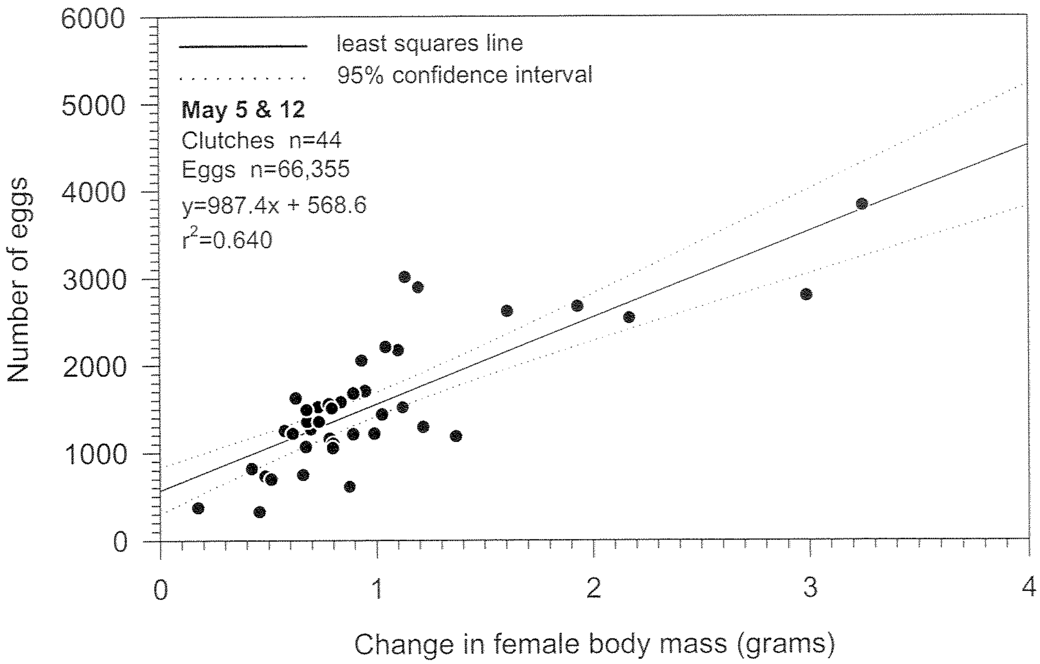


Figure 7. Correlation between change in female Rio Grande silvery minnow body mass and number of eggs spawned by individual dates.



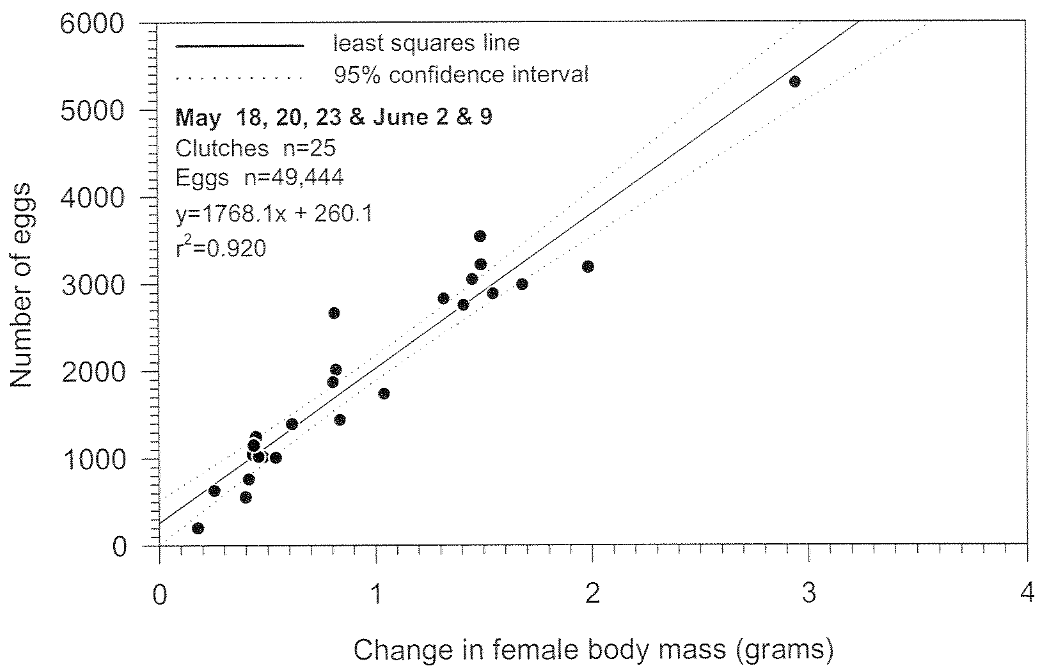


Figure 8. Relationship between change in female Rio Grande silvery minnow body mass and number of eggs spawned by combined trials.

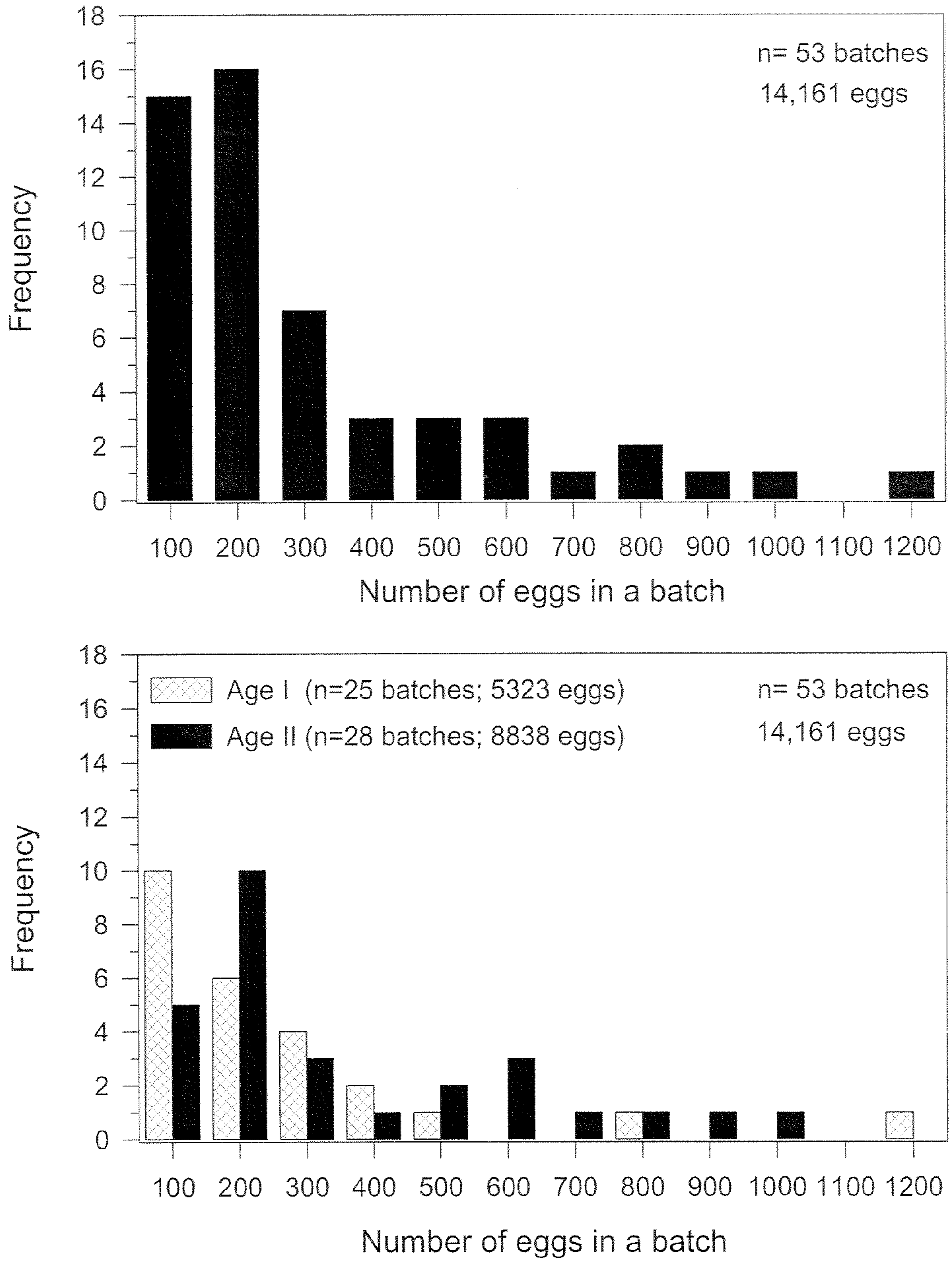


Figure 9. Frequency distribution of the number of Rio Grande silvery minnow eggs produced per batch.

Over 58% (n=31) of all egg batches contained 200 or fewer eggs. There was a gradual decline in frequency of larger batches of eggs with only three batches (5.6%) containing >801 eggs (Figure 9). Examination of the batch production by age class demonstrated a trend in Age I fish of decreasing frequency with increasing batch size. About 92% of the egg-batches of Age I fish contained <501 eggs. Age II Rio Grande silvery minnow demonstrated a trend similar to that of Age I fish except that the greatest number of Age II batches contained between 101-200 eggs. About 32% of Age II batches contained >401 eggs compared with 12% for Age I fish.

Discussion

This investigation augmented previous field and laboratory studies on the reproductive behavior of Rio Grande silvery minnow. The data from this research program collaborated several of our previous hypotheses and, when integrated with field studies, provided a better overall understanding of the life history and ecology of this and other plains stream fishes. The information from these studies should provide a better understanding of the relationships of flow and water management practices on this species.

Our previous laboratory research documented that Rio Grande silvery minnow is a pelagic spawner producing semi-buoyant eggs that developed in 24-48 hours. The current investigation revealed that reproduction in Rio Grande silvery minnow consisted of multiple spawning events with between 100-200 eggs expelled per spawn and the fecundity of an Age II female being over 5,000 eggs. The duration between spawning events and high level of spawning achieved by some individuals, especially during later trials, suggested that this species had the capability of releasing all eggs within a relatively short time frame. This ability, in combination with this species apparent schooling behavior, suggested a high probability for synchronous spawning within populations in selected reaches of the Rio Grande.

We suspected that environmental cues such as increasing flow and temperature incited female Rio Grande silvery minnow to cycle eggs into the last stage of development (ripe egg). The duration between initial stimulation and maturation of oocytes to ripe eggs appears to be about 6-8 hours. This time period is based primarily on our laboratory observations of the time between hormone injection and spawning. We believe that when favorable environmental stimuli persisted, the developmental cycling of eggs ultimately reached a level whereby, under natural conditions, spawning resulted in the release of virtually all eggs. This event may occur over one or several days dependant upon prevailing environmental conditions. It is during this period that the majority of all spawning by individuals and Rio Grande silvery minnow population is presumed to occur. This does not preclude the possibility of protracted spawning by a selected few individuals.

These assumptions were collaborated by collections of larval Rio Grande silvery minnow during a concurrent field study. Length-frequency histograms of larval Rio Grande silvery minnow did not depict the presence of several different age cohorts. Such cohorts, if present, would be readily identifiable due to the rapid larval growth of this species. Additional collaboration of synchronous spawning was provided by the virtual disappearance of such a large proportion of Age I and Age II Rio Grande silvery minnow after spawning (runoff). Protracted spawning would result in the post-runoff survival of a large number of female silvery minnow and those individuals would maintain elevate GSI levels. Neither of these two criteria have been observed in field collections of Rio Grande silvery minnow.

The data demonstrated a strong temporal difference in correlation between change in body mass and the number of eggs produced. The data generated from trials 8 and 9 yielded the highest correlation coefficient suggesting that those females needed very little stimulation to induce spawning. Conversely, trials 1 and 2, which were conducted one month earlier than 8 and 9, resulted in the least significant correlations (change in body mass-egg number). These low r^2 values appear to suggest that several of these individuals were not as physiologically ready to spawn as those fish in the later trials.

These differences may reflect the temporal variance in developmental stages of eggs. Less developed eggs, which were smaller in diameter and presumably occurred in earliest trials, would be more numerous per gram of body mass versus eggs in later stages of development. As such, there was greater variance in measures on fishes with more immature eggs as compared to those with more developed oocytes. Studies of egg developmental stages and diameter in Rio Grande silvery minnow throughout the breeding season might provide additional insight to these variances.

The lack of strong correlation between length and number of eggs produced seemed, initially, counter-intuitive. Fishery literature is replete with references of the positive length-fecundity correlations (Potts and Wooton, 1984). In such comparisons, fecundity is determined by counting the number of oocytes in the body cavity. In our investigation, we regressed the actual number of eggs spawned versus female length. The former value (number of eggs) did not and was not purported to represent the potential fecundity of the female. For that value to represent fecundity required that the female expel all eggs that would normally be laid during the spawning season. While that was not the norm, there were several individuals that appeared, based on post-spawning morphological examination, to have achieved high levels of spawning. Those individuals provided the most valid estimates, from this study, of silvery minnow potential fecundity.

Discharge in the Middle Rio Grande during the spawning period of Rio Grande silvery minnow (May-June) is primarily snowmelt runoff at which time peak annual velocity and volume are generally achieved. The current Middle Rio Grande release pattern does not necessarily represent a natural hydrograph as flow is modulated by dams and reservoirs operated under the covenant of the Rio Grande Compact. Management of spring runoff generally serves to reduce its magnitude (=flood control) and may concurrently extend its duration. Lotic water temperatures during spring runoff are also affected by flow manipulations. Natural spatial and temporal changes in Middle Rio Grande water temperatures may artificially increase or decrease depending on discharge. The manipulations of flow and temperature probably has a synergistic effect on Middle Rio Grande fishes and the impacts of these practices on the historic native fish fauna of the Middle Rio Grande may have been significant.

Examination of 1993-1995 field collected Rio Grande silvery minnow demonstrated increasing GSI values that generally corresponded with periods of spring runoff. The downstream gradient of increasing GSI values during this period also correlated with increasing water temperatures. These data provide field documentation of the relationship between discharge and temperature and spawning of Rio Grande silvery minnow.

Manipulations of flow through dams is frequently employed as a management tool whose purpose is to induce or increase success of spawning by rare fishes (e.g., Colorado River, Colorado squawfish and humpback chub). Selective control of flow during spring runoff in the Middle Rio Grande may result in an increase in population levels of Rio Grande silvery minnow. Any experimental manipulations of Middle Rio Grande spring discharge would require monitoring efforts designed to ascertain the affects of the modified flow on the reproductive biology of Rio Grande silvery minnow populations in each of the four reaches.

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