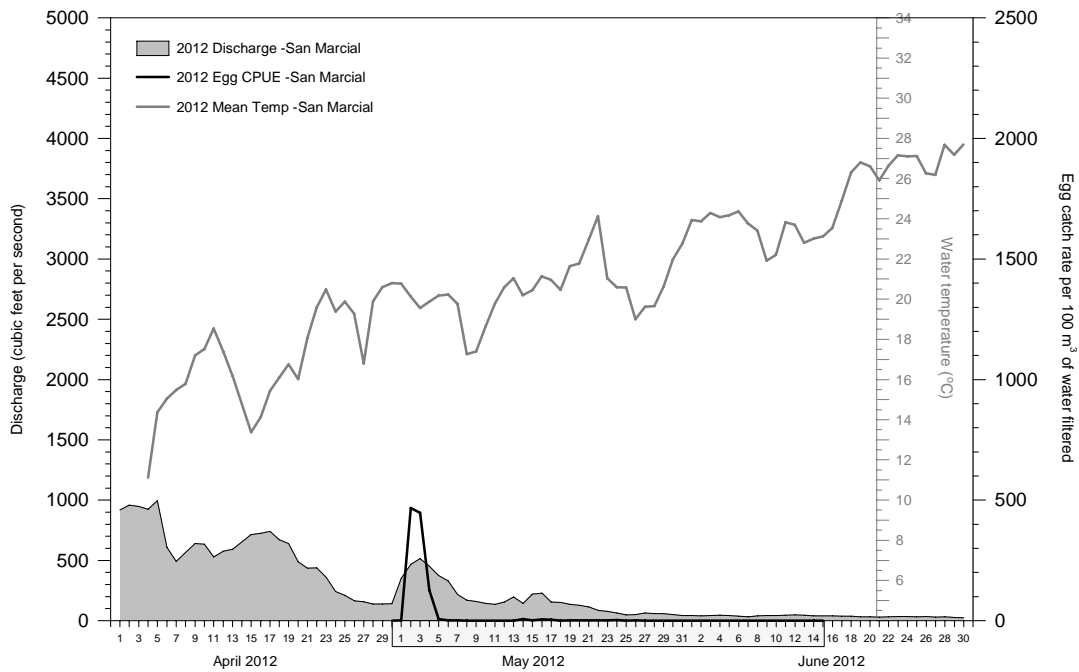


**SPAWNING PERIODICITY OF RIO GRANDE SILVERY MINNOW
DURING 2012**

**A MIDDLE RIO GRANDE ENDANGERED SPECIES
COLLABORATIVE PROGRAM FUNDED RESEARCH PROJECT**



Robert K. Dudley, Adam L. Barkalow, and Steven P. Platania
American Southwest Ichthyological Researchers, L.L.C.
800 Encino Place NE
Albuquerque, NM 87102-2606

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Upper Colorado Regional Office
125 South State Street, Room 6107
Salt Lake City, UT 84138-1102

prepared by:

Robert K. Dudley, Adam L. Barkalow, and Steven P. Platania
American Southwest Ichthyological Researchers, L.L.C.
800 Encino Place NE
Albuquerque, NM 87102-2606

submitted to:

U. S. Bureau of Reclamation
555 Broadway NE, Suite 100
Albuquerque, NM 87102-2352

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EXECUTIVE SUMMARY

Systematic monitoring of the reproductive output of Rio Grande silvery minnow at multiple sites in the Middle Rio Grande was first conducted in 1999 and has continued annually (except 2005) since 2001. Previous studies demonstrated May and June as the primary period of spawning activity. The 2012 study was structured to monitor the temporal reproductive output of Rio Grande silvery minnow in the downstream-most river reach (San Acacia), where the majority of the population currently persists.

Sampling at the San Marcial Site was conducted from 30 April through 15 June (47 days). The cumulative volume of water at the San Marcial Site was 45,719 m³. A total of 12,398 Rio Grande silvery minnow eggs was collected from 1 May to 26 May during 2012. Daily egg catch rates ranged between 0.17 and 466.71 eggs per 100 m³ of water sampled ($n = 1$ and $n = 6,691$ respectively). During the study, the overall egg catch rate at the San Marcial Site was 27.12 eggs per 100 m³ of water sampled. The number of eggs estimated to be transported downstream over the duration of the study was 12,471,939. The highest number of eggs estimated to be transported downstream in a single day was 5,619,124 (3 May 2012).

Analysis of reproductive output revealed a significant difference ($F = 8.70$; $p < 0.0001$) among mean values of catch rate (#/100 m³) over the period of record (2002 to 2004 and 2006 to 2012). The following pair-wise comparisons were significant ($p < 0.05$) over the period of record (2002 vs. 2006; 2006 vs. 2007; 2007 vs. 2010). The natural log-transformed mean egg catch rate (standardized for discharge) was highest in 2002 (5.86), followed by 2007 (4.77), 2011 (4.10), 2008 (3.22), 2003 (2.89), 2012 (2.79), 2009 (2.57), 2010 (2.24), 2006 (1.44), and 2004 (0.96).

A comparison between log-transformed egg catch rate (standardized for discharge) and the percentage increase in mean daily discharge two days prior to egg collection yielded a highly significant ($F = 38.17$; $p < 0.0001$) relationship. The significant San Marcial Site regression model ($y = 3.19 + (2.69 \times 10^{-2})(x)$) was derived from 157 observations and yielded a modest value for the coefficient of determination ($R^2 = 0.20$). Subsequent annual regression analyses of this relationship revealed elevated R^2 values (range = 0.35 to 0.65), with significant p -values, in years with lower flows (i.e., 2003, 2006, 2001, and 2012). In contrast, years with adequate observations ($N > 5$) and higher flows (i.e., 2007, 2009, and 2010) yielded very low R^2 values (range = 0.00 to 0.03) along with non-significant p -values.

While some spawning events occurred at cool water temperatures ($< 15^\circ\text{C}$), these only occurred during low flows. At warmer water temperatures (ca. 19 to 26°C), there was a gradual decline in the flow at which spawning was observed (i.e., generally coinciding with low June discharge). There was a mottled pattern of spawning intensity across the range of discharge-temperature combinations. The highest densities of eggs were collected at the San Marcial Site between about 19 to 23°C, coinciding with either low flows (ca. 200 to 1,000 cfs) or high flows (ca. 1,700 to 2,300 cfs).

These results suggest that spawning intensity may be related to some combination of discharge and water temperature conditions. Large increases in flow over a relatively short period (from two days to one day prior to egg collection) seemed particularly related to increased spawning activity in Rio Grande silvery minnow. This relationship was especially robust during years when flows in May and June were relatively low, which led to more dramatic increases in flow following spring runoff. Increased flow leads to a series of changes to the physical dynamics of the river (e.g., increased water velocities/depths in some areas and augmented or new "flooded" low velocity habitats in other areas) and changing water chemistry conditions (e.g., increased turbidity and salinity levels) that could be important spawning cues associated with rising flows. While increases in discharge appear to be the primary cue for spawning, water temperature seems to be an important factor as well. Spawning appears to occur over a wide range of mean daily water temperatures (ca. 13 to 26°C) but with the majority occurring over a narrower range of temperatures (ca. 17 to 23°C).

Population trends lend support to the observation that substantial numbers of eggs, and presumably larvae, are being transported downstream every year. In support of these observations, the highest densities of juvenile Rio Grande silvery minnow are most frequently found in the southern reaches of the Middle Rio Grande. The few exceptions to this trend (i.e., higher densities of juveniles found in upstream reaches) have almost always occurred following years when flows were very low in the San Acacia Reach, resulting in moderate to substantial river drying and loss of fish in that area.

Despite the seemingly large number of Rio Grande silvery minnow propagules transported downstream every year, some portion do remain upstream. It is likely that the proportion of individuals retained and successfully recruited upstream is related to the complexity of instream habitat conditions and the long-term availability of nursery habitat. Years with elevated and extended spring runoff conditions appear to create the favorable habitat conditions required for the successful recruitment of early life stages of Rio Grande silvery minnow.

The potential association between Rio Grande silvery minnow CPUE, from the Population Monitoring Program (see Dudley and Platania, 2012), and the spring spawning magnitude was examined over the appropriate period of record (2002-2004 and 2006-2012). However, both the July and October CPUE values for Rio Grande silvery minnow in the San Acacia Reach yielded non-significant relationships ($p > 0.6$) with the spring spawning magnitude as measured at the San Marcial Site. The number of eggs collected at a particular sampling site is likely a result of the complex interrelationships among spring runoff conditions (e.g., extended periods with elevated flows vs. low flows accompanied by river drying), the spatial distribution of upstream population concentrations, egg development time (i.e., faster at higher water temperatures), and local egg transport efficiencies (e.g., decreased habitat complexity may result in increased egg transport). As it is unlikely that eggs collected near the downstream terminus of the range of Rio Grande silvery minnow would correlate to population trends in upstream areas, additional upstream sampling sites that minimally include coverage in all three reaches would provide the needed resolution to adequately investigate these potential relationships. Additional years of data will hopefully further elucidate these subtle relationships and lend insight to the causal mechanisms resulting in the successful recruitment of Rio Grande silvery minnow.

The timing, magnitude, and duration of spring flows all seem to be influencing the patterns of spawning periodicity of Rio Grande silvery minnow. Early spring flows (e.g., late March/early April) don't appear to result in successful spawning/recruitment of Rio Grande silvery minnow (based on subsequent collections of larval fish in population monitoring efforts) but spawning appears to peak during May in most years. While a single magnified spawning event often occurs during low flow years, spawning appears to be more protracted during years with higher and extended flows. These later conditions also consistently result in higher October CPUE values, indicating that a more proactive management of spring flows could be essential to successfully recovering this species.

Increased flows in May apparently resulted in an elevated spawning effort during the 2012 study period. Rio Grande silvery minnow appear to have had a modest year for spawning but it is possible that poor recruitment in 2012 (apparently as a result of a truncated spring runoff followed by persistently low summer flows) could translate into decreased numbers of reproductively capable females available to spawn in the spring of 2013. Populations of Rio Grande silvery minnow appear to have declined since 2009 and the lack of an adequately high spring runoff (high magnitude over an extended duration) combined with summer drying in 2012 could result in further population declines. The loss of individuals from downstream reaches during river drying events is particularly problematic as these are the areas that most frequently and consistently support the highest densities of Rio Grande silvery minnow. The future conservation status of Rio Grande silvery minnow appears dependent on ensuring appropriate flow and habitat conditions during the spawning and early recruitment phases of this species, while also allowing upstream recolonization by individuals transported downstream.

INTRODUCTION

The reach of the Rio Grande between Cochiti Dam and Elephant Butte Reservoir (Middle Rio Grande) has been greatly modified over the last 50 years; this has alternatively led to aggradation, degradation, armoring, and narrowing of the river channel in different portions of the reach (Lagasse, 1985). This section of the river flows through the massive Rio Grande rift and historically resulted in a wide floodplain within the sparsely vegetated Rio Grande valley. Extensive braiding of the river through the relatively linear Rio Grande rift valley was common as it flowed over shifting sand and alluvium substrata; flow in the Middle Rio Grande was generally perennial except during times of severe or extended drought (Scurlock, 1998).

The Middle Rio Grande was relatively shallow throughout most of the year because of regionally low precipitation levels (Gold and Dennis, 1985) but was subjected to periods of high discharge. Flows were generally highest during the annual spring snow melt runoff (April-June). However, intense localized rainstorms (monsoonal events that generally occur in July and August) often caused severe flooding and were important in maintaining perennial flow throughout the summer. The cyclic pattern of drought and flooding over mobile substrata likely helped to promote the active interaction between the river and its floodplain. Historically, the Middle Rio Grande in many ways possessed all of the characteristics distinctive of a dynamic semi-arid river ecosystem.

The reduced species diversity typical of semi-arid ecosystems was also reflected in the depauperate ichthyofaunal composition of the Middle Rio Grande. Despite the reduced overall species richness of the Rio Grande, the river supported numerous native cyprinids that were endemic to this drainage (Platania and Altenbach, 1998). However, many of the endemic pelagic-spawning cyprinids that historically occupied the Rio Grande basin have been extirpated (speckled chub, *Macrhybopsis aestivalis*, Rio Grande shiner, *Notropis jemezianus*, and Rio Grande bluntnose shiner, *Notropis simus simus*) or have become extinct (phantom shiner, *Notropis orca*) over the past century (Bestgen and Platania, 1990). Rio Grande silvery minnow, *Hybognathus amarus*, is the only extant pelagic-spawning cyprinid in the Middle Rio Grande (Bestgen and Platania, 1991; Platania, 1991).

This group of pelagic-spawning cyprinids shared several life-history characteristics. All were small (generally <90 mm SL), short-lived (ca. 2-5 years), fishes that occupied mainstem habitats. In addition to these shared traits, all five species were members of a reproductive guild of fishes that spawn semibuoyant eggs (Platania and Altenbach, 1998). Reproduction in this guild of fishes is characterized by the production of non-adhesive eggs that, upon expulsion from the female, swell rapidly with water and become nearly neutrally buoyant. Spawning is generally associated with increases in discharge, such as spring run-off or summer rainstorms. The eggs are about 1.6 mm in diameter shortly after spawning but quickly expand (ca. 3.0 mm) and are passively transported downstream during development. Egg hatching time is temperature dependent, but usually occurs in 24-48 hours (Platania, 2000). Recently hatched larval fish are potentially subject to additional passive downstream transport for several days (ca. 3 to 5 days) until development of the gas bladder. This physiological development corresponds with a shift in swimming behavior as larvae are able to actively avoid higher velocity habitats to some extent.

The 4-7 days necessary for propagules to attain the developmental stage necessary to control their horizontal movements allows for potentially considerable downstream transport of eggs and larvae in the Middle Rio Grande. As has been well documented for other aquatic organisms, it is necessary for at least some portion of the drifting propagules to settle in appropriate nearby low-velocity habitats or move upstream to maintain viable populations (Speirs and Gurney, 2001). Downstream transport distance of the progeny of Rio Grande silvery minnow is dependent on a variety of factors including flow magnitude and duration, water temperature, and channel morphology (Dudley and Platania, 2007). Historically, there were no permanent barriers to upstream dispersal of fishes in the Middle Rio Grande. There are currently three instream diversion structures between

Cochiti Dam and Elephant Butte Reservoir that act as barriers to upstream movement of fishes and fragment the once continuous range of the Rio Grande silvery minnow.

Population monitoring efforts over the past decade have documented vast changes (i.e., order of magnitude increases and decreases) in the abundance of Rio Grande silvery minnow within the fragmented reaches of the Middle Rio Grande (Dudley and Platania, 2012). Recent monitoring efforts (Dudley and Platania, 2012) show that the October density of Rio Grande silvery minnow was significantly lower ($p < 0.05$) in 2011 than in recent years (e.g., 2007, 2008, and 2009) but not significantly higher ($p > 0.05$) than in 2002 and 2003 (years with the lowest densities ever recorded). In October 2011, the San Acacia Reach yielded the highest density of Rio Grande silvery minnow and the lowest density of this species was observed in the Angostura Reach.

Systematic monitoring of the reproductive output of Rio Grande silvery minnow at several sites in the Middle Rio Grande was first conducted in 1999 (Platania and Dudley, 2000). The 1999 monitoring effort involved collecting and quantifying catch rate of Rio Grande silvery minnow eggs at several Middle Rio Grande sites during the relatively short spawning period of this species. Limited Rio Grande silvery minnow egg collecting efforts were also conducted at selected sites in the Middle Rio Grande (Platania and Hoagstrom, 1996) and in the Low Flow Conveyance Channel (Smith, 1998, 1999) from 1996 to 1999. These latter samples provide information on the magnitude of reproduction during certain times and for specific sites. However, consistent monitoring throughout the spawning season produces the most reliable measure of the duration and magnitude of Rio Grande silvery minnow reproductive output. The first site-specific sampling effort to document the magnitude of the reproductive effort of Rio Grande silvery minnow occurred daily throughout May and June 2001 (Platania and Dudley, 2002) at a location near the southern end of the San Acacia Reach of the Middle Rio Grande (River Mile 58.8). Monitoring of the reproductive effort of Rio Grande silvery minnow also occurred daily at this site in May and June 2002 (Platania and Dudley, 2003), 2003 (Platania and Dudley, 2004), and 2004 (Platania and Dudley, 2005). More intensive monitoring efforts were conducted from 2006 to 2008 (Platania and Dudley, 2006, 2007, 2008) and resulted in the sampling of the Angostura, Isleta, and San Acacia reaches of the Middle Rio Grande. While monitoring of the Rio Grande silvery minnow reproductive effort occurred annually in the Isleta and San Acacia reaches from 2009-2011, only the San Acacia Reach was monitored in 2012.

The spatial spawning periodicity study conducted herein is a continuation of the long-term (2001-2004 and 2006-2012) systematic Rio Grande silvery minnow reproductive monitoring research activity. The primary objective of this study is to provide data that will enable characterization of the timing, duration, and magnitude of Rio Grande silvery minnow reproduction in the San Acacia Reach of the Middle Rio Grande. Additional objectives include assessing differences in Rio Grande silvery minnow spawning magnitude among sampling years; examining the relationships among discharge, water temperature, and spawning magnitude; and determining the potential association between spawning magnitude and Rio Grande silvery minnow CPUE from the Population Monitoring Program. Long-term monitoring of the reproductive effort of Rio Grande silvery minnow provides insight to potential factors affecting annual reproductive output, remains necessary for ongoing recovery efforts, and helps facilitate effective management decisions in the Middle Rio Grande.

Institutional background and considerations

Monitoring the reproductive effort of Rio Grande silvery minnow was identified as a requirement of the 29 June 2001 Programmatic Biological Opinion of the Effects of Actions Associated with the U. S. Bureau of Reclamation's, U. S. Army Corps of Engineers', and Non-Federal Entities Discretionary Actions related to Water Management on the Middle Rio Grande, New Mexico as authored by the U. S. Fish and Wildlife Service. This work was part of an ongoing effort to document changes in the distribution and abundance of the federally endangered Rio Grande silvery

minnow. This research effort provided an assessment of the reproductive output (eggs) for Rio Grande silvery minnow within the Middle Rio Grande and specifically addressed the task: "Evaluate the status and trend of the Rio Grande silvery minnow" as identified by the MRGESACP.

The Rio Grande silvery minnow Recovery Plan (U. S. Fish and Wildlife Service, 2010) also outlined a key research objective (1.1.1 Improve understanding of the relationship between environmental factors and spawning by the Rio Grande silvery minnow) that was addressed through this research. This investigation provided an assessment of the relative magnitude of Rio Grande silvery minnow spawning effort. This project was also a central component of the Rio Grande silvery minnow propagation and genetics research efforts, both requirements of the 29 June 2001 Programmatic Biological Opinion (see "Project Objectives" 2 and 3).

In 2002-2003, MRGESACP members met and discussed Rio Grande low flow issues and impacts of the hydrological conditions on Rio Grande silvery minnow. The dismal 2002-2003 snow pack in the Rio Grande headwaters meant there would not be a natural spring flow spike in 2003 in the Middle Rio Grande. Personnel from the MRGESACP decided to create an artificial flow spike during mid-May 2002, using reservoir storage, to initiate a spawn by Rio Grande silvery minnow. As 2003 climatic conditions were similar to those experienced in 2002, an artificial flow spike was also created in the Rio Grande in 2003. Snow pack runoff and ambient flow conditions in 2004 were sufficient enough that, for the first time in two years, an artificial flow spike was not deemed necessary. However, the timing and magnitude of flows have been modified to some degree in most years since 2004 with the intent of inundating within-channel and floodplain habitats to the maximum extent possible.

STUDY AREA

The principal area of interest in the Middle Rio Grande is the reach between the outflow of Cochiti Reservoir and inflow to Elephant Butte Reservoir; this area encompasses the known range of Rio Grande silvery minnow in the Middle Rio Grande (Figure 1). Five upstream reservoirs and numerous irrigation diversion dams regulate flow in the Middle Rio Grande. Cochiti Reservoir has been operational since 1973, is located 76 km upstream of Albuquerque, and is the primary flood control reservoir that regulates flow in the Middle Rio Grande. Reach names are taken from the diversion structure at the upstream boundary of that reach of river. In the Cochiti Reach (between Cochiti Dam and Angostura Diversion Dam), the Rio Grande flows through Cochiti, Santo Domingo, and San Felipe pueblos, respectively.

The reproductive effort of Rio Grande silvery minnow has, in the past, been sporadically determined at selected collecting localities in the Middle Rio Grande. From 2001 to 2004 and in 2012, sampling efforts were restricted to a single San Acacia Reach collection location. The San Acacia Reach of the Middle Rio Grande is about 56 miles (91 km) long, extending from the apron of San Acacia Diversion Dam to the head of Elephant Butte Reservoir. Sections of this reach are characterized by a wide and braided river channel, sand substrate, high suspended sediment load, and a broad variety of aquatic mesohabitats. Conversely, some segments in this reach are relatively narrow and result in increased water velocity and decreased habitat heterogeneity. The 12 mile (19 km) reach of the Rio Grande downstream of San Marcial Railroad bridge crossing is confined to a channel that is about 50 m wide. Substrate in this segment of the river is predominately sand and braiding of the channel is uncommon except under conditions of relatively low flow.

Given the downstream drift of the eggs, the original location of the collecting activities was selected so as to maximize the number of eggs collected and potentially rescue eggs destined to drift into Elephant Butte Reservoir where, if hatched, larvae would be subjected to a wide array of nonnative predators. This site was located near the downstream-most portion of the San Acacia

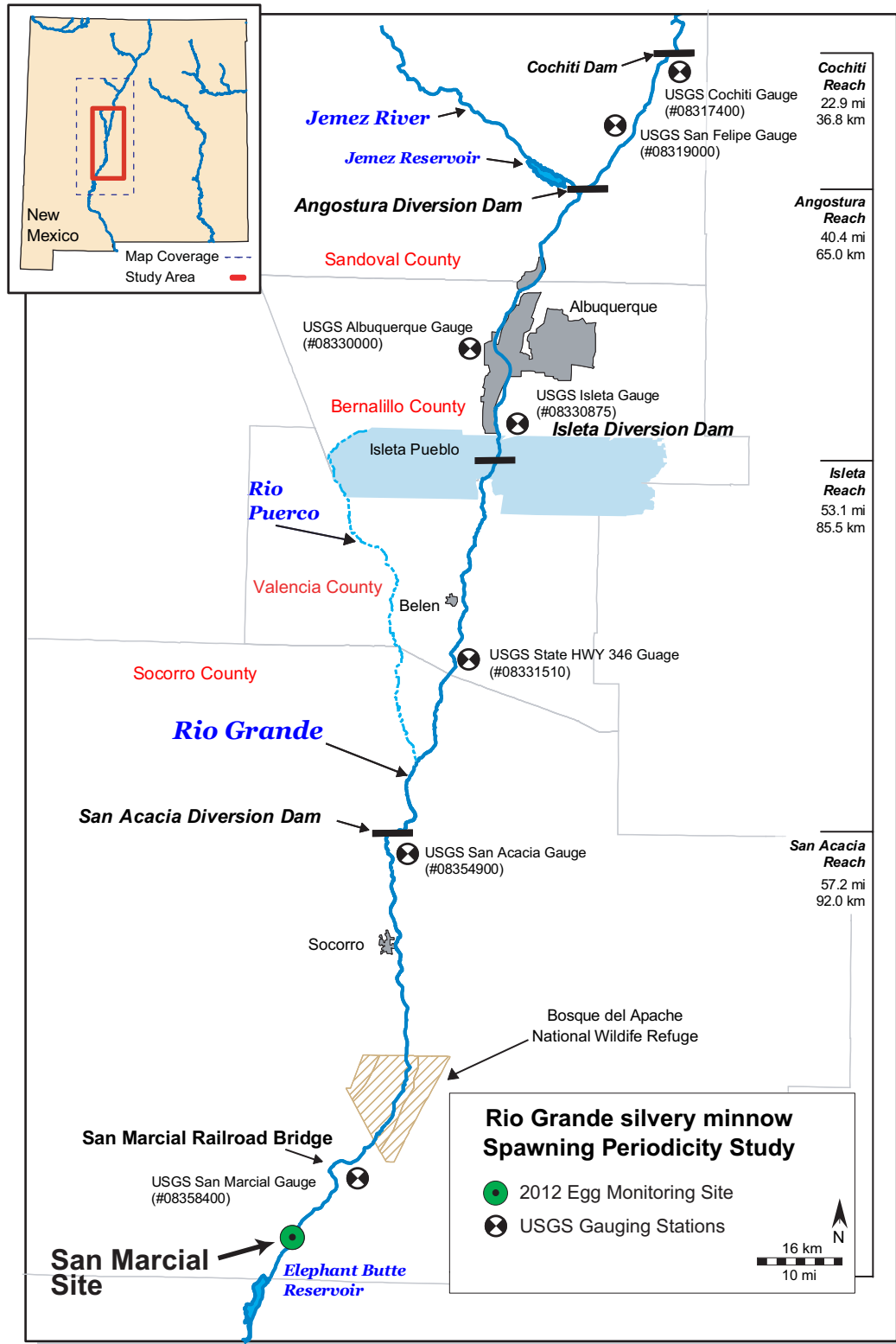


Figure 1. Map of the Middle Rio Grande, New Mexico, and the 2012 study site location.

Reach (River Mile 55.0). The sampling site is downstream of a U. S. Geological Survey stream gauging station located near San Marcial, New Mexico (# 08358400), which is the nearest upstream San Acacia Reach gauge. Discharge in the San Acacia Reach was highly variable during 2012 (Figure 2). In addition to easy accessibility and favorable river conditions (i.e., wide river channel, current being carried through a single river channel, gently sloped banks, moderate gradient), the only means of vehicle access to this site was gated and could be secured. This area has been sampled annually from 2001-2004 and from 2006-2012.

MATERIALS AND METHODS

The egg collecting device, developed specifically for the collection of large numbers of live and undamaged semibuoyant fish eggs (Moore Egg Collector; MEC), was the only sampling apparatus used in this project (Altenbach et al., 2000). Numerous modifications have been made to the collecting gear, since the original publication detailing the construction and operation of the MEC (Altenbach et al., 2000), that have resulted in increased effectiveness and efficiency of the MEC (i.e., greater catch rate per sampling period). Catch rate of Rio Grande silvery minnow eggs in the Middle Rio Grande was determined following the sampling protocol described in Altenbach et al. (2000). A mechanical flow-meter was attached to the MEC so that volume of water filtered could be calculated and catch rate per unit of water determined. The catch-per-unit-effort (CPUE) of drifting eggs was calculated as the total number of eggs collected \cdot volume of water sampled⁻¹ \cdot 100 (i.e., N [eggs] \cdot 100 m³ water⁻¹). The total number of eggs passing a sampling site in a 24 hour period was estimated by using egg sampling data from the site (over a 4 hour period) and flow data from the nearest upstream USGS gauging station (i.e., (number of eggs collected / (volume of water sampled / volume of water available)) \cdot 6).

Previous studies demonstrated May and June as the primary period of Rio Grande silvery minnow reproductive activity (Dudley and Platania, 2011). The normal sampling regime in 2012 was composed of an intensive four-hour daily sampling effort. Eggs were not staged (i.e., determining approximate time from spawning) as this would require substantial laboratory work outside of the current core objectives of this study. Staging eggs would give a general idea of drift time but extrapolating to drift distance would be subject to a series of simplifying assumptions. Also, determining drift distance is a complex modeling exercise of which eggs are only a component (i.e., eggs can be present in the drift for about one day but larvae can be present in the drift for about another three days post-hatching). Two MEC's were operated so as to increase the volume of water sampled per unit of time. Research personnel worked daily at the San Marcial Site from 30 April through 15 June 2012.

Volumetric determination of the number of Rio Grande silvery minnow eggs collected, as employed in 2001, lacked the rigor necessary for effective evaluation of the relative level of spawning by this species. Changes initiated in the 2002 sampling protocol were instituted to increase the amount and utility of the information acquired from this research activity. One result was that the sampling protocols incorporated in 2002 included direct counts of all eggs collected. The aforementioned differences in egg catch rate determination between 2001 and post-2001 studies preclude use of 2001 data for quantitative or statistical comparison with data from subsequent years. There have not been changes in the sampling methodology for quantitative determination of egg catch rates since 2002.

Rio Grande silvery minnow egg CPUE values are (in part) dependent on flow conditions thereby precluding unadjusted comparison of inter-annual catch rates (e.g., higher flow volume will result in lower CPUE since the number of eggs in the water column remains constant). Catch rate was standardized (CPUE_S) based on mean daily discharge (Q) to account for these differences,

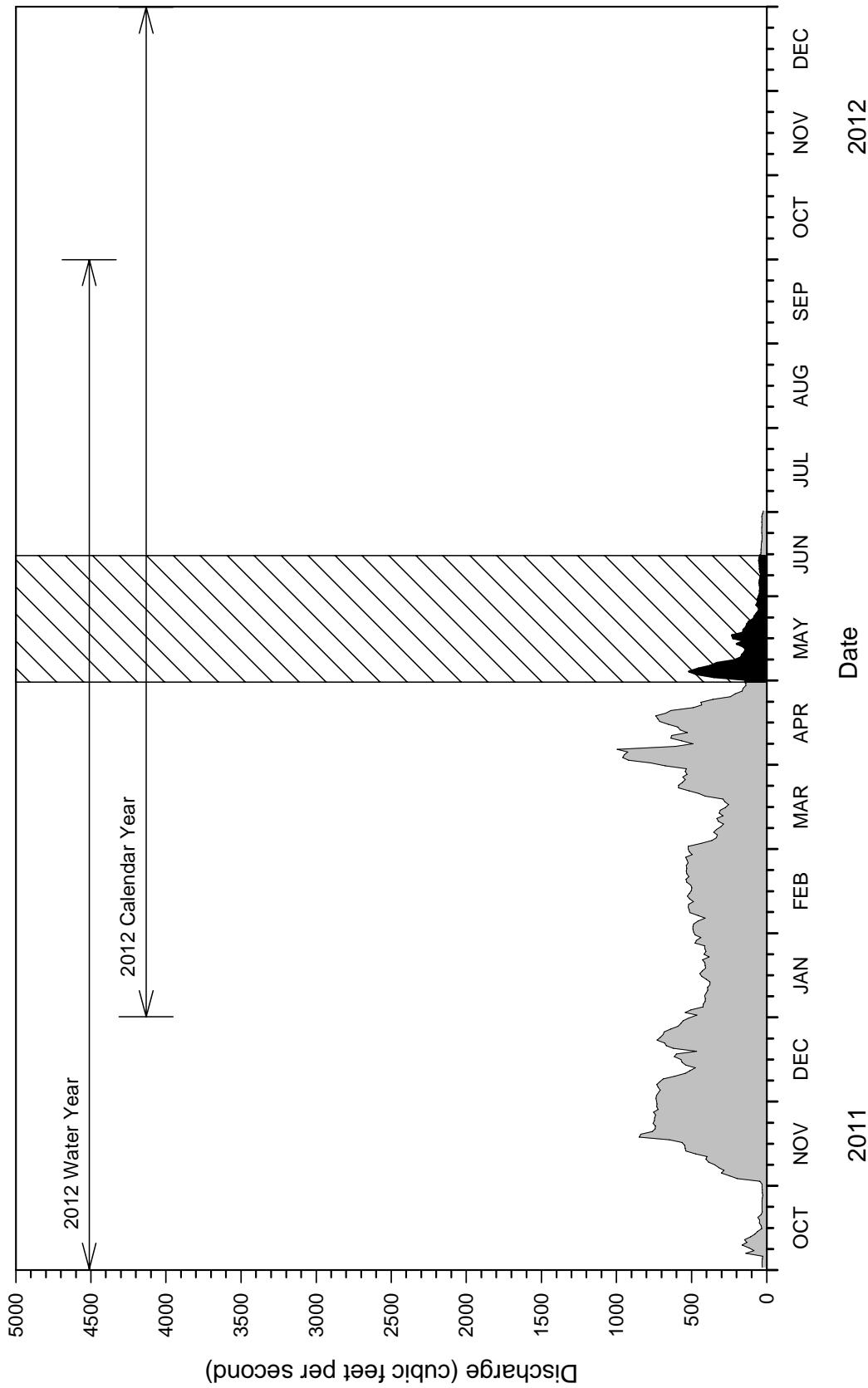


Figure 2. Hydrograph of the Rio Grande, New Mexico, at the San Marcial Gauging Station before, during, and after the 2012 study period. Cross-hatching indicates annual study period.

using the formula: $CPUE_S = CPUE \cdot Q$. All USGS discharge data presented graphically or analyzed statistically in this report are provisional and subject to change.

Assumptions of normality in annual Rio Grande silvery minnow egg catch rates were evaluated using the Shapiro-Wilk test. Critical values of W were calculated and significant differences were assessed using a goodness-of-fit procedure. The 2002-2004 and 2006-2012 egg catch rate time-series distributions were compared with a normal distribution using the Shapiro-Wilk test. To meet normality assumptions, all data were log-transformed ($X' = \ln(X + 1)$). Normal quantile plots of data were also examined for notable departures from Lilliefors's confidence bounds.

The log-transformed CPUE_S values were compared among years to determine general differences in spawning magnitude. Differences among independent samples were tested using ANOVA (or Welch's ANOVA when variances were heteroscedastic) to detect differences in values of CPUE_S among years. Differences among sample means were evaluated based on the critical value of F for the particular sample size. Multiple pair-wise comparisons were made using the Tukey-Kramer HSD procedure.

Linear regression modeling was used to determine the strength of the relationships among log-transformed CPUE_S values and percentage change in mean daily discharge (MD_{day}) at one day and two days prior to egg collection. For example, if eggs were collected on day 15, the one day formula would be $(MD_{15} - MD_{14}) / MD_{14} * 100$ and the two day formula would be $(MD_{14} - MD_{13}) / MD_{13} * 100$. Regression models were developed for the San Marcial Site using data from May and June over the period of record. A negative or positive trend in spawning effort was defined as occurring when the slope of the regression was significantly different ($p < 0.05$) from zero. A similar approach was employed to determine the relationship among log-transformed CPUE_S values and mean values of log-transformed July and October CPUE ($\#/100 \text{ m}^2$) of Rio Grande silvery minnow in the San Acacia Reach (Dudley and Platania, 2012).

A modified filtering screen to separate drifting debris from Rio Grande silvery minnow eggs was developed and tested for the MEC in 2009. The screen was designed to allow the passage of much of the very fine particulate debris while preventing the passage of drifting eggs. Experimental tests revealed that the modified screen was consistently more efficient at sampling a larger volume of water than was the old screen over the same time period (Platania and Dudley, 2009). All MEC's were fitted with the modified screen for sampling conducted in 2012.

Water temperature was recorded by a temperature logging device deployed close to the study site and programmed to record hourly water temperature. Hourly water temperature data from the primary temperature logger were presented in this report as mean, minimum, and maximum daily water temperatures. Data from past spawning periodicity studies were also included for comparative purposes.

RESULTS

Hydrology

Flows in the Middle Rio Grande have fluctuated dramatically among years since the beginning of this study in 2001 (Figures 3 to 7). A drought that enveloped the study region in 2000 was somewhat interrupted in 2004 by a moderate snow pack and a wetter than normal April. These precipitation events helped but did not replenish the already diminished water reserves in upstream reservoirs. Despite the presence of a more normal spring runoff in 2004, elevated flows persisted for only few weeks in May and had declined notably by the beginning of June. Snow pack runoff in 2005 was larger (greater magnitude and duration) than any of the previous four study years (egg sampling was not conducted in 2005). Conversely, flow in the Rio Grande during 2006 (prior to 27

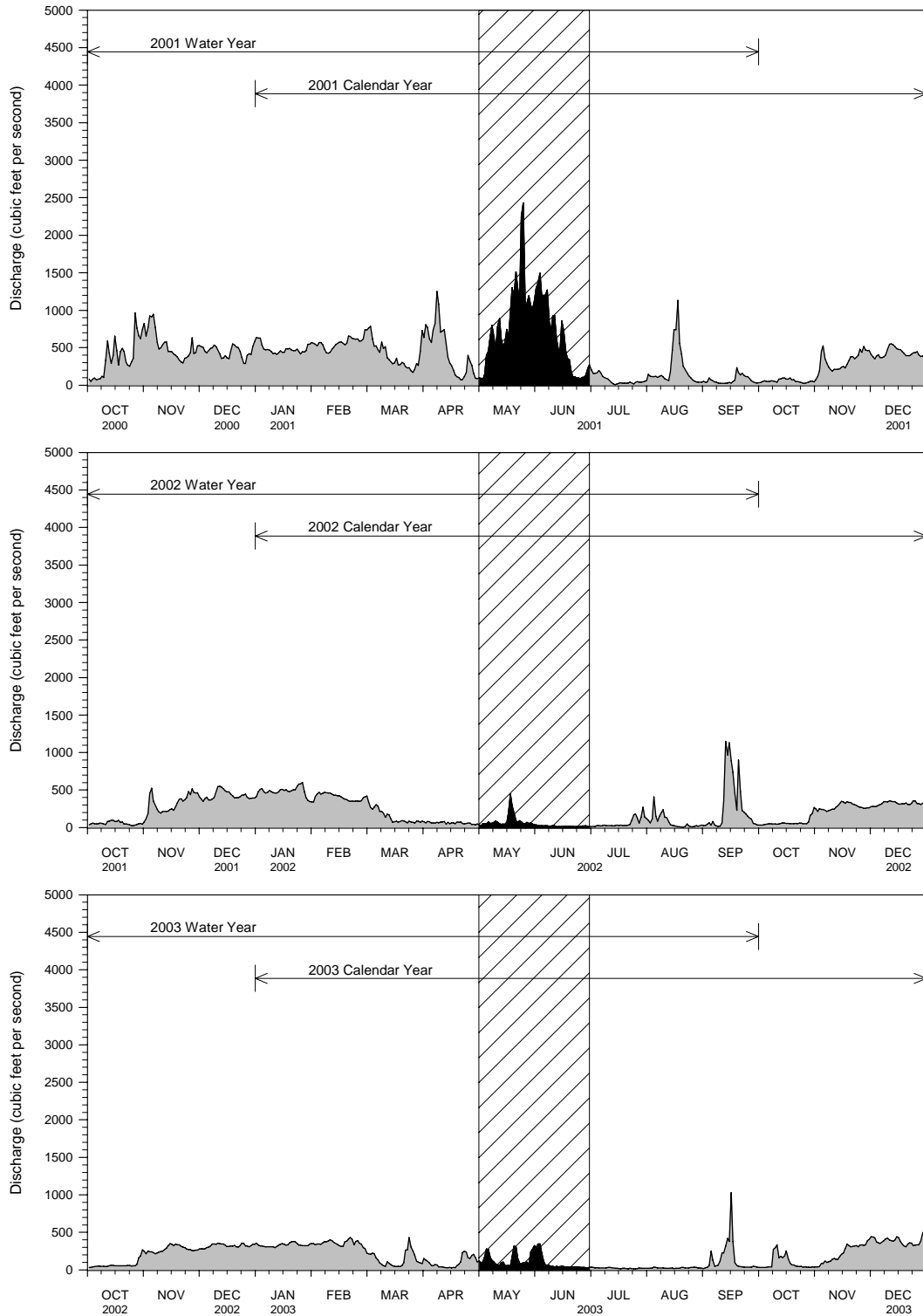


Figure 3. Annual hydrographs of the Rio Grande, New Mexico, at the San Marcial Gauging Station before, during, and after the 2001-2003 Rio Grande silvery minnow spawning periodicity study periods. Cross-hatching indicates annual study periods.

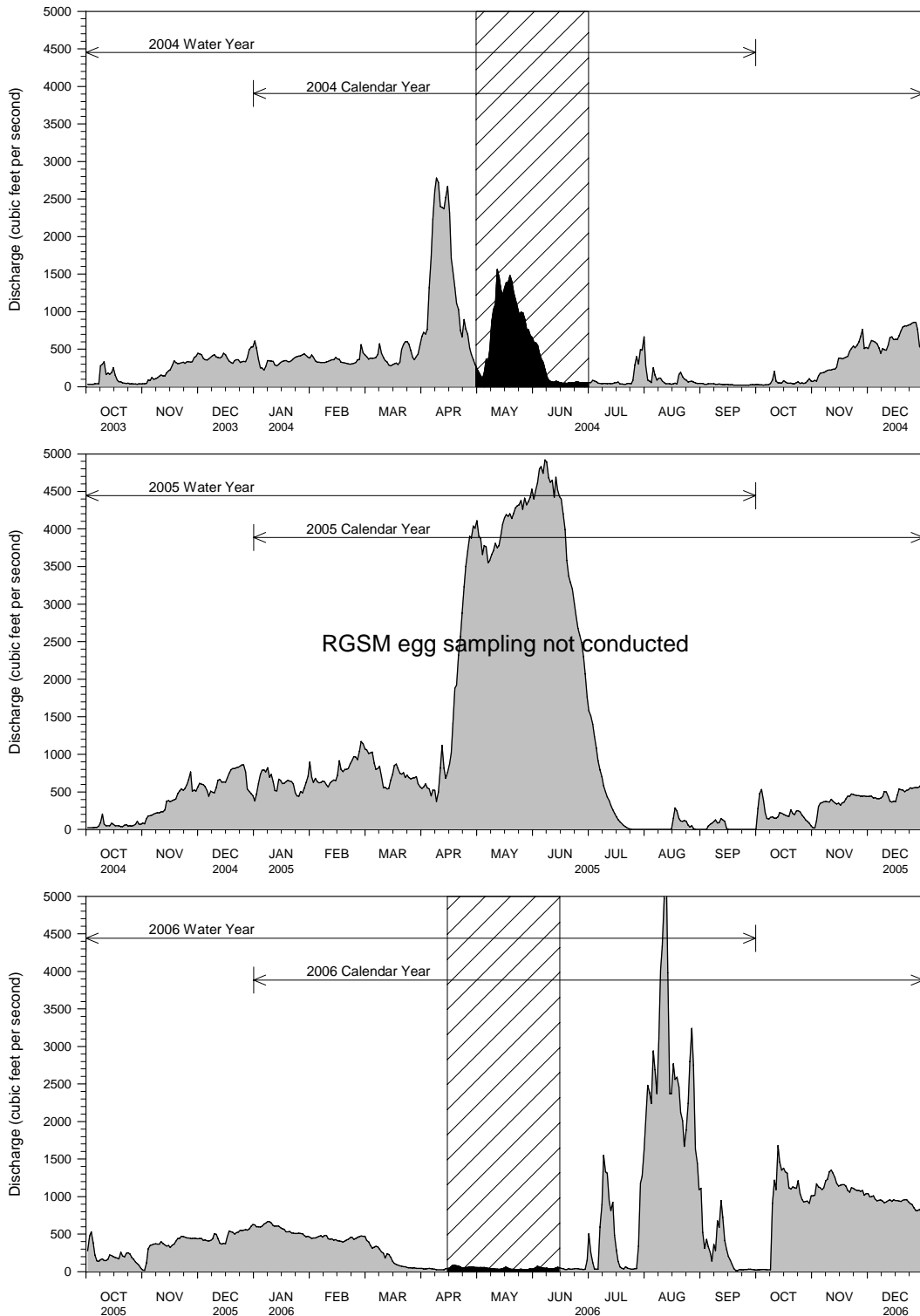


Figure 4. Annual hydrographs of the Rio Grande, New Mexico, at the San Marcial Gauging Station before, during, and after the 2004 and 2006 Rio Grande silvery minnow spawning periodicity study periods. Cross-hatching indicates annual study periods. Sampling was not conducted in 2005.

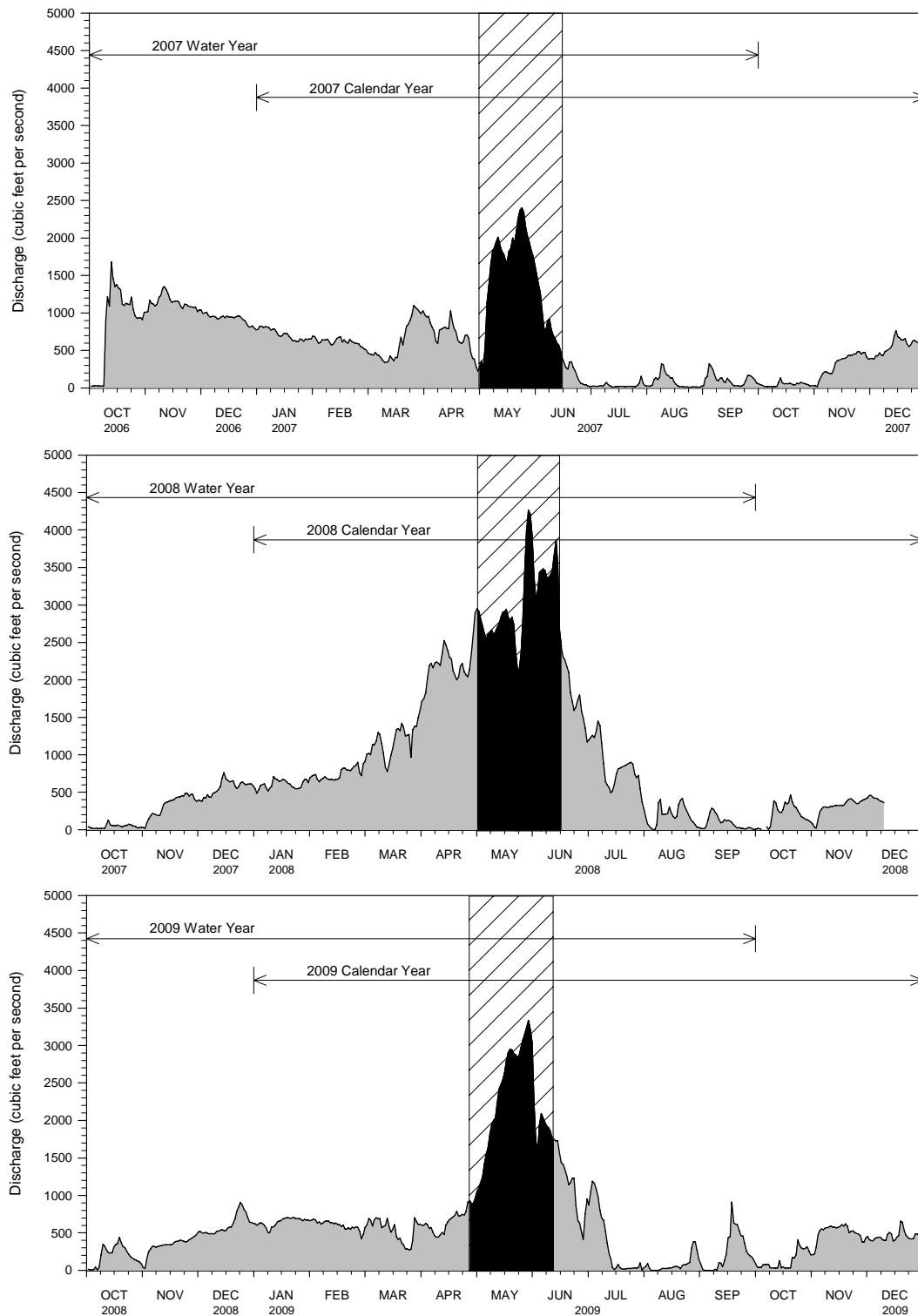


Figure 5. Annual hydrographs of the Rio Grande, New Mexico, at the San Marcial Gauging Station before, during, and after the 2007-2009 Rio Grande silvery minnow spawning periodicity study periods. Cross-hatching indicates annual study periods.

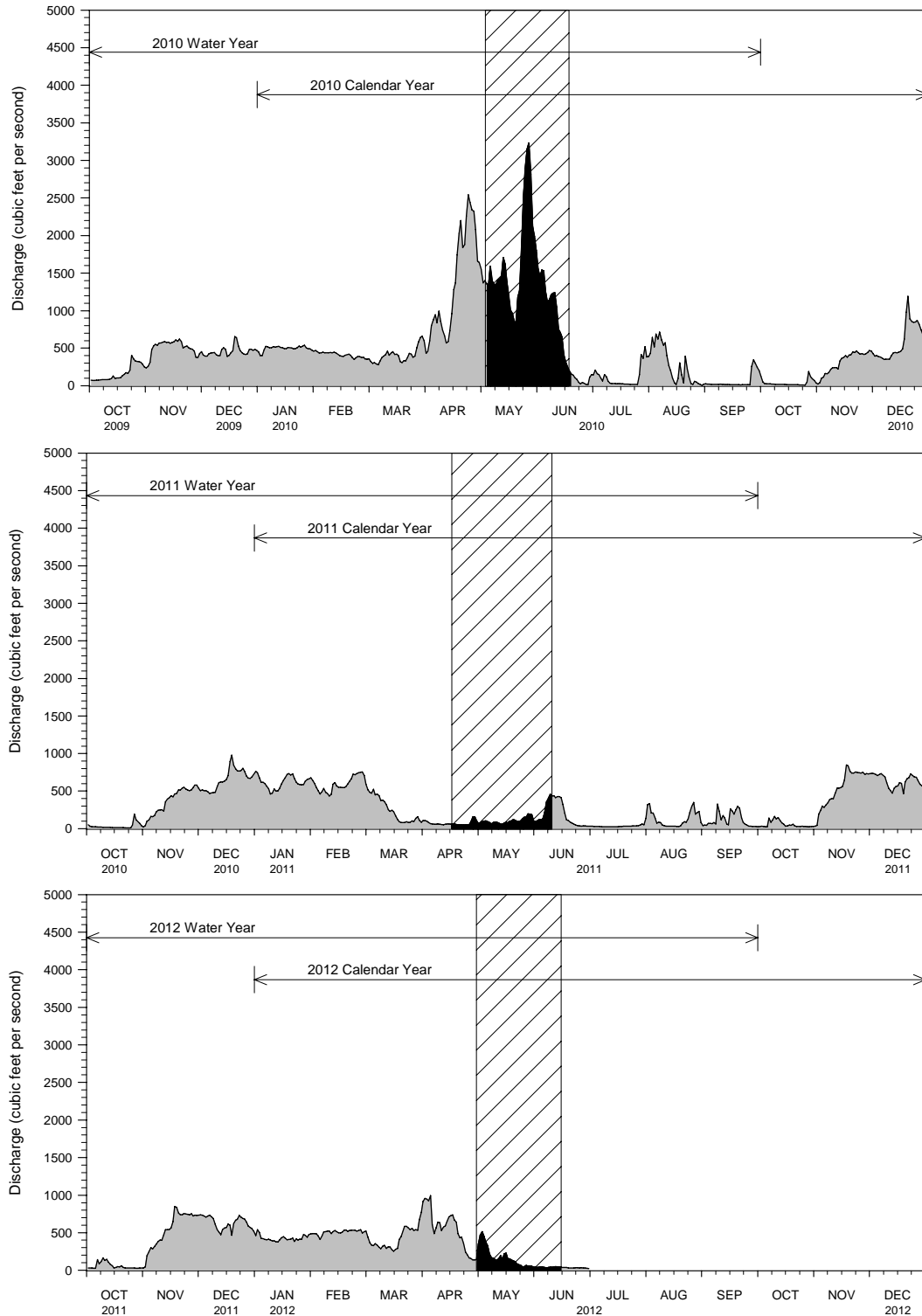


Figure 6. Annual hydrographs of the Rio Grande, New Mexico, at the San Marcial Gauging Station before, during, and after the 2010-2012 Rio Grande silvery minnow spawning periodicity study periods. Cross-hatching indicates annual study period.

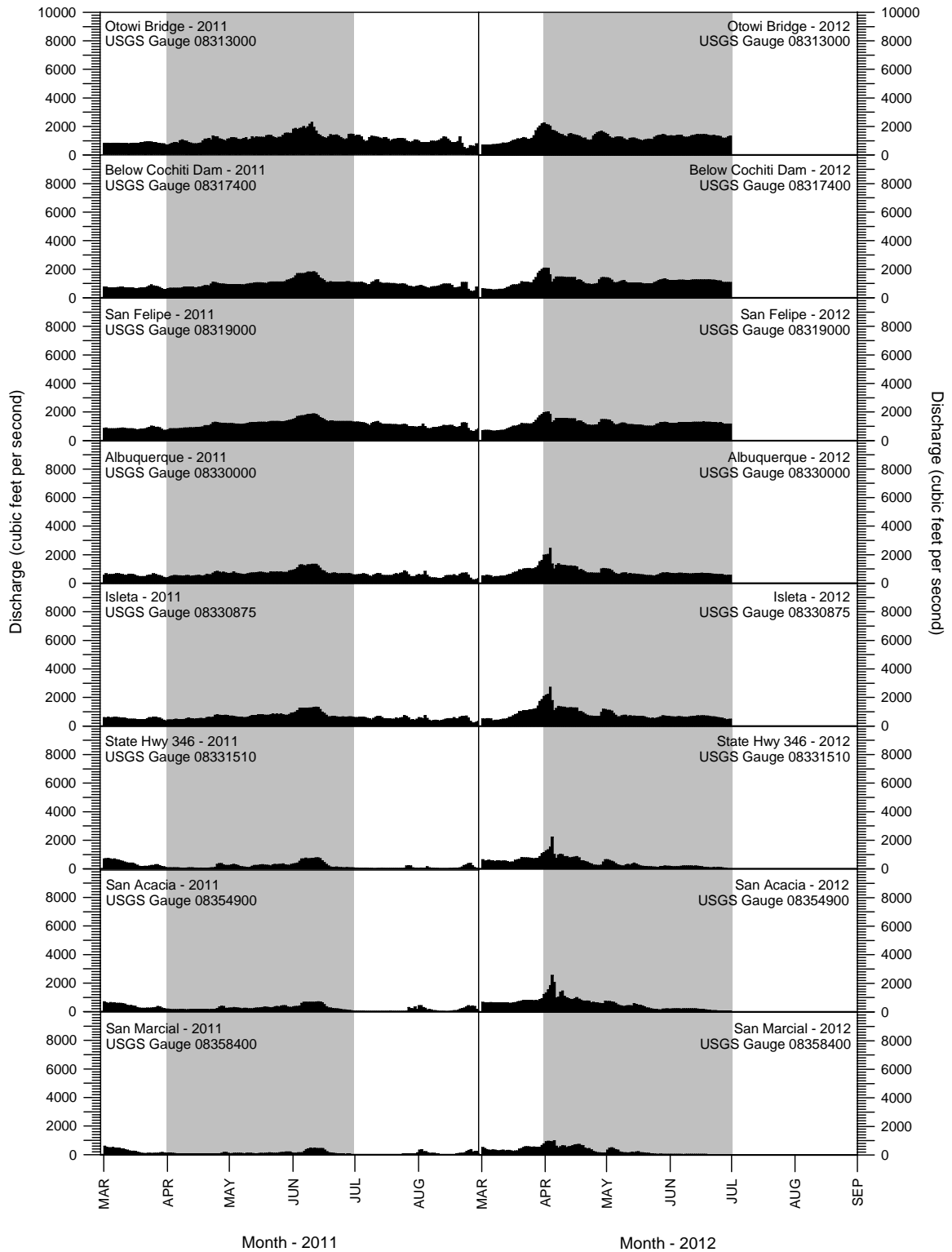


Figure 7. Rio Grande discharge from March through August 2011 and March through June 2012 at seven U. S. Geological Survey Gauging Stations (see Figure 1). The Otowi Bridge gauge is not shown in Figure 1 but is provided for reference. Gray rectangles indicate peak months for Rio Grande silvery minnow spawning activity.

June) was extremely low because of minimal spring snowmelt runoff. Spring flows were markedly higher from 2007 to 2010 as compared with 2006, but returned to very low conditions during the 2011 and 2012 study periods.

Discharge in the Rio Grande at the San Marcial Railroad Bridge Crossing (USGS Gauge 08358400) during the 2012 water year closely mirrored nearly all of the upstream gauges (except at a reduced magnitude). From 01 April to 30 June 2012, daily discharge in the Rio Grande at the San Marcial Gauge ranged from 22 to 995 cfs (mean = 252.8 cfs, SE = 28.7). Flows peaked on 5 April (995 cfs) and then declined steadily throughout April. May began with a small increase in flow, from 141 cfs on 30 April to a peak of 514 cfs on 3 May. The remainder of the 2012 spawning season was marked by very low flow conditions and reached the minimum flow of 22 cfs on 30 June 2012 (Figure 8).

Water temperature

Mean daily water temperature at the San Marcial Site fluctuated from about 20°C to 25°C in May but then steadily rose to nearly 28°C by late June (Figure 9). Temperature extremes (minimums and maximums) followed different trajectories over the course of the study. In general, there was less diurnal fluctuation during May and more diurnal fluctuation during June. This pattern seemed to be mostly reflective of the extremely low flows recorded during June. Maximum daily water temperature exceeded 30°C on 3 June and nearly reached 36°C by the end of June. Minimum daily water temperature ranged from about 15°C to 20°C from May until mid-June but began to exceed 20°C on a more regular basis by the end of June. During the entire study period (30 April to 15 June), water temperature ranged from a low of 13.8°C (28 May) to a high of 31.9°C (6 June) at the San Marcial Site.

Comparison of 2001 to 2011 water temperatures at the San Marcial Site during May and June demonstrated only modest differences in mean daily water temperature trends across this period (Figures 10 to 12). However, there were notable day-to-day differences in mean water temperatures among years (e.g., 13.8°C [3 May 2011] vs. 21.7°C [3 May 2007]). The minimum daily water temperatures were consistently the lowest in low flow years (e.g., 2002, 2003, 2006, 2011, and 2012). Maximum water temperatures were also consistently the highest during these same years (2002, 2003, 2006, 2011, and 2012) as compared with other years of the study. The general trend for minimum temperatures was a slow steady increase through May and June. In contrast, maximum temperatures generally rose slowly in May and then rose rapidly at some point during June (i.e., coinciding with the reduction in flow following spring runoff). There were sometimes notable differences in the day-to-day maximum water temperatures among years (e.g., 18.2°C [19 May 2008] vs. 30.5°C [19 May 2006]) but the difference in day-to-day minimum water temperatures were not as pronounced. The principal reason for these patterns, besides ambient temperature, was the volume of water in the river channel during the respective study periods. The relatively high flows present in 2001, 2004, and 2007 to 2010 (May to mid-June) served to ameliorate water temperatures and minimize diel variation. Conversely, the very low flow conditions present throughout most of the spawning period in 2002, 2003, 2006, 2011, and 2012 typically resulted in large daily temperature fluctuations.

Spatial spawning periodicity

Sampling at the San Marcial Site was conducted from 30 April through 15 June (47 days). The cumulative volume of water at the San Marcial Site was 45,719 m³ (Table 1). A total of 12,398 Rio Grande silvery minnow eggs was collected from 1 May to 26 May during 2012 (Table 2). Daily egg catch rates ranged between 0.17 and 466.71 eggs per 100 m³ of water sampled (n = 1 and n =

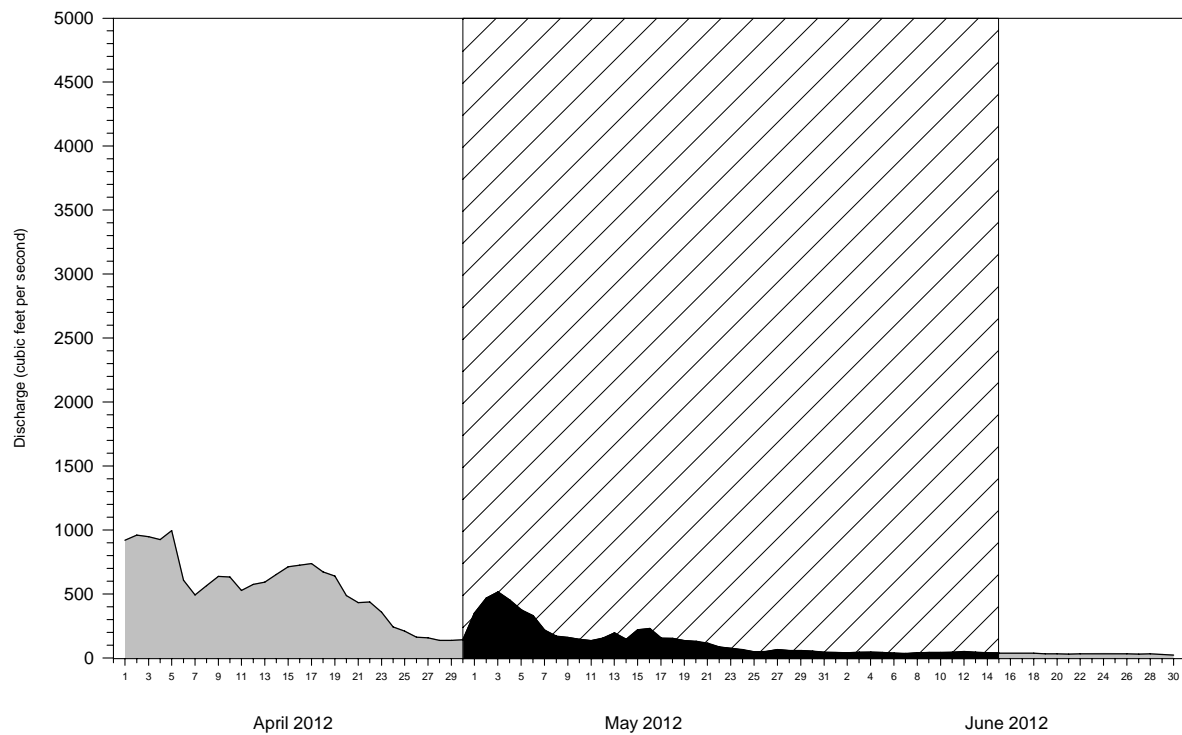


Figure 8. April-June 2012 hydrograph (dark gray) of the Rio Grande, New Mexico, at the San Marcial Gauging Station. Cross-hatching indicates annual study period.

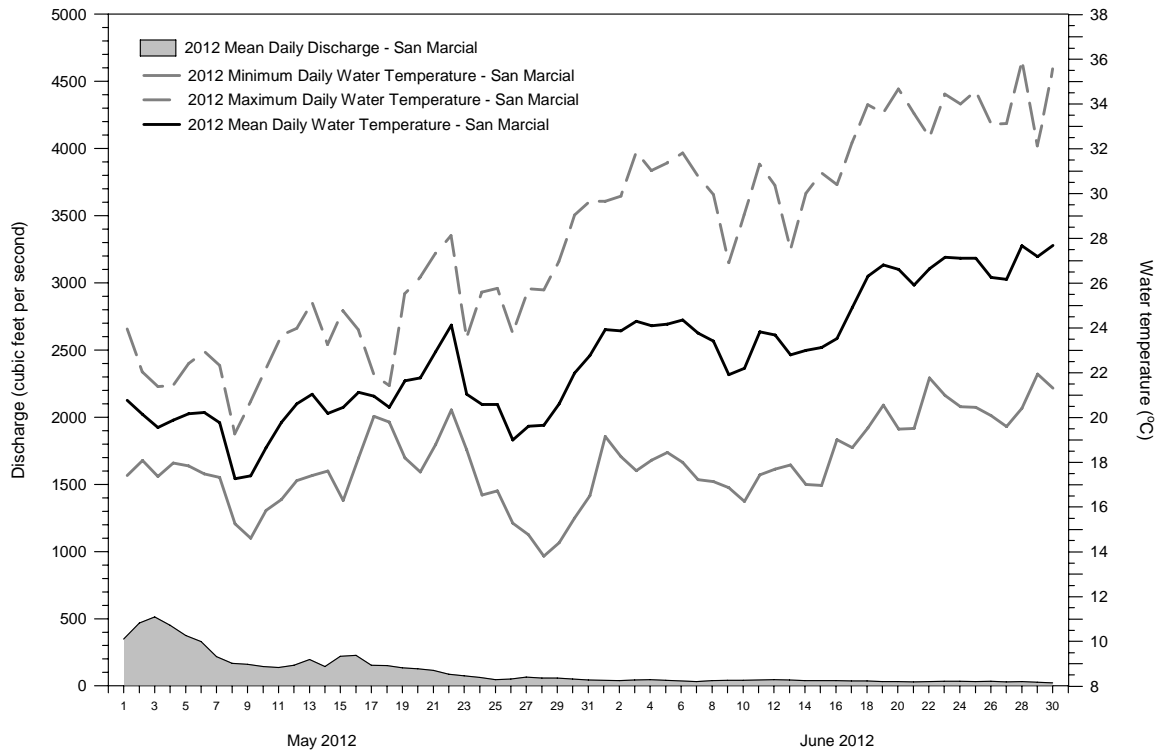


Figure 9. Daily water temperatures (mean, minimum, and maximum) at the San Marcial Site in 2012. Mean daily discharge at the San Marcial Gauging Station is shown in light gray.

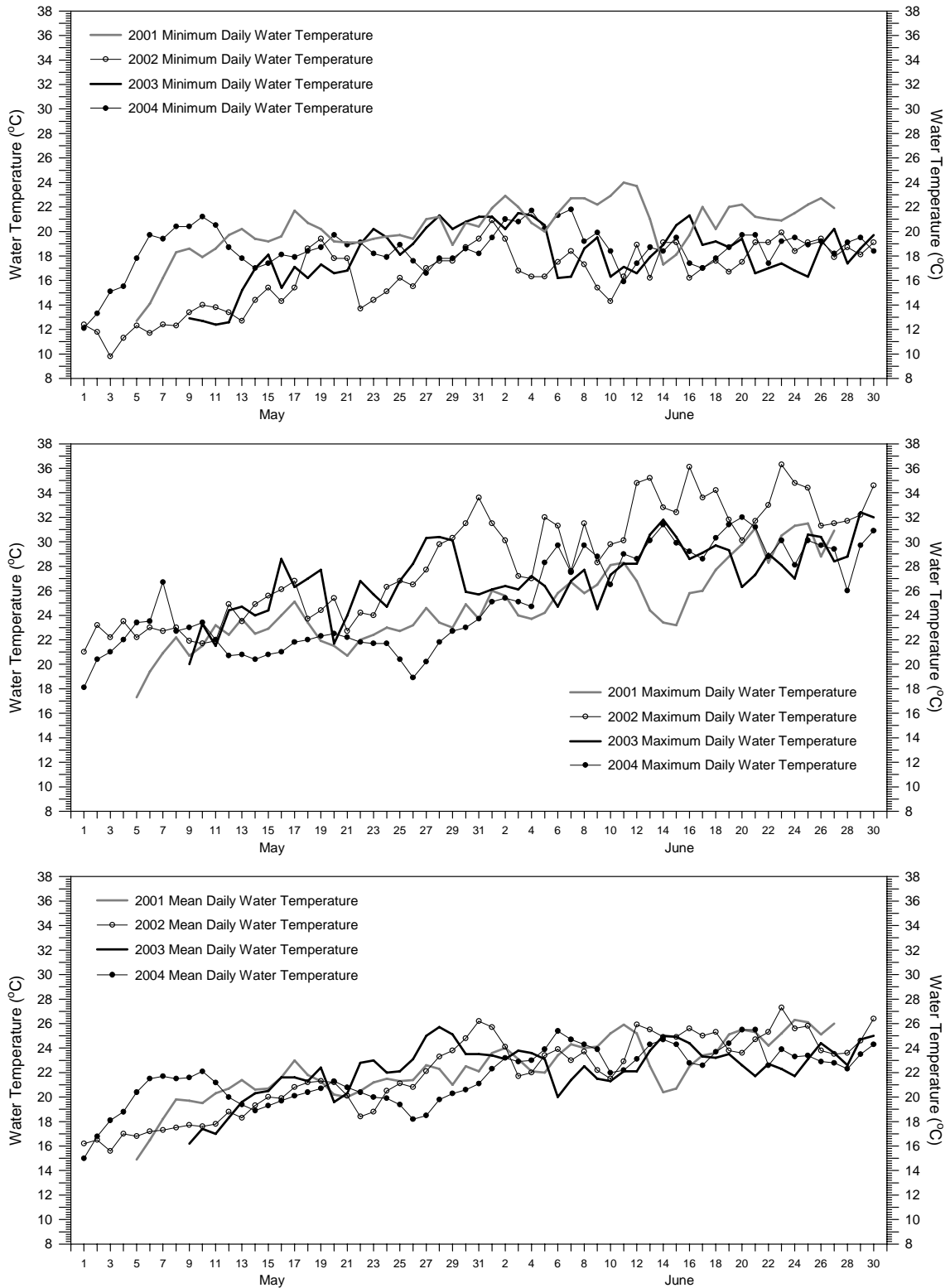


Figure 10. Annual minimum, maximum, and mean daily water temperatures at the San Marcial Site during the 2001-2004 Rio Grande silvery minnow spawning periodicity study periods.

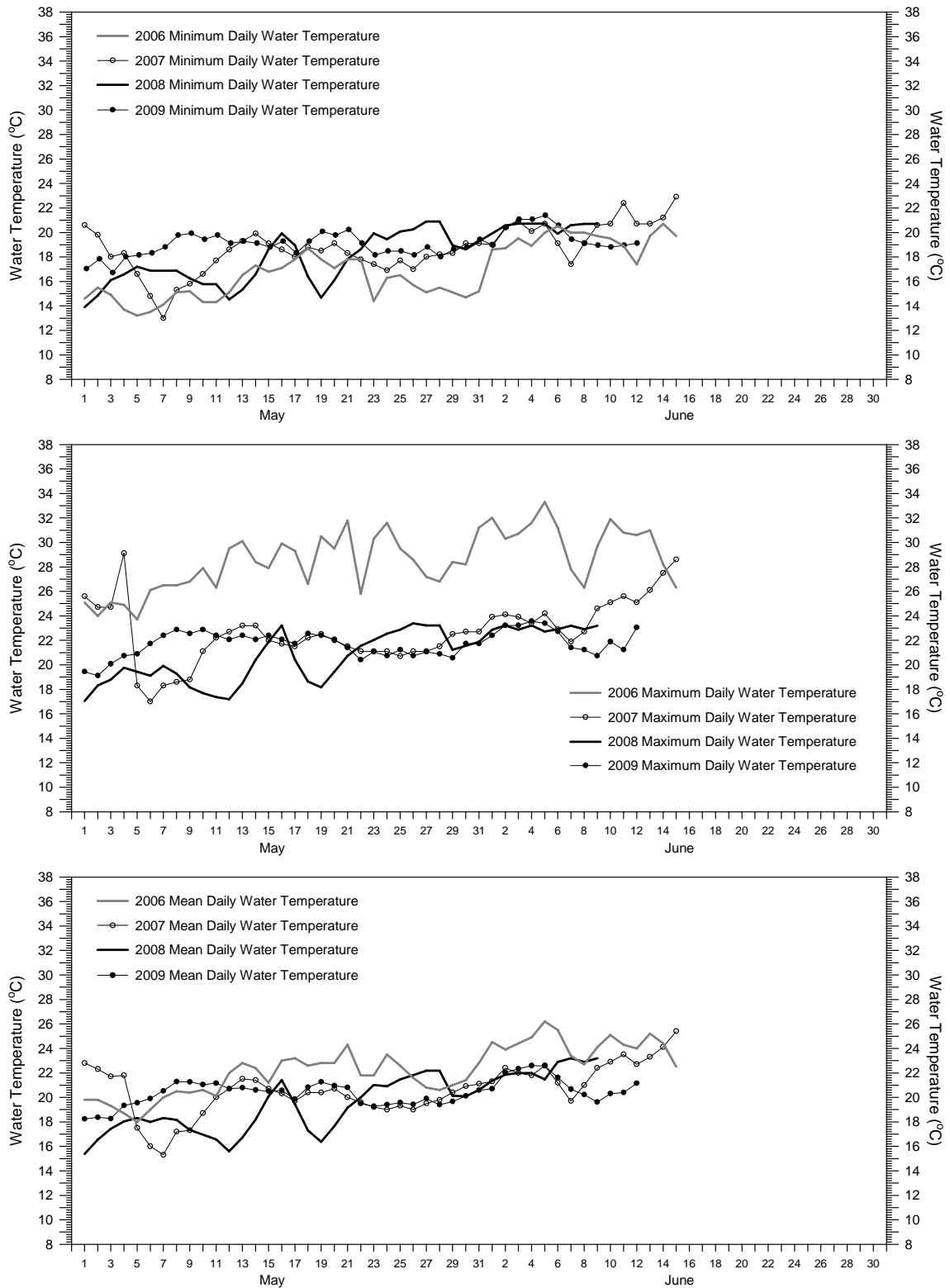


Figure 11. Annual minimum, maximum, and mean daily water temperatures at the San Marcial Site during the 2006-2009 Rio Grande silvery minnow spawning periodicity study periods.

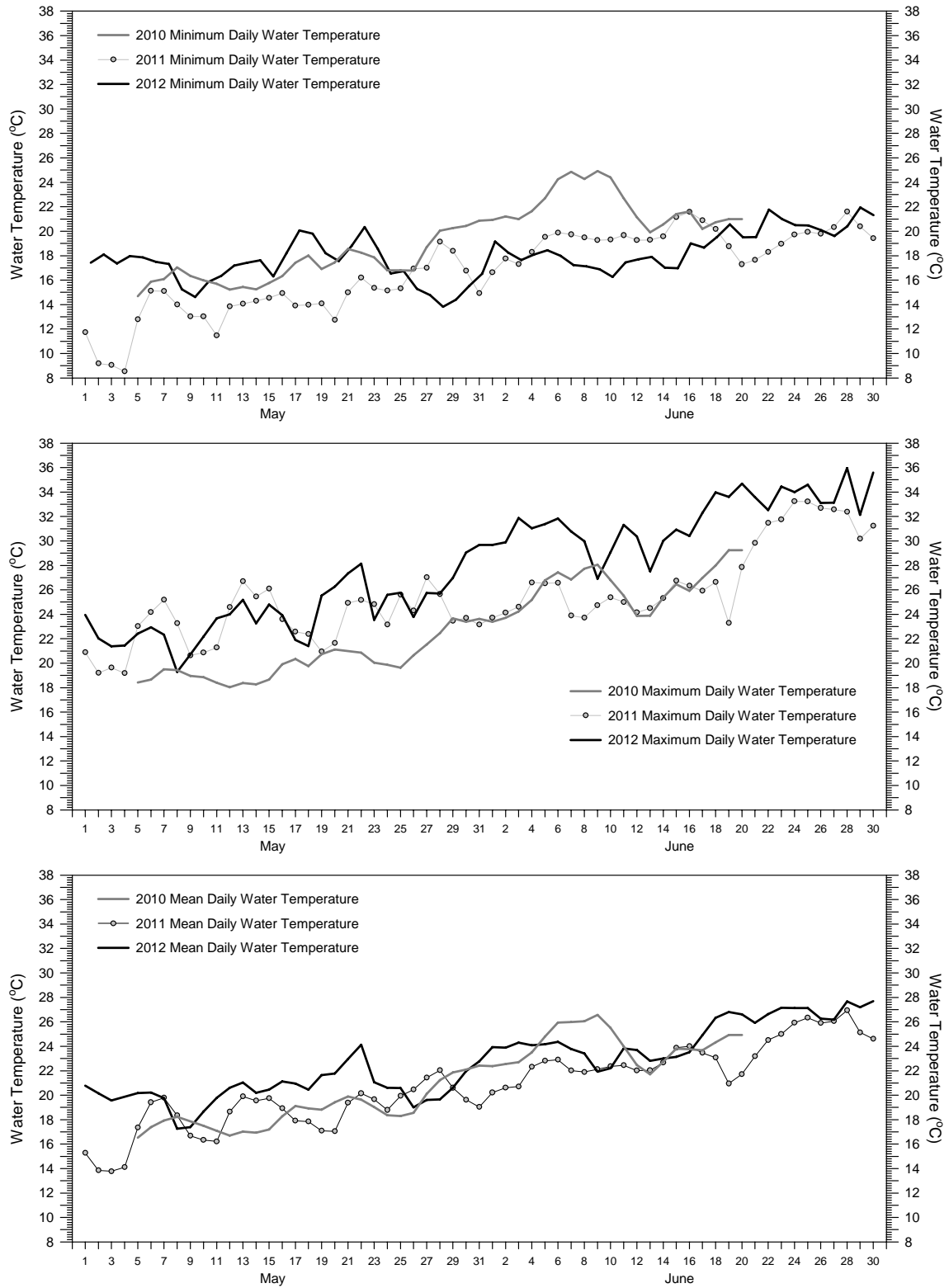


Figure 12. Annual minimum, maximum, and mean daily water temperatures at the San Marcial Site during the 2010-2012 Rio Grande silvery minnow spawning periodicity study periods.

Table 1. Summary of 2012 sampling effort for Rio Grande silvery minnow eggs.

SAMPLING INFORMATION		NUMBER OF EGGS	EGG CATCH RATE ¹	VOLUME OF WATER SAMPLED (M ³)	NUMBER OF DAYS SAMPLED	DATES SAMPLED	
SYSTEM:	SITE					START	STOP
RIO GRANDE:	SAN MARCIAL	12,398	27.12	45,718.88	47	30 APR 2012	15 JUN 2012

¹ Value based on number of Rio Grande silvery minnow eggs collected per 100 m³ of water sampled for all sampling days.

Table 2. Number of Rio Grande silvery minnow eggs collected per day. Table does not include dates that eggs **were not** collected.

LOCATION : RIVER MILE :	SAN MARCIAL RM 55.0
1-May-12	10
2-May-12	6,691
3-May-12	3,952
4-May-12	1,433
5-May-12	104
6-May-12	3
7-May-12	2
14-May-12	69
15-May-12	9
16-May-12	49
17-May-12	33
19-May-12	3
20-May-12	8
21-May-12	2
22-May-12	7
23-May-12	9
24-May-12	13
26-May-12	1
TOTALS	12,398

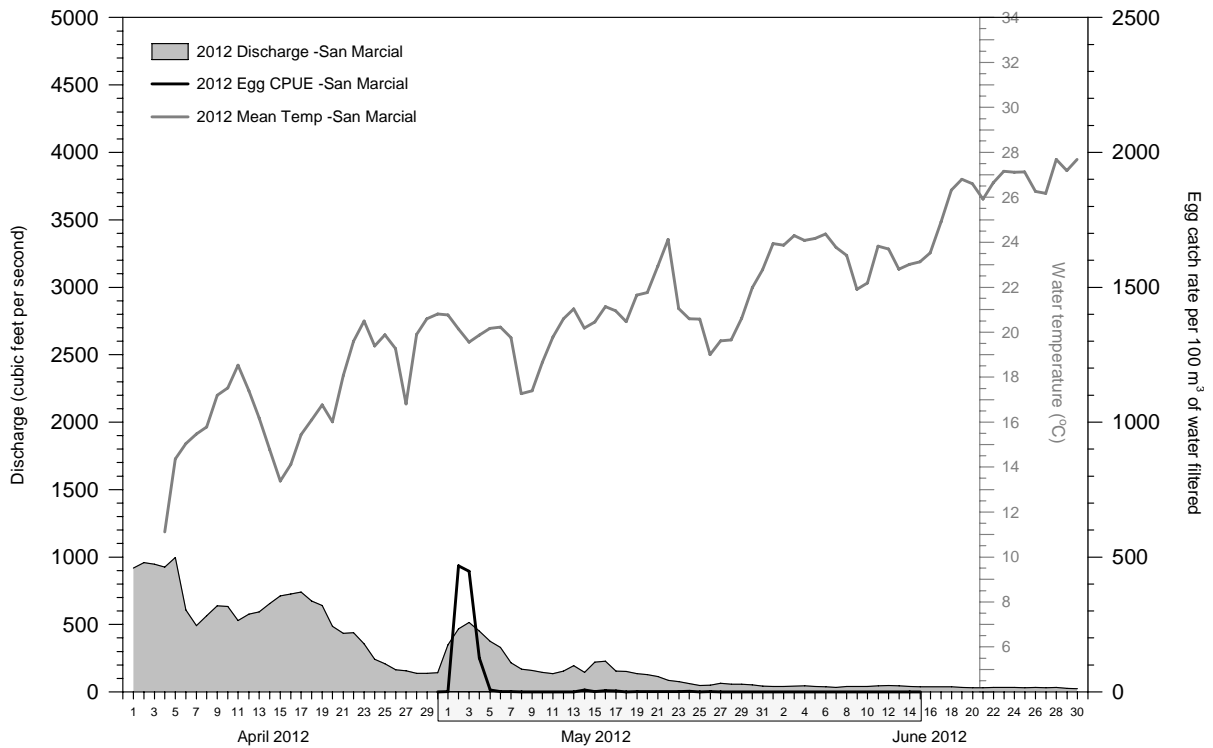


Figure 13. Mean daily discharge, mean daily egg catch rate, and mean daily water temperature during the 2012 Rio Grande silvery minnow spawning periodicity study period (sampling period is highlighted in gray along the abscissa axis).

6,691 respectively). During the study, the overall egg catch rate at the San Marcial Site was 27.12 eggs per 100 m³ of water sampled (Figure 13).

The volume of water sampled at the San Marcial Site constituted only a small fraction of the total volume available (i.e., nearly two orders of magnitude lower or about 1%) over each daily four-hour sampling period (Table 3). The number of eggs estimated to be transported downstream over the duration of the study was 12,471,939. The highest number of eggs estimated to be transported downstream in a single day was 5,619,124 (3 May 2012).

Comparison of 2002-2004 and 2006-2012 studies

Despite substantial interannual differences in Rio Grande silvery minnow egg catch rate metrics (Table 4), there were several similarities apparent regarding the timing of reproduction during 2002-2004 and 2006-2011. Based on the results of data taken from all years of the project, there was an extended duration of spawning (April-July). However, most spawning consistently occurred during the early to middle portion of May over the period of record. One notable exception to this pattern occurred in 2011 when peak spawning coincided with elevated flows in early June. The start of the spawning season varied across years and generally occurred later in upstream reaches.

Mean daily water temperatures during the initial and peak spawning events were relatively similar among years (Figures 14-17). In general, mean daily water temperatures ranged from 17 to 20°C during peak spawning events. However, spawning frequently occurred at elevated water temperatures (> 20°C) during years with relatively lower flows (e.g., 2002, 2003, 2006, 2011, and 2012). Mean daily water temperatures ranged between 19.0°C and 24.1°C during spawning in 2011 at the San Marcial Site.

Statistical analyses among all years were made using data from the San Marcial sampling locality since that site was consistently sampled since 2002. Analysis of reproductive output revealed a significant difference ($F = 8.70$; $p < 0.0001$) among mean values of catch rate (# / 100m³) over the period of record (2002 to 2004 and 2006 to 2012). The following pair-wise comparisons were significant ($p < 0.05$) over the period of record (2002 vs. 2006; 2006 vs. 2007; 2007 vs. 2010). The log-transformed mean egg catch rate (standardized for discharge) was highest in 2002 (5.86), followed by 2007 (4.77), 2011 (4.10), 2008 (3.22), 2003 (2.89), 2012 (2.79), 2009 (2.57), 2010 (2.24), 2006 (1.44), and 2004 (0.96) (Figure 18). Linear regression of the mean values of log-transformed July and October CPUE (#/100 m²) of Rio Grande silvery minnow in the San Acacia Reach and log-transformed egg catch rates at San Marcial (2002-2004, 2006-2012) yielded a non-significant relationships ($R^2 = 0.00$; $F = 0.00$; $p = 0.96$ and $R^2 = 0.03$; $F = 0.18$; $p = 0.68$, respectively; Figure 19).

A comparison between log-transformed egg catch rate (standardized for discharge) and the percentage increase in mean daily discharge from one day prior to egg collection at the San Marcial Site yielded a non-significant ($F = 0.59$; $p = 0.44$) relationship. However, a comparison between log-transformed egg catch rate (standardized for discharge) and the percentage increase in mean daily discharge from two days to one day prior to egg collection yielded a highly significant ($F = 38.17$; $p < 0.0001$) relationship (Figure 20). The significant San Marcial Site regression model ($y = 3.19 + (2.69 \times 10^{-2})(x)$) was derived from 157 observations and yielded a modest value for the coefficient of determination ($R^2 = 0.20$). Subsequent annual regression analyses of this relationship (Table 5) revealed elevated R^2 values (range = 0.35 to 0.65), with significant p -values, in years with lower flows (i.e., 2003, 2006, 2001, and 2012); the relationship in 2002 yielded a low albeit non-significant p -value ($p = 0.06$). In contrast, years with adequate observations ($N > 5$) and higher flows (i.e., 2007, 2009, and 2010) yielded very low R^2 values (range = 0.00 to 0.03) along with non-significant p -values.

Table 3. Volume of water sampled (4 hours), volume of water available (4 hours), and number of Rio Grande silvery minnow eggs estimated to be transported downstream of sampling location (24 hours) by date. Table does not include dates that eggs *were not* collected.

DATA TYPE: LOCATION:	VOLUME SAMPLED (ft ³) SAN MARCIAL	VOLUME AVAILABLE (ft ³) SAN MARCIAL	ESTIMATED EGGS TRANSPORTED SAN MARCIAL
1-May-12	63,190	5,025,600	4,772
2-May-12	50,629	6,710,400	5,320,951
3-May-12	31,234	7,401,600	5,619,124
4-May-12	40,338	6,494,400	1,384,279
5-May-12	54,904	5,371,200	61,045
6-May-12	51,772	4,752,000	1,652
7-May-12	41,530	3,110,400	899
14-May-12	35,178	2,073,600	24,403
15-May-12	25,409	3,153,600	6,702
16-May-12	35,685	3,268,800	26,931
17-May-12	35,112	2,203,200	12,424
19-May-12	34,613	1,929,600	1,003
20-May-12	41,175	1,828,800	2,132
21-May-12	41,713	1,627,200	468
22-May-12	41,660	1,224,000	1,234
23-May-12	42,129	1,080,000	1,384
24-May-12	29,867	892,800	2,332
26-May-12	20,800	705,600	204
TOTALS	716,938	58,852,800	12,471,939

Table 4. Catch rates of Rio Grande silvery minnow eggs by year, site, and category (mean daily, maximum daily, and maximum sample).

SAMPLING INFORMATION			MEAN DAILY CATCH RATE ¹ (# / 100 M ³)	MAXIMUM DAILY CATCH RATE (# / 100 M ³)	MAXIMUM SAMPLE CATCH RATE (# / 100 M ³)
YEAR:	SITE				
2002	RIO GRANDE:	SAN MARCIAL	1,622.04	14,222.00	96,558.00
2003	RIO GRANDE:	SAN MARCIAL	34.85	476.00	1,027.00
2004	RIO GRANDE:	SAN MARCIAL	0.07	0.09	0.22
2005			Not Sampled	Not Sampled	Not Sampled
2006	RIO GRANDE:	ALBUQUERQUE	3.47	19.15	22.64
2006	RIO GRANDE:	SEVILLETA	4.50	44.88	53.82
2006	RIO GRANDE:	SAN MARCIAL	30.81	289.33	621.97
2007	RIO GRANDE:	ALBUQUERQUE	0.34	0.94	1.43
2007	RIO GRANDE:	SEVILLETA	13.00	147.13	158.56
2007	RIO GRANDE:	SAN MARCIAL	11.55	106.12	201.55
2008	RIO GRANDE:	ALBUQUERQUE	0.32	1.33	2.65
2008	RIO GRANDE:	SEVILLETA	5.70	76.87	136.31
2008	RIO GRANDE:	SAN MARCIAL	1.76	5.10	7.67
2009	RIO GRANDE:	SEVILLETA	3.86	24.42	38.72
2009	RIO GRANDE:	SAN MARCIAL	1.33	8.05	8.57
2010	RIO GRANDE:	SEVILLETA	0.49	5.57	10.8
2010	RIO GRANDE:	SAN MARCIAL	1.10	9.47	13.5
2011	RIO GRANDE:	SEVILLETA	49.23	850.63	1,157.38
2011	RIO GRANDE:	SAN MARCIAL	173.14	2,334.93	2,458.75
2012	RIO GRANDE:	SAN MARCIAL	59.29	466.71	631.49

¹ Catch rate determinations in this table are not corrected for discharge and only incorporate daily sample totals that contained Rio Grande silvery minnow eggs.

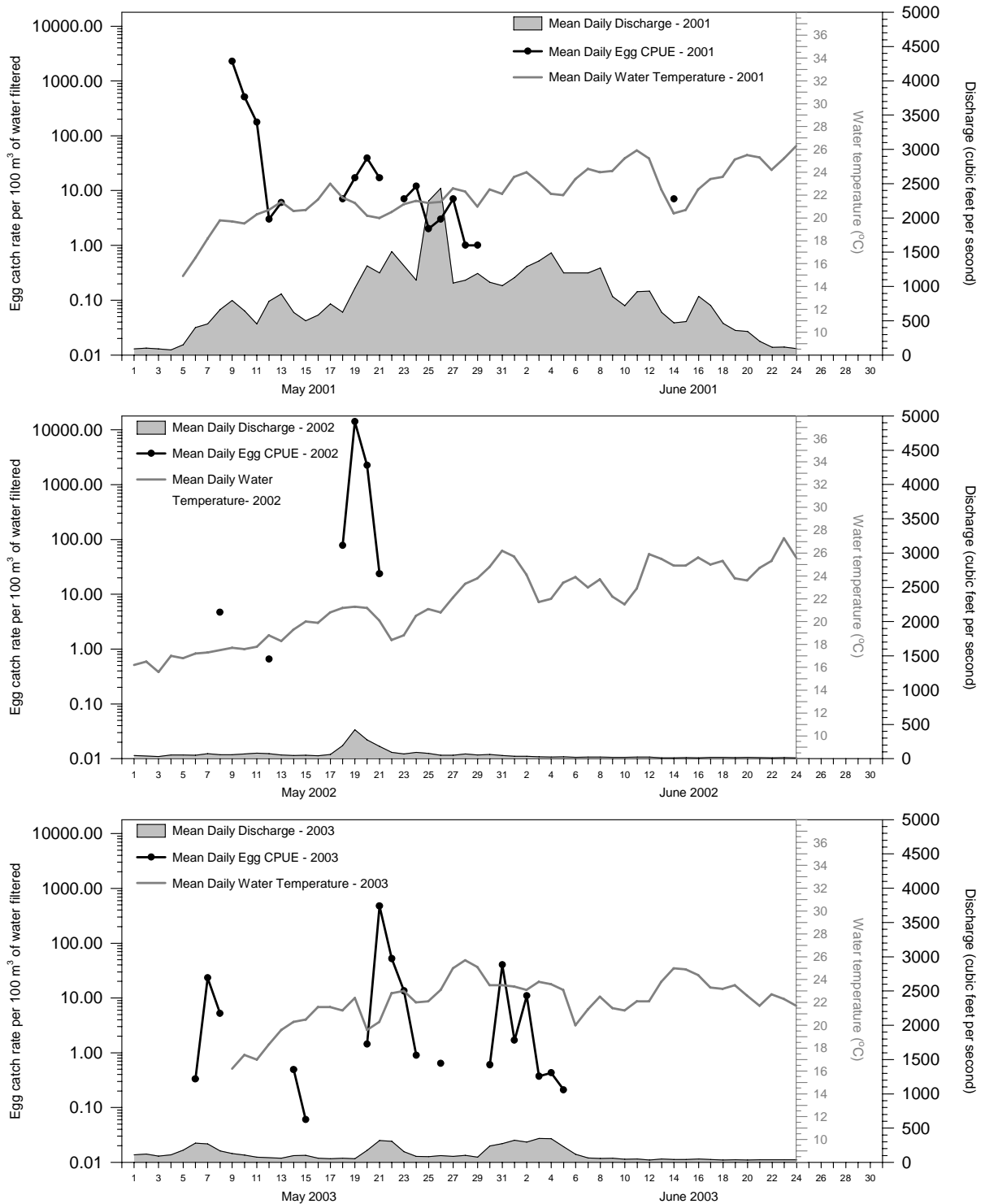


Figure 14. Mean daily discharge, mean daily egg catch rate, and mean daily water temperature during the 2001-2003 Rio Grande silvery minnow spawning periodicity study periods at the San Marcial Site. Note that the Y-axis for egg catch rate is a log-scale.

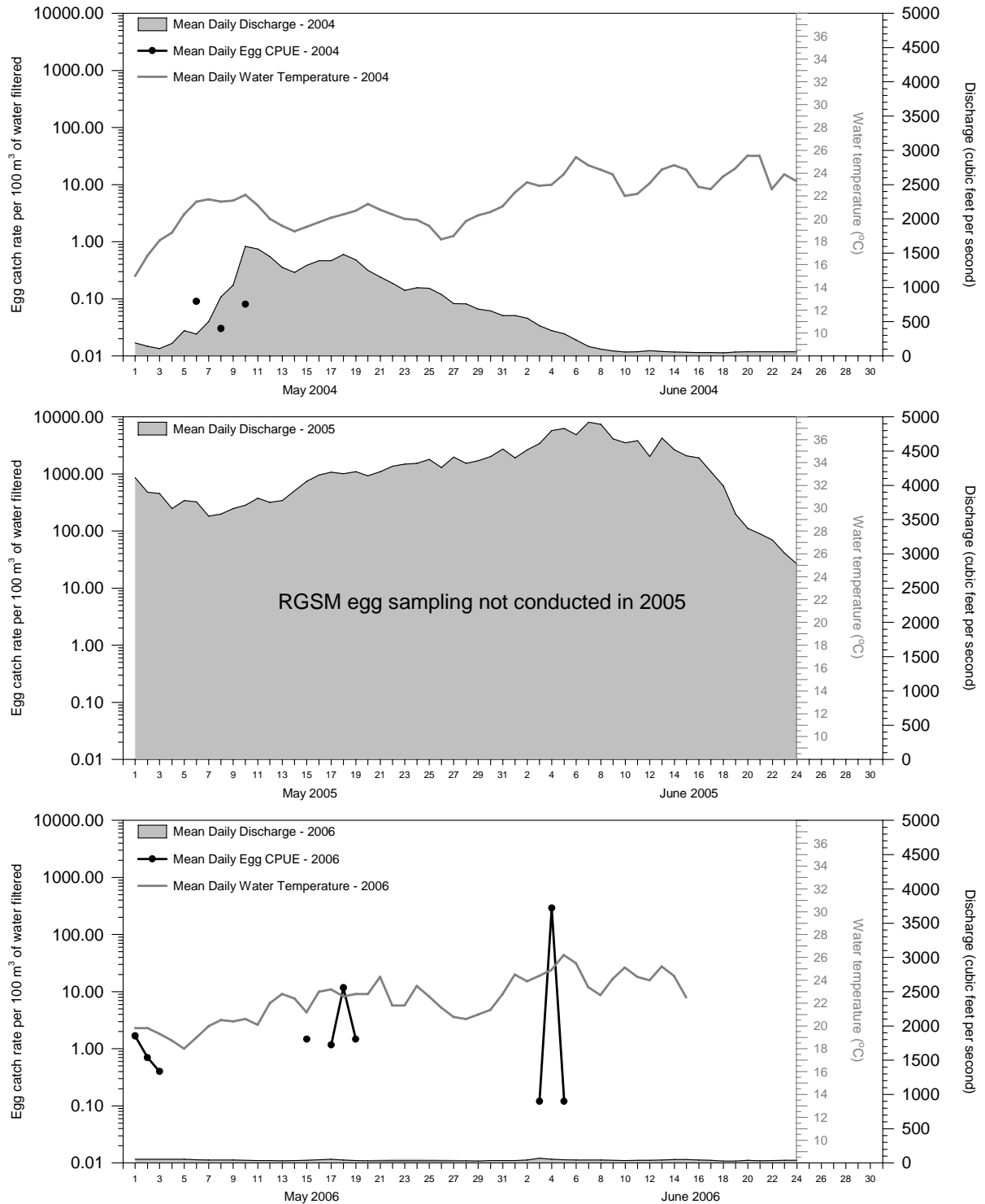


Figure 15. Mean daily discharge, mean daily egg catch rate, and mean daily water temperature during the 2004 and 2006 Rio Grande silvery minnow spawning periodicity study periods at the San Marcial Site. Note that the Y-axis for egg catch rate is a log-scale. Sampling was not conducted in 2005.

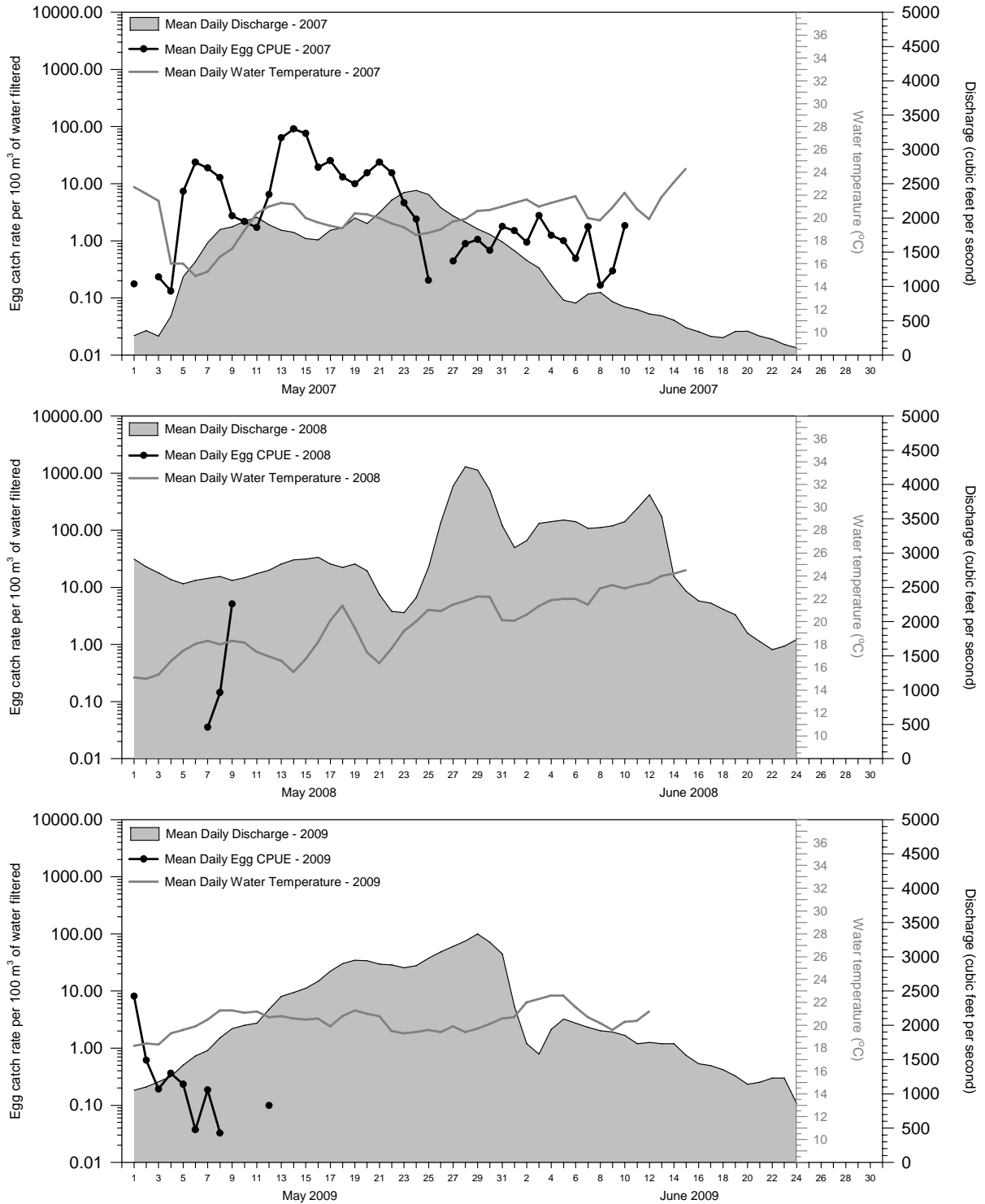


Figure 16. Mean daily discharge, mean daily egg catch rate, and mean daily water temperature during the 2007-2009 Rio Grande silvery minnow spawning periodicity study periods at the San Marcial Site. Note that the Y-axis for egg catch rate is a log-scale.

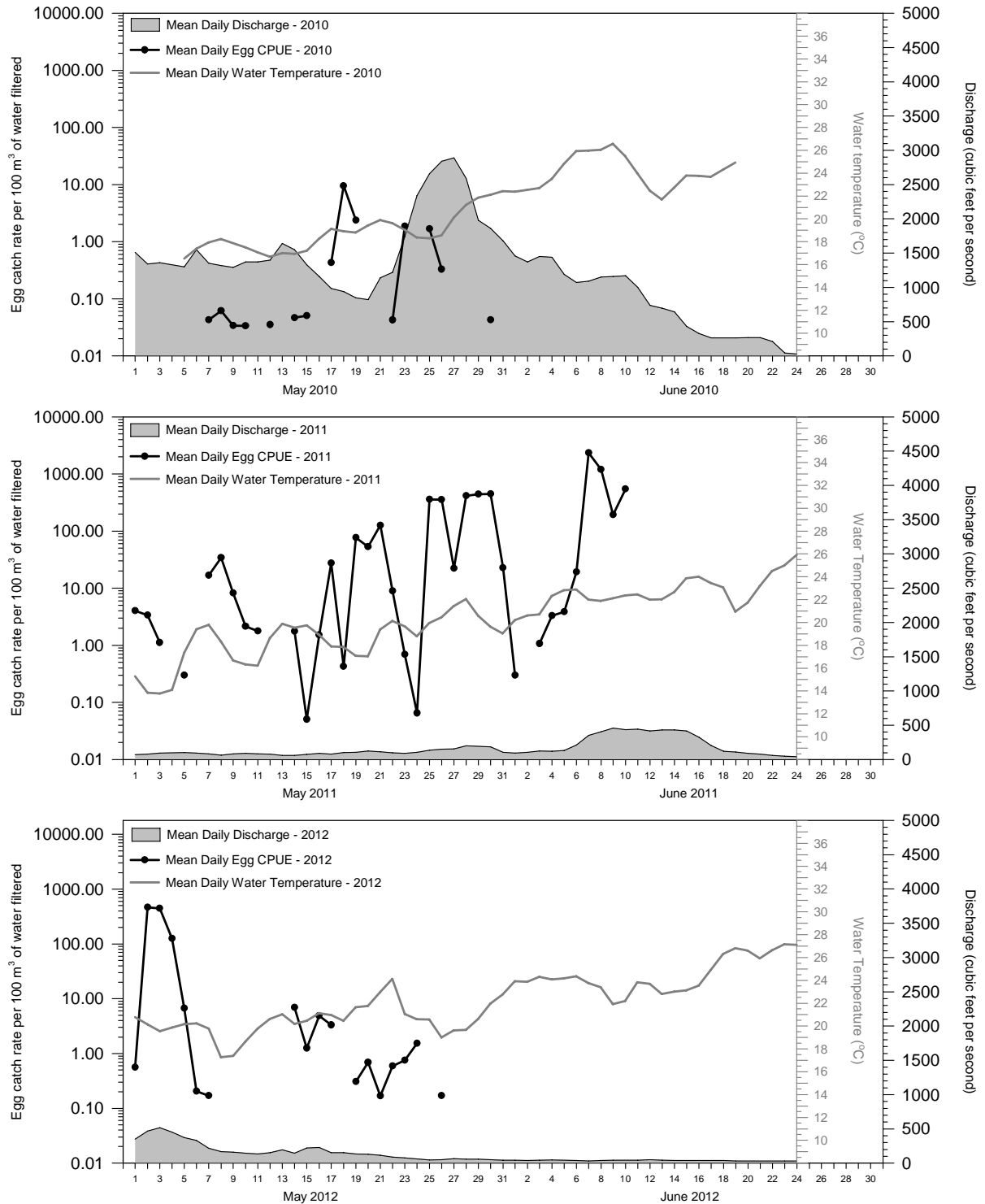


Figure 17. Mean daily discharge, mean daily egg catch rate, and mean daily water temperature during the 2010-2012 Rio Grande silvery minnow spawning periodicity study periods at the San Marcial Site. Note that the Y-axis for egg catch rate is a log-scale.

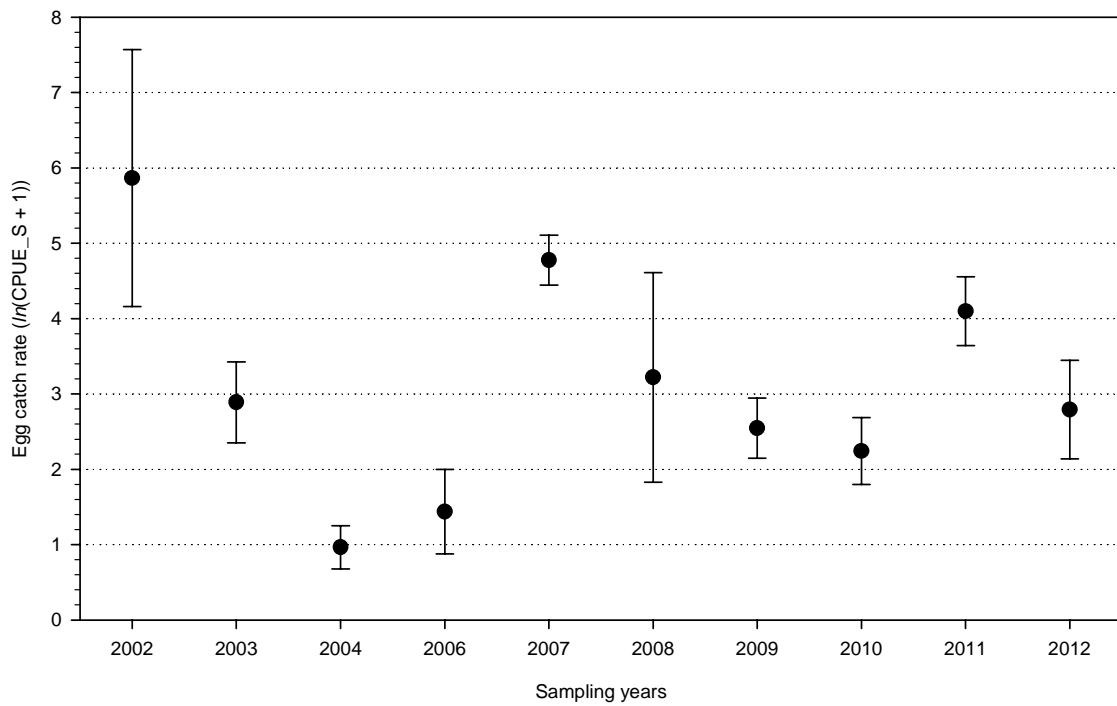


Figure 18. Log-transformed egg densities (standardized for discharge) at the San Marcial Site over the period of record. Solid circles indicate means and capped-bars represent the standard error. Dotted horizontal lines represent different orders of magnitude.

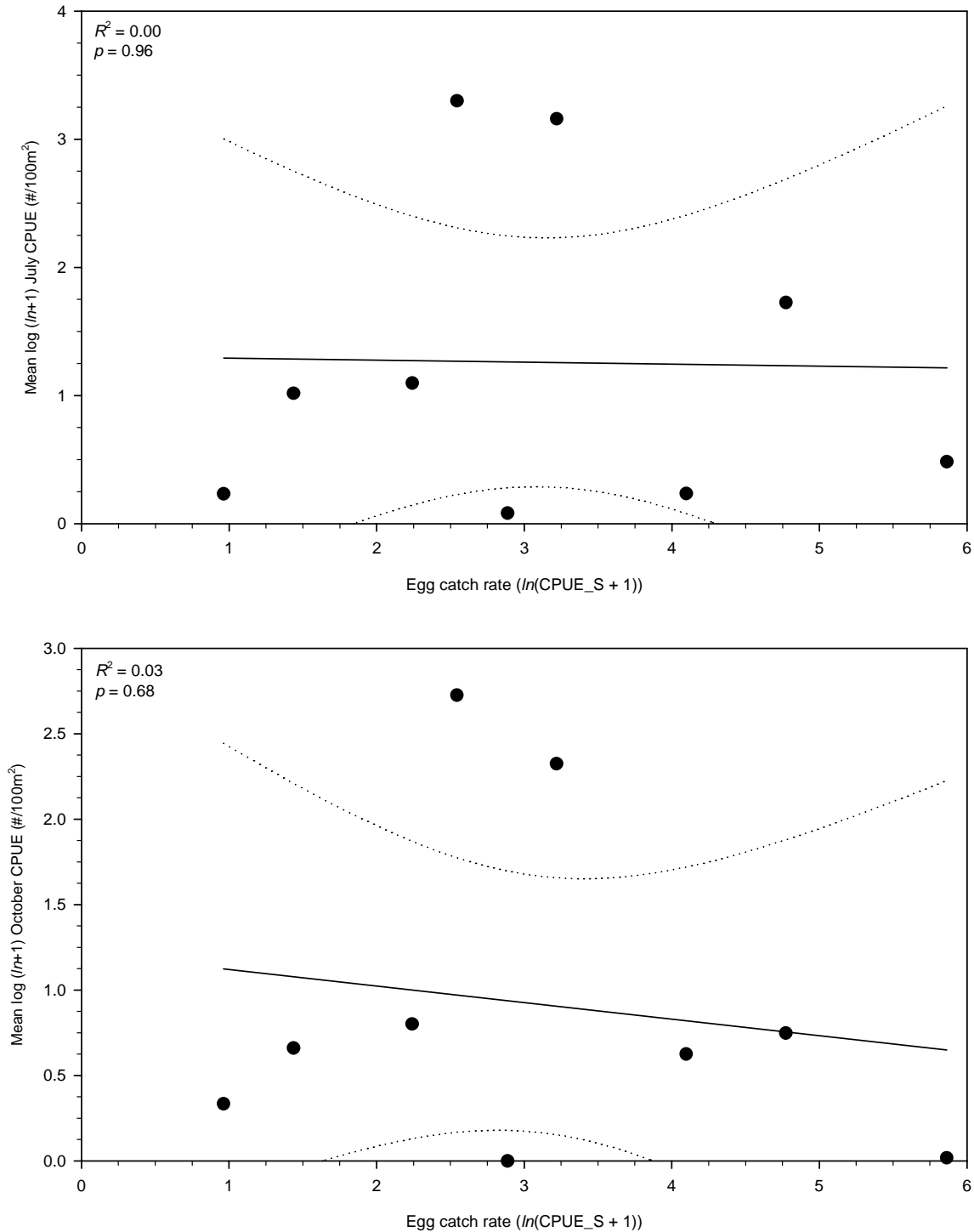


Figure 19. Regression analysis of mean values of log-transformed July and October CPUE (#/100 m²) of Rio Grande silvery minnow in the San Acacia Reach and log-transformed egg densities (standardized for discharge) at the San Marcial Site over the period of record. Graph shows regression line (solid) and 95% confidence intervals (dotted).

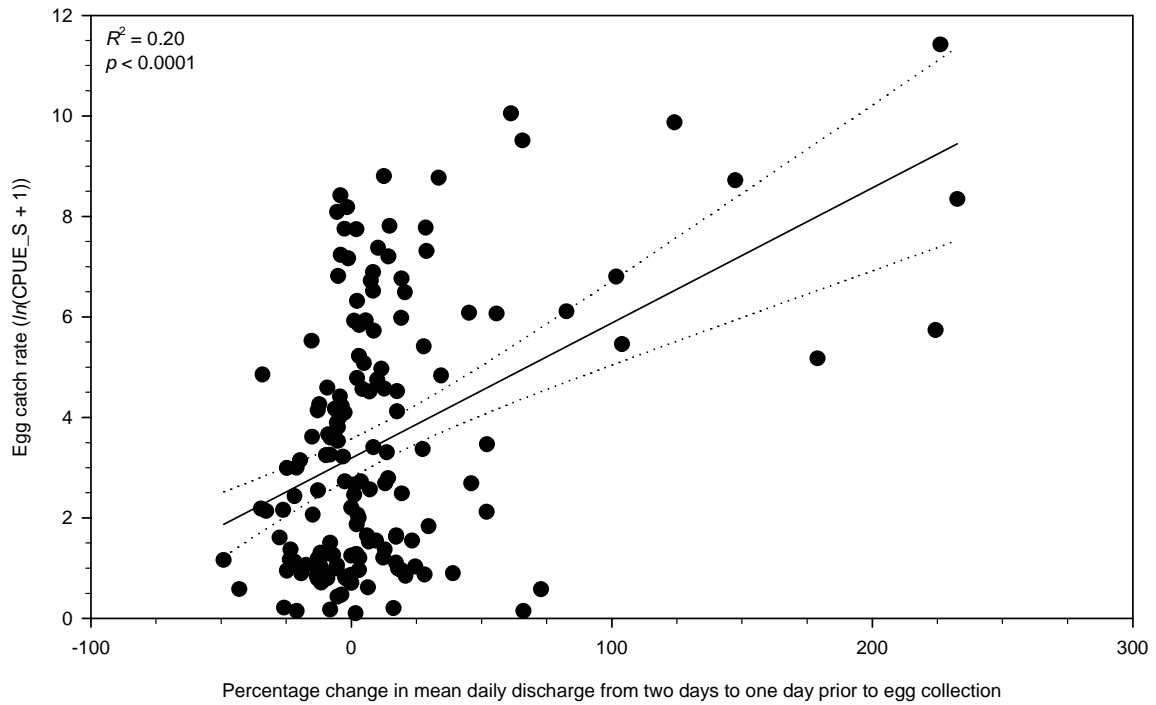
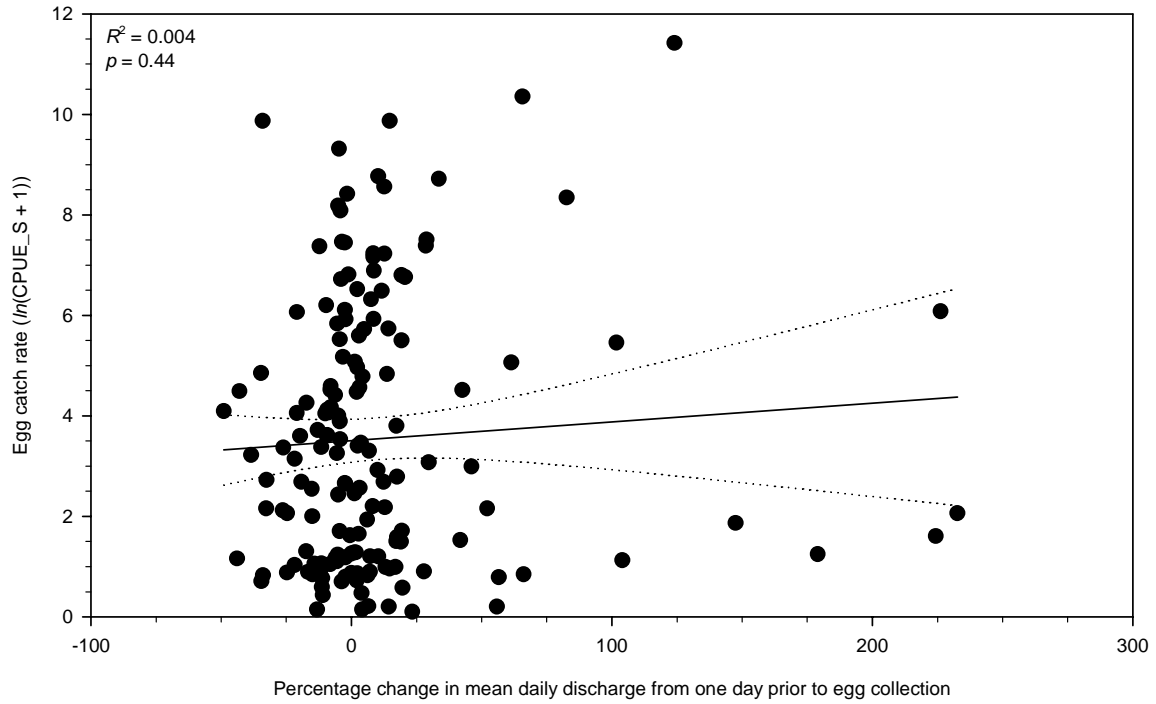


Figure 20. Regression analysis of log-transformed egg densities (standardized for discharge) and percentage change in mean daily discharge one day and two days prior to egg collection at the San Marcial Site during May and June over the period of record. Graph shows regression line (solid) and 95% confidence intervals (dotted).

Table 5. Summary of annual regression analyses of log-transformed egg densities (standardized for discharge) and percentage change in mean daily discharge from two days to one day prior to egg collection at the San Marcial Site during May and June. Mean discharge for May-June is an average of all mean daily discharge values during those months.

SAMPLING YEAR (MAY-JUNE)	R^2	F-RATIO	p-VALUE	NUMBER OF OBSERVATIONS	MAXIMUM DISCHARGE (MAY-JUNE)	MEAN DISCHARGE (MAY-JUNE)
2002	0.62	6.56	0.06	6	446	54.43
2003	0.61	24.64	<0.0001	18	347	110.59
2004	0.24	0.32	0.67	3	1,560	524.28
2005	NA	NA	NA	NA	4,920	3,951.64
2006	0.65	15.13	<0.01	10	151	40.98
2007	0.03	0.98	0.33	39	2,400	1,113.15
2008	0.50	1.00	0.50	3	4,260	2,717.38
2009	0.00	0.01	0.91	9	3,560	2,191.00
2010	0.00	0.01	0.92	15	3,230	1,145.80
2011	0.35	18.46	<0.0001	36	456	148.79
2012	0.52	17.67	<0.001	18	514	108.07

The complex relationships among discharge, water temperature, and log-transformed egg densities at the San Marcial Site are presented as a contour plot (Figure 21). The colored space shown on the graph illustrates the boundary of all possible discharge/temperature combinations, resulting in an observed spawning event, over the period of record. While some spawning events occurred at cool water temperatures (< 15°C), these only occurred during low flows. At warmer water temperatures (ca. 19 to 26°C), there was a gradual decline in the flow at which spawning was observed (i.e., generally coinciding with low June discharge). There was a mottled pattern of spawning intensity across the range of discharge-temperature combinations. The highest densities of eggs were collected at the San Marcial Site between about 19 to 23°C, coinciding with either low flows (ca. 200 to 1,000 cfs) or high flows (ca. 1,700 to 2,300 cfs).

DISCUSSION

As rivers have become increasingly fragmented, one factor limiting the recolonization of upstream reaches and imperiling pelagic spawning cyprinids is the downstream transport of reproductive products below barriers or displacement into highly degraded downstream riverine habitats and reservoirs (Dudley and Platania, 2007). The negative impacts of dam-related modifications of flow and habitat on Great Plains stream cyprinids that employ drifting eggs and larvae as an early life history strategy have been well documented (Stanford and Ward, 1979; Cross et al., 1983; Cross et al. 1985, Cross and Moss, 1987; Winston et al., 1991; Luttrell et al., 1999). In the Middle Rio Grande, large numbers of eggs of the federally endangered Rio Grande silvery minnow have been estimated to be transported past downstream collecting sites (Dudley and Platania, 2011). The downstream transport of this reproductive effort from upstream sources is potentially one factor that led to the apparent loss of Rio Grande silvery minnow from the Cochiti Reach and to its decline in the Angostura Reach in the Middle Rio Grande (Platania and Altenbach, 1998).

In addition to the problems created by river fragmentation, habitat simplification (caused by flow regulation, bank armoring etc.) also appears to contribute to the downstream displacement of the Rio Grande silvery minnow reproductive effort. The closure of Cochiti Dam resulted in a greatly reduced passage of fine sediments through the Middle Rio Grande which has, in turn, contributed to channel degradation, armoring, and narrowing (Lagasse, 1985). Arroyos, backwaters, and other "nursery habitats" may result in increased upstream retention of eggs and larvae because their off-channel location often results in negligible water velocities (Porter and Massong, 2004a; Porter and Massong, 2004b; Pease et al., 2006). The reduction in the number and size of low velocity mesohabitats has likely reduced egg retention in upper reaches of the Middle Rio Grande.

Since Rio Grande silvery minnow is the only extant species of the previously discussed reproductive guild in the Middle Rio Grande, the species-specific identification of any semibuoyant egg collected during this study was unambiguous. The only other fish eggs that we have captured in the Middle Rio Grande during this and previous investigations were those of the common carp, *Cyprinus carpio*. Fortunately, there are numerous differences between eggs of these species that aid in identification. As the eggs of common carp are adhesive, there are usually small pieces of particulate matter attached to the chorion. Additionally, common carp eggs are smaller and more opaque than Rio Grande silvery minnow eggs, and the eyes of carp embryos become pigmented very early in development. Conversely, the eggs of Rio Grande silvery minnow are clear, non-adhesive, smooth, large, and the embryos lack discernible pigment.

Prior to spawning, there appears to be a steady increase in the gonadosomatic index (GSI) values of Rio Grande silvery minnow during early spring (Platania and Altenbach, 1996). As the GSI value is ratio of gonad weight to body weight, higher GSI values indicate an increased readiness to

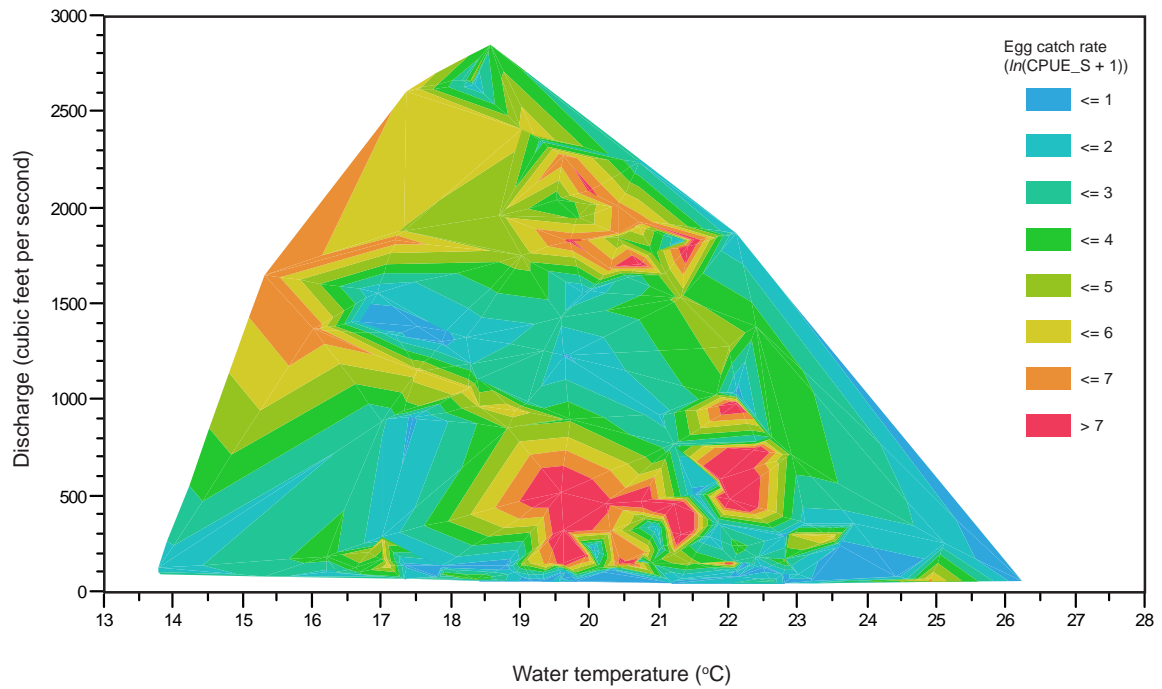


Figure 21. Contour plot of discharge, water temperature, and log-transformed egg densities (standardized for discharge) at the San Marcial Site over the period of record.

spawn. Field collections (1993 to 1995) indicated that the increase in GSI values generally corresponded with the gradually increasing flows of spring runoff along with the gradually increasing water temperatures of the river (Platania and Altenbach, 1996). It is possible that some combination of factors (e.g., extended photoperiod and increasing water temperatures) could be the initial trigger for GSI values to increase in the early spring and that the steadily increasing flows of spring runoff contribute to this effect, especially during higher flow years.

Spawning of Rio Grande silvery minnow and other members in its reproductive guild appears to be triggered by specific environmental cues (Platania and Altenbach, 1998). These fishes often spawned shortly after increases in flow during spring and summer months. Egg catch rates in the Pecos River and Rio Grande appear most closely related to flow increases as opposed to absolute water volume. This relationship has been observed throughout the Middle Pecos River from early-May until late-September. Spawning was closely correlated to sharp increases in flow from local rainstorms and egg catch rates would drop as soon as flows began to drop. This sequential pattern (increased flow, increased spawning, decreased flow, decreased spawning) occurred throughout the summer in the Pecos River, NM. By late-September, the association between spawning and flow was minimal, indicating the end of the reproductive season for the five members of the reproductive guild that occupy the Pecos River.

The results of this study suggest that the number of eggs produced in the Middle Rio Grande appears to be related to some combination of discharge and water temperature conditions. Large increases in flow over a relatively short period (from two days to one day prior to egg collection) seemed particularly related to increased spawning activity of Rio Grande silvery minnow. This relationship was especially robust during years when flows in May and June were relatively low (i.e., 2002, 2003, 2006, 2001, and 2012), which led to more dramatic increases in spawning following relatively brief increases in flow. However, the apparent propensity of Rio Grande silvery minnow to spawn shortly after these flow increases could have negative consequences on recruitment success in low flow years if appropriate nursery habitats do not persist over an extended period. In contrast, there often appeared to be a more protracted spawning period during higher flow years (i.e., 2004 and 2007 to 2010), primarily occurring during periods of elevated flow in May. While peak spawning by Rio Grande silvery minnow generally occurred soon after the initiation of spring runoff (often during the first two weeks of May), extended spawning while appropriate nursery habitats persist could lead to increased recruitment success.

Elevated flows lead to a series of changes to the physical dynamics of the river (e.g., increased water velocities/depths in some areas and augmented or new "flooded" low velocity habitats in other areas) that could be important spawning cues associated with rising flows. Additionally, there are frequently changes in water chemistry that accompany flow increases, particularly when large amounts of sediment are carried into the river from formerly dry shoreline areas, eroding banks, or flowing arroyos. The increased sediment load results in increased turbidity levels (decreased water clarity), slightly decreased water temperatures, and can lead to substantial increases in salinity levels. For example, three-fold increases/decreases in salinity levels (at the same sampling site) are regularly documented as a result of different flow conditions during spring/summer population monitoring surveys. It is possible the Rio Grande silvery minnow are spawning as a result of some combination of changing habitat and water chemistry conditions that result from these increased flow events.

While increases in discharge appear to be the primary cue for spawning, water temperature seems to be an important factor as well. Spawning appears to occur over a wide range of mean daily water temperatures (ca. 13 to 26°C) but with the majority occurring over a narrower range of temperatures (ca. 17 to 23°C). However, this interaction is quite complex and varies among years and reaches. Also, sampling has focused on May through mid-June as part of this project but spawning has been documented from late March into late June (Platania and Dudley, 2000). The

mean daily water temperatures during these extended periods were at the limits of the range at which spawning has been documented (i.e., 13 to 26°C).

It is possible that this range of spawning temperatures is even broader, particularly at warmer water temperatures, but there have been no longer-duration systematic studies conducted to document this possibility. Despite the lack of field spawning studies earlier or later in the year, experimental water temperature treatments on eggs and larvae of Rio Grande silvery minnow revealed that mortality was notably higher at 15°C or 30°C as compared with 20°C or 25°C (Platania, 2000). It is likely that individuals spawned earlier in the year (e.g., April) or later in the year (e.g., July), when water temperatures are at the presumed limits of their spawning range, have an increased rate of mortality. However, those individuals that are spawned slightly earlier in the year might have an increased chance of early survival as compared to those spawned later in the year since there would presumably be reduced competitive pressure from other early stage larval fishes, which are generally less abundant in May as compared with June or July (Pease et al., 2006).

There are, however, multiple factors that affect survival of Rio Grande silvery minnow throughout the spring and summer, including numerous abiotic (e.g., flow, temperature, water quality) and biotic (e.g., temporal/spatial resource availability, competitive interactions, predation pressure) factors. Genetic analyses of wild Rio Grande silvery minnow eggs and adults suggested that survival was highly variable, leading to large differences in reproductive success among individuals (Osborne et al., 2005). In fact, the broad range of conditions that result in Rio Grande silvery minnow reproduction could indicate that there is no "perfect" spawning cue (i.e., combination of abiotic/biotic conditions) that would result in a consistently higher chance of early survival and recruitment into the population. The closest combination of favorable conditions, based on the last decade of spawning studies, appears to be increased flows that occur with appropriately warm water temperatures. While increased flows can, and often do, lead to the creation of new or the expansion of existing larval fish nursery habitats and presumably higher recruitment success, there is no guarantee that flows will continue to rise or remain stable after spawning. Sometimes flow in the river will briefly increase and then return to low levels either as a result of changes in ambient temperature (affecting the rate of snowmelt) or as a result of short-term precipitation events. The young that are produced as a result of these flow events are often subjected to biotic and physical conditions that may preclude their successful growth and survivorship, particularly during summer months. Excessively elevated water temperatures (>30°C) in the Rio Grande, caused by warm ambient conditions and low flows, may reduce the hatching success of newly spawned eggs and survival of larvae. In addition to high water temperatures and possibly poor water quality, the likelihood of intra- and inter-specific interactions (e.g., predator-prey or competition) would be expected to increase during low flows as available aquatic habitat decreases. The complex interactions among abiotic and biotic variables in the early survival, growth, and recruitment of Rio Grande silvery minnow continue to give new insight to the patterns of their spatial spawning periodicity over time.

Linear regression analyses of October densities of Rio Grande silvery minnow from 1993-1997, 1999-2011 revealed significant associations with hydraulic variables (Dudley and Platania, 2012). The relationships that explained the most variation in mean density were number of days with discharge >2,000 cfs or >3,000 cfs (as measured at the Albuquerque gauge) and increased mean daily discharge (as measured at the San Marcial gauge). The physical conditions produced by prolonged and elevated flows result in overbank flooding of vegetated areas, formation of inundated habitats within the river channel, and creation of shoreline and island backwaters. Shallow low-velocity habitats (e.g., shoreline pools, backwaters, overbank floodplains etc.) are well known to be essential for the successful recruitment of early life history stages of many freshwater fish species throughout the world (for review see Welcomme, 1979). It is quite likely that similar processes are important for the successful survival and recruitment of the Middle Rio Grande ichthyofaunal community, including early life stages of Rio Grande silvery minnow (Pease et al., 2006; Turner et al.,

2010).

Efforts were made to estimate the number of eggs transported downstream of each sampling site based on the total number of eggs collected, volume of water sampled, volume of water available to be sampled, and duration of sampling. This approach required several simplifying assumptions including: 1) eggs were approximately evenly distributed within the volume of water passing the sampling site, 2) eggs collected during a four hour period of sampling in a given day approximately represented the rate at which eggs were transported downstream of the site during that day, and 3) the volume of water at the nearest upstream USGS station approximately represented the volume of water passing the sampling site. While these assumptions seem reasonable, it is likely that some unquantified error is introduced into the calculations through these extrapolations. Even with modest violations of these assumptions, the number of eggs estimated to be transported downstream would likely still be quite high. These results indicate a substantial downstream transport of drifting eggs at San Marcial Site in the Middle Rio Grande despite the seemingly modest numbers collected in individual MECs. However, it is unknown what proportion of these eggs was viable since determining egg viability was not an objective of this study.

The total number of Rio Grande silvery minnow eggs collected was generally obtained through direct counting of individual propagules in the field. This direct counting method worked for nearly all sampling days during the spawning season. However, there was an occasional need to preserve egg samples when the total number of eggs collected exceeded our ability to accurately count them while also effectively operating the MECs. This threshold was exceeded when more than about 1,000 eggs were collected every hour. While these elevated spawning events occurred only a few times, the need to accurately quantify the number of eggs was particularly crucial since these events compose the vast majority of the total spawning effort within a given year.

While we did not use estimates of the number of eggs collected during this study (i.e., only actual counts), we had employed a volumetric estimation of the number of eggs in 2001. Since 2002, we have only used actual counts because we found that volumetric determination of the number of Rio Grande silvery minnow eggs collected lacked the rigor necessary for effective evaluation of the relative level of spawning by this species. Based on several trials conducted in 2011, we also determined that time-based estimates of the number of eggs collected were even more problematic than volumetric estimates and consistently resulted in overestimated total egg counts. Time-based estimates of egg catch rate on 5 June 2011 were derived by enumerating the eggs collected during a given period (five-minutes) and extrapolated for the desired collecting period (i.e., two and six hours). All eggs collected during that sample date were retained and individually enumerated in the laboratory. Comparison of the time-based estimates of the eggs with the actual number of eggs retained revealed that the time-based estimates were nearly an order of magnitude higher than actual counts. These problems were most exacerbated when the number of eggs was higher than could be counted accurately and perhaps led to an excited tendency to overestimate (or round up) the number of eggs collected. While these issues could be partially addressed through training or replication to essentially "calibrate" the time-based (or volumetric) estimates to actual numbers, it is clear that for this study there is a need to count individual eggs to accurately evaluate the level of spawning by Rio Grande silvery minnow.

The total number of eggs collected at a site, from multiple MECs and over an extended daily sampling period, has been combined for the purposes of this report. The variation in egg catch rates among MECs and different sequential periods in a single day was minimal compared to the variation among sampling days. The primary purpose in sampling with two MECs over an extended time period was to filter an adequate volume of water to both detect the presence of eggs and to obtain an accurate estimate of the level of spawning over time. The volume of water currently sampled daily at each of the sampling sites is very high, primarily because of the use of the modified and more efficient sampling screens.

Population trends lend support to the observation that substantial numbers of eggs (and presumably larvae) are being transported downstream every year. In support of these observations, the highest densities of juvenile Rio Grande silvery minnow are most frequently found in the southern reaches of the Middle Rio Grande (Dudley and Platania, 2012). This trend was first noted over two decades ago (Bestgen and Platania, 1991), before Rio Grande silvery minnow was listed as a federally endangered species, and it persists to the present time. The few exceptions to this trend (i.e., higher densities of juveniles found in upstream reaches) have almost always occurred following years when flows were very low in the San Acacia Reach, resulting in moderate to substantial river drying and loss of fish in that area.

Despite the seemingly large number of Rio Grande silvery minnow propagules transported downstream every year, some portion do remain upstream (Dudley and Platania, 2007; Widmer et al., 2012). It is likely that the proportion of individuals retained and successfully recruited upstream is related to the complexity of instream habitat conditions and the long-term availability of nursery habitat. Since successful growth and survival of Rio Grande silvery minnow from the egg to the juvenile stage requires about six weeks (Platania, 1995), the persistence of these nursery habitats throughout this critical phase of development could lead to improved recruitment success.

Increased flows in May apparently resulted in an elevated spawning effort during the 2012 study period. However, summer monsoonal rainstorms (during late June and early July) that sometimes help maintain flow throughout the study area were largely absent in 2012, resulting in critically low flows during the post-spawning period. Rio Grande silvery minnow appear to have had a modest year for spawning but it is possible that poor recruitment in 2012 (apparently as a result of a truncated spring runoff followed by persistently low summer flows) could translate into decreased numbers of reproductively capable females available to spawn in the spring of 2013.

The potential association between Rio Grande silvery minnow CPUE, from the Population Monitoring Program (see Dudley and Platania, 2012), and the spring spawning magnitude was examined over the appropriate period of record (2002-2004 and 2006-2012). However, both the July and October CPUE values for Rio Grande silvery minnow in the San Acacia Reach yielded non-significant relationships ($p > 0.6$) with the spring spawning magnitude as measured at the San Marcial Site. These preliminary findings suggest that the number of eggs produced in the river during spring does not appear to be strongly related to the abundance of Rio Grande silvery minnow that survive to either July or October. It is likely that the physical conditions of the river immediately following spawning (e.g., extended periods with elevated flows vs. low flows accompanied by river drying) are more important than the quantity of eggs in determining the success of annual recruitment. Additionally, eggs collected at the San Marcial Site represented individuals that were going to be transported farther downstream, possibly into Elephant Butte Reservoir, and therefore might not contribute substantially to the San Acacia Reach population. Further, the number of Rio Grande silvery minnow eggs collected at a particular sampling site is likely a result of the complex interrelationships among the spatial distribution of upstream population concentrations, egg development time (i.e., faster at higher water temperatures), and local egg transport efficiencies (e.g., decreased habitat complexity will likely result in increased egg transport (Dudley and Platania, 2007; Widmer et al., 2012)). As it is unlikely that eggs collected near the downstream terminus of the range of Rio Grande silvery minnow would correlate to population trends in upstream areas, additional upstream sampling sites that minimally include coverage in all three reaches would provide the needed resolution to adequately investigate these potential relationships. Additional years of data will hopefully further elucidate these subtle relationships and lend insight to the causal mechanisms resulting in the successful recruitment of Rio Grande silvery minnow.

Populations of Rio Grande silvery minnow appear to have declined since 2009 and the lack of an adequately high spring runoff (high magnitude over an extended duration) combined with summer drying in 2012 could result in further population declines. The loss of individuals from

downstream reaches during river drying events is particularly problematic as these are the areas that most frequently and consistently support the highest densities of Rio Grande silvery minnow. The future conservation status of Rio Grande silvery minnow appears dependent on ensuring appropriate flow and habitat conditions during the spawning and early recruitment phases of this species, while also allowing upstream recolonization by individuals transported downstream.

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