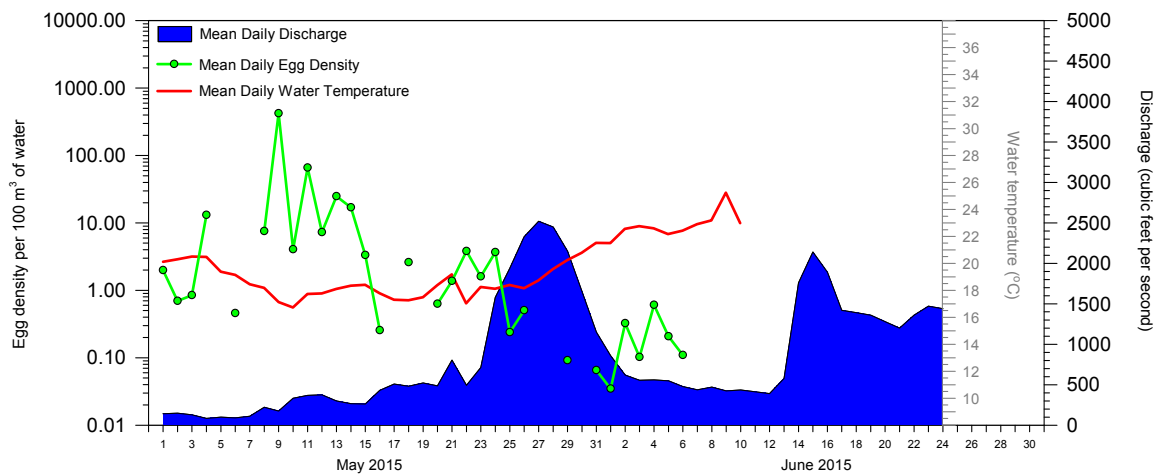
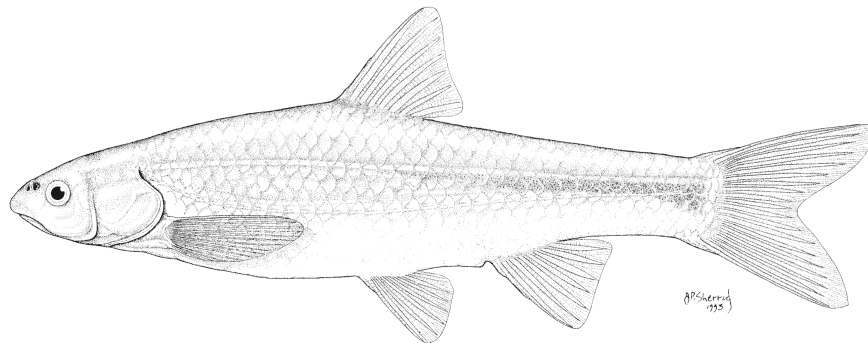


**MONITORING OF THE RIO GRANDE SILVERY MINNOW REPRODUCTIVE EFFORT DURING 2015
IN THE RIO GRANDE AND SELECTED IRRIGATION CANALS**

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



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25 September 2015

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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 has been conducted annually since 1999. Previous studies demonstrated May and June as the primary period of spawning activity. The 2015 study was a continuation of the long-term monitoring effort in the downstream-most river reach, just upstream of Elephant Butte Reservoir. Additionally, five upstream sites (three in the Isleta Reach and two in the San Acacia Reach) provided data on the entrainment of Rio Grande Silvery Minnow eggs into the Middle Rio Grande irrigation canal network from 2013–2015.

Rio Grande Silvery Minnow mixture-model estimates ($E(x)$), using standardized egg passage rate data ($E_p = \text{eggs} \cdot \text{second}^{-1}$) from 2003–2015, were highest in 2011 (5.40×10^1) and lowest in 2004 (9.62×10^{-4}). Values of E_p (i.e., daily estimates of the number of eggs passing the site per second) are indicative of the relative spawning intensity across years, corrected for annual differences in flow magnitude. There was a decline in the densities of eggs collected from 2011–2013, followed by an increase in 2014. The estimated values of $E(x)$ did not differ significantly ($P > 0.05$) from 2014 (5.41×10^0) to 2015 (7.05×10^{-1}).

General linear models of Rio Grande Silvery Minnow mixture-model egg passage rate estimates (Δ (δ) and μ (μ)) revealed that variation in δ and μ was only weakly predicted by changes in hydraulic variables (allowing for random effects) over the study period (2003–2015). The top model ($\delta(\text{Year}) \mu(\text{Year})$) received over 99% of the AIC_C weight (w_i). The next four models, which accounted for < 1% of the cumulative w_i , were primarily related to the interaction among δ , μ , and hydraulic variables representing elevated spring flows in the Angostura Reach. No models relating to the interaction among δ , μ , and previous year October fish density data received appreciable values of w_i (i.e., no models with $w_i > 0.01\%$). Thus, prolonged high flows during spring were most predictive of increased egg passage rate estimates of Rio Grande Silvery Minnow over the period of study.

Logistic regression modeling of Rio Grande Silvery Minnow egg presence-absence data revealed strong associations with the percentage change in mean daily discharge just prior to egg collection ($X^2 = 25.32$ and $P < 0.001$). The probability of collecting eggs was 0.83 during a 100% increase in mean daily discharge. An even more substantial increase in flow (e.g., Δ discharge = 200%) was predicted to have a correspondingly higher probability of collecting eggs (0.97).

While Rio Grande Silvery Minnow eggs were collected at only one of the three canal-monitoring sites during 2015, eggs were documented at all three river-monitoring sites. The mixture-model was used to estimate the egg passage rate at the Isleta ($E(x) = 0.43$), San Acacia ($E(x) = 0.60$), and San Marcial ($E(x) = 0.71$) sites; there were no differences ($P > 0.05$) among these estimates. The estimated egg passage rate at the Isleta Site was significantly higher ($P < 0.05$) than at the Belen ($E(x) = 0.00$) or Peralta sites ($E(x) = 0.01$). The estimated proportion of eggs entrained and water diverted in the Isleta Reach was 0.02 and 0.16, respectively. The estimated egg passage rate at the San Acacia Site was significantly higher ($P < 0.05$) than at the Socorro Site ($E(x) = 0.00$). The estimated proportion of eggs entrained and water diverted in the San Acacia Reach was 0.0 and 0.0, respectively.

Rio Grande Silvery Minnow spawning intensity appears strongly related to rapid increases in river flows during spring. Increased flow leads to a series of changes to the physical dynamics of the river (e.g., increased water velocities) and changing water chemistry conditions (e.g., increased turbidity/salinity) that could be important spawning cues. While increases in discharge appear to be the primary cue for spawning, water temperature seems to be an important factor as well with majority of spawning occurring over a relatively narrow range of temperatures (ca. 17 to 23°C).

While Rio Grande Silvery Minnow has declined markedly throughout its range since 2009, the modest spring runoffs of 2014 and 2015 resulted in increased spawning intensity as compared with 2013. Recruitment success during summer and fall of 2015 will likely be highly dependent on local flow conditions during spring and summer, respectively. The loss of individuals from downstream reaches during river drying events is particularly pertinent as these areas consistently support the highest occurrence and density levels of Rio Grande Silvery Minnow. The future conservation status of Rio Grande Silvery Minnow appears strongly dependent on consistently ensuring appropriate seasonal flow and habitat conditions to support the crucial spawning and early recruitment phases of this species.

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).

Historically, 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 snowmelt 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 was characteristic 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 are 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, to some extent, 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, and these larvae can 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 Rio Grande Silvery Minnow.

Population monitoring efforts over the past two decades (see Dudley et al., 2015) 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. Recent monitoring

efforts (Dudley et al., 2015) demonstrated that the October density of Rio Grande Silvery Minnow was significantly lower ($P < 0.05$) from 2012–2014 than in recently past years (e.g., 2010 and 2011) and was similar to record lows documented during another drought period (i.e., 2002 and 2003).

Systematic monitoring of the reproductive output of Rio Grande Silvery Minnow was first conducted in 1999 and included sampling in all three reaches of the Middle Rio Grande (Platania and Dudley, 2000). This comprehensive monitoring effort involved quantifying the occurrence and density of Rio Grande Silvery Minnow eggs from nine sites; spawning was documented from late March to late June of 1999. 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. 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 2006–2008 (Platania and Dudley, 2006, 2007, 2008) and resulted in the sampling of the Angostura, Isleta, and San Acacia reaches of the Middle Rio Grande. Recent monitoring of the Rio Grande Silvery Minnow reproductive effort in these three reaches has been conducted by a variety of organizations, including ASIR, and has occurred annually with some minor exceptions, from 2009–2015.

The spatial spawning periodicity study described herein is a continuation of the long-term systematic Rio Grande Silvery Minnow reproductive monitoring research study. The primary objectives of this study are to characterize the timing, duration, and magnitude of Rio Grande Silvery Minnow reproduction and assess the entrainment of eggs into canals in the Isleta and San Acacia reaches 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, fish density, and spawning magnitude; and assessing spatial spawning patterns from multiple canal and river sites in the Isleta and San Acacia reaches. Long-term monitoring of the reproductive effort of Rio Grande Silvery Minnow provides insight to potential factors affecting annual reproductive output, remains relevant and necessary for ongoing recovery efforts, and provides managers with timely scientific information on which to base key natural resource management decisions in the Middle Rio Grande.

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 were taken from the diversion structure at the upstream boundary of that reach of river. There were three sampling sites in both the Isleta Reach (Isleta Diversion Dam to San Acacia Diversion Dam) and the San Acacia Reach (San Acacia Diversion Dam to inflow of Elephant Butte Reservoir).

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. A wide and braided river channel, sand substrate, high sediment load, and a broad variety of aquatic mesohabitats characterize sections of this reach. 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 Reach (River Mile 55.0). The sampling site is downstream of a U.S. Geological Survey stream gaging station located near San Marcial, New Mexico (# 08358400), which is the nearest upstream San Acacia Reach gage. In addition to easy accessibility and favorable river conditions (e.g., 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 2006–2015.

Additionally, five new sites have been sampled since 2013 for the purpose of assessing spatial spawning patterns in the Isleta and San Acacia reaches. Three sampling sites were added in the Isleta Reach while two sampling sites were added in the San Acacia Reach. In the Isleta Reach, two sampling sites were added in canals (Belen High Line Canal and Peralta Canal) and one site was added in the river just downstream of Isleta Diversion Dam. In the San Acacia Reach, one site was established in the Socorro Main Canal and one in the river just downstream of San Acacia Diversion Dam. These additional sampling sites not only allowed for a more detailed assessment of spatial spawning patterns over a nearly 200 km reach of the Rio Grande but also enabled a direct comparison between canal and river monitoring localities in the two downstream-most reaches of the study area.

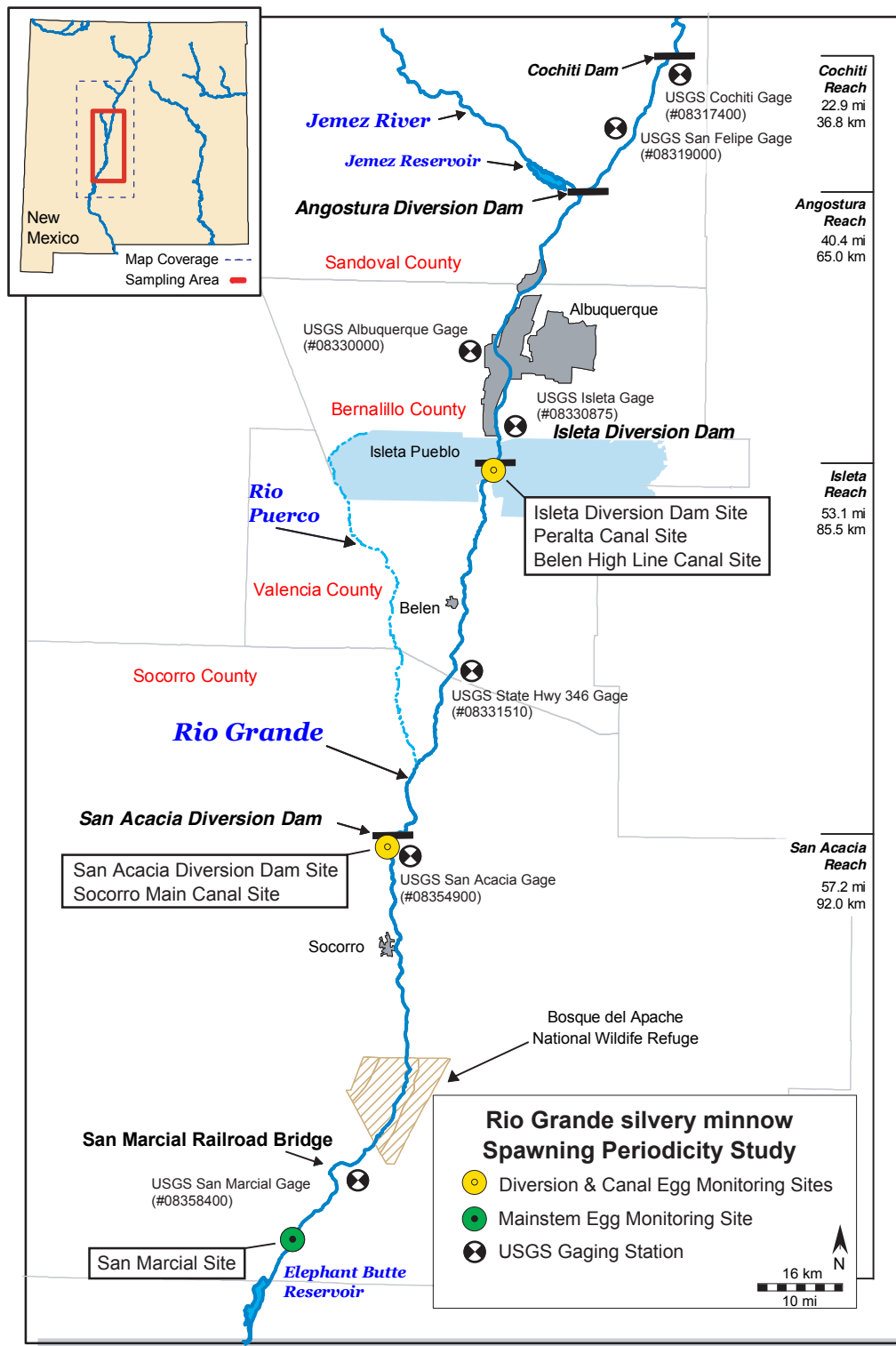


Figure 1. Map of the Middle Rio Grande, New Mexico, and the 2015 study site locations.

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 efficiency of the MEC (i.e., greater volume of water sampled). Density 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 density of eggs per unit of water determined. The density of drifting eggs (D) was calculated as the total number of eggs (n) collected relative to the total volume of water (V) sampled, using the formula: $D = ((n / V) \cdot 100)$. The total number of eggs passing a sampling site in one day (E) was estimated by using egg sampling data from the site and mean daily discharge ($Q = \text{m}^3 / \text{s}$) from the nearest upstream USGS gaging station, using the formula: $E = ((D / 100) \cdot Q \cdot 86,400 \text{ s})$.

Previous studies demonstrated May and June as the primary period of Rio Grande Silvery Minnow reproductive activity (Dudley and Platania, 2014). The normal sampling regime in 2015 consisted of an intensive daily sampling effort at each sampling site. 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. Two MECs were used at the San Marcial Site and one MEC was used at the other five sampling sites.

Rio Grande Silvery Minnow egg density values are (in part) dependent on flow conditions, thereby precluding unadjusted comparison of inter-annual densities. For example, higher flow volume will result in lower density assuming the number of eggs in the water column remains constant. Egg density (D) was standardized to a downstream passage rate (E_p) based on mean daily discharge (Q) to account for these differences, using $E_p = ((D / 100) \cdot Q)$. Values of E_p (i.e., eggs $\cdot \text{second}^{-1}$) are indicative of the relative spawning intensity among years, corrected for inter-annual differences in flow magnitude. All USGS and MRGCD discharge data presented graphically or analyzed statistically in this report are provisional and subject to change.

Volumetric determination of the number of eggs collected, as employed in 2001, lacked the rigor necessary to evaluate the relative level of spawning. Changes initiated in the 2002 sampling protocol (e.g., direct counts of all eggs collected) were instituted to increase the amount and utility of the information acquired from this research study. However, the continuous sampling protocols employed in 2002, during peak spawning events, were not used in subsequent years. The data collected in 2002 were also highly skewed, making them unsuitable for computing a valid estimate of $E(x)$ (described below). The aforementioned differences between pre-2002 and post-2002 studies precluded use of 2001–2002 data for quantitative or statistical comparison with data from subsequent years. There have not been changes to the sampling methodology for quantitative determination of egg densities since 2003, which justified the statistical methods used to analyze the long-term dataset (2003–2015).

Mixture models (e.g., combining a binomial distribution with a lognormal distribution) have been shown to be particularly effective for modeling ecological data with multiple zeros (White, 1978; Welsh et al., 1996; Fletcher et al., 2005; Martin et al., 2005). Long-term Rio Grande Silvery Minnow spawning data (2003–2015) were analyzed using PROC NLMIXED (SAS, 2015), a numerical optimization procedure, by fitting a mixture model consisting of the binomial and lognormal distributions using the methods outlined in White (1978). Egg passage rate data [E_p], during the most common sampling period (1 May to 10 June), were used for the analysis. Logistic regression was used to model the probability that eggs were collected on any given day, and the lognormal model was used to model the distribution of E_p given that eggs were collected. Models provided four parameter estimates for each year (δ = probability of egg occurrence, μ = mean of the lognormal E_p distribution, σ = standard deviation of the lognormal E_p distribution, and $E(x)$ = estimate of E_p).

General linear models were used to incorporate covariates to model δ , μ , and σ where a logit link was used for δ and log links were used for μ and σ . In the simplest case with no covariates and no random effects, this model can be considered a zero-inflated lognormal model. Covariates considered for

modeling spawning data included sampling year (Year) and various hydraulic variables at USGS Gages (#08330000 [ABQ; Rio Grande at Albuquerque, NM] and #08358400 [SAN; Rio Grande Floodway at San Marcial, NM]). Maximum discharge (ABQmax) and days exceeding threshold discharge values in 1,000 cfs increments (days > 1,000 [ABQ>1,000], 2,000 [ABQ>2,000], 3,000 [ABQ>3,000], and 4,000 [ABQ>4,000] cubic feet per second, cfs) represented the typical range of spring runoff conditions (May–June). The onset of lower flows (i.e., first day with discharge < 200 cfs after 1 June [SAN1stday<200]), mean daily discharge (SANmean), and lower threshold discharge values (days < 200 [SAN<200] and < 100 [SAN<100] cfs) represented some general characteristics of low flow conditions during the previous irrigation season (March–October) of each sampling year. Additionally, the estimated density of Rio Grande Silvery Minnow $E(x)$ from the previous October of each sampling year (Dudley et al., 2015) was included as a covariate (RGSM) in the model. Fixed effects models for each covariate were linear models ($\beta_0 + \beta_1 \times \text{covariate}$) with the corresponding link function. These fixed effects assume that variation in the data is explained by the covariate. That is, for δ , there is no over-dispersion or extra-binomial variation, and for μ , no extra variation provided beyond the constant σ model. Random effects models were also considered for δ and μ to provide additional variation around the fitted line where a normally distributed random error with mean zero and non-zero standard deviation is used to explain deviations around the fitted covariate. Adaptive Gaussian quadrature as described in Pinheiro and Bates (1995) was used to integrate out these random effects in fitting the model.

Goodness-of-fit statistics (logLike = -2[log-likelihood] and AIC_C = Akaike's information criterion [Akaike, 1973] for finite sample sizes) were generated to assess the relative fit of data to various models among all years sampled. Lower values of AIC_C indicate a better fit of the data to the model. Models were ranked by AIC_C values and the top ten models, based on AIC_C weight (w_i), were presented. As environmental covariates were only used to model a single parameter (δ or μ), potential issues of multicollinearity were avoided. Further, AIC_C model selection ranks single-variable models appropriately even if variables are highly correlated (i.e., resulting w_i values would be similar).

Logistic regression modeling was used to determine the relationship between the probability of collecting eggs and the percentage change in mean daily discharge from two days to one day prior to egg collection, using long-term sampling data (2003–2015). This was to allow time for the discharge changes occurring at the San Marcial gage to reach the San Marcial Site (ca. 25 km downstream). This metric best represented the approximate change in mean daily discharge that occurred just prior to spawning. Regression models were developed for the San Marcial Site using all data over the period of record. The associated 95% confidence intervals of the modeled regression line were constructed using inverse predictions of discharge across the range of modeled egg collection probabilities (SAS, 2007).

Estimated egg passage rates in the river were compared to those in the canals at similar longitudinal locations, using data collected since 2013. The proportion of eggs entrained into the canals ($E(x)_{EC}$) was computed based on the total of the estimated egg passage rates in the canals ($E(x)_C$) and the total of the estimated egg passage rates from both the river and the canals ($E(x)_{C+R}$), using the formula: $E(x)_{EC} = E(x)_C \cdot E(x)_{C+R}^{-1}$. Similarly, the proportion of water diverted into the canals (Q_{DC}) was computed based on the total of the average discharge in the canals (Q_C) and the total of the average discharge in both the river and the canals (Q_{C+R}), using the formula: $Q_{DC} = Q_C \cdot Q_{C+R}^{-1}$. The average discharge in the river and the canals was calculated based on mean daily discharge values during May.

A modified filtering screen to separate drifting debris from Rio Grande Silvery Minnow eggs was developed and tested for the MEC in 2009. Experimental tests revealed that the modified screen was more efficient at sampling a larger volume of water than was the old screen but that the egg density estimates were very similar (Platania and Dudley, 2009). Thus, all MECs have been fitted with the modified screen since 2009.

Temperature-logging devices were deployed at each study site to record hourly water temperatures. Each data logger was approximately located in the middle of the water column and attached to a steel post. Data loggers have a published $\pm 0.21^\circ\text{C}$ level of accuracy over 0°C to 50°C and a resolution of about 0.02°C over the same range of temperatures. The stability (drift) of this device is 0.1°C per year and use generally did not exceed three years. If data loggers became buried in the substrate or were no longer submerged in the water column, corrective measures were taken and invalid data were not included in further analysis. Mean daily water temperature data from canal and river monitoring sites were presented graphically for comparative purposes.

RESULTS

Hydrology (2001–2015)

Flows in the Middle Rio Grande have fluctuated dramatically among years since the beginning of this study in 2001 (Figure 2 and Appendix A). 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 supplemented 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 a 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 June) was extremely low because of minimal spring snowmelt runoff. Spring flows were markedly higher from 2007–2010 as compared with 2006, but returned to very low conditions from 2011–2013 with some improvement in 2014–2015. During the 2015 sampling efforts, flows at the San Marcial Gage ranged from 76 to 2,520 cfs (mean = 579.4 cfs). Flows peaked on 27 May 2015 (2,520 cfs) at San Marcial but quickly dropped below 500 cfs by early June.

Spawning Periodicity (2001–2015)

Despite substantial inter-annual differences in Rio Grande Silvery Minnow spawning metrics at San Marcial (Table 1), there were several similarities apparent regarding the timing of reproduction over the study period (Figure 3 and Appendix B). Based on the results of data taken from all years of the project, spawning was found to occur in April, May, and June. While the frequency and duration of spawning were highly variable among years, the highest numbers of eggs were consistently collected during a relatively short period in May. Two notable exceptions to this pattern occurred in 2006 and 2011, respectively, when peak spawning coincided with elevated flows in early June. During 2015, mean daily water temperature at the San Marcial Site fluctuated from about 17°C to 22°C in May but consistently remained above 21°C during June.

Mean daily water temperatures during the initial and peak spawning events were relatively similar among years. In general, mean daily water temperatures ranged from about 17 to 22°C during peak spawning events. However, spawning occurred across a wide range of water temperatures over the period of study. Mean daily water temperatures ranged between 16.7°C and 22.8°C during days when eggs were collected at the San Marcial Site in 2015.

Rio Grande Silvery Minnow mixture-model estimates ($E(x)$), using standardized egg passage rate data (E_p) from 2003–2015, revealed notable differences among sampling years (Figure 4). Standardized egg passage rates were highest in 2011 (5.40×10^1) and lowest in 2004 (9.62×10^{-4}). There was a steady decline in the densities of eggs collected from 2011–2013, followed by an increase in 2014. The estimated values of $E(x)$ did not differ significantly ($P > 0.05$) from 2014 (5.41×10^0) to 2015 (7.05×10^{-1}). Simple estimates of mean densities, using the method of moments, were similar to values of $E(x)$ over time.

General linear models of Rio Grande Silvery Minnow mixture-model egg passage rate estimates (Delta (δ) and Mu (μ)) revealed that variation in μ and δ was only weakly predicted by changes in hydraulic variables (allowing for random effects) over the period of study (2003–2015). The top model ($\delta(\text{Year}) \mu(\text{Year})$) received over 99% of the AIC_C weight (w_i) and had an AIC_C value of 1,146.02 (Table 2). The next four models, which accounted for < 1% of the cumulative w_i , were primarily related to the interaction among δ , μ , and hydraulic variables representing elevated spring flows in the Angostura Reach (e.g., ABQmax and ABQ>2,000). No models relating to the interaction among δ , μ , and previous year October fish density data received appreciable values of w_i (i.e., no models with $w_i > 0.01\%$). Thus, prolonged high flows during spring were most predictive of increased egg passage rate estimates of Rio Grande Silvery Minnow over the period of study.

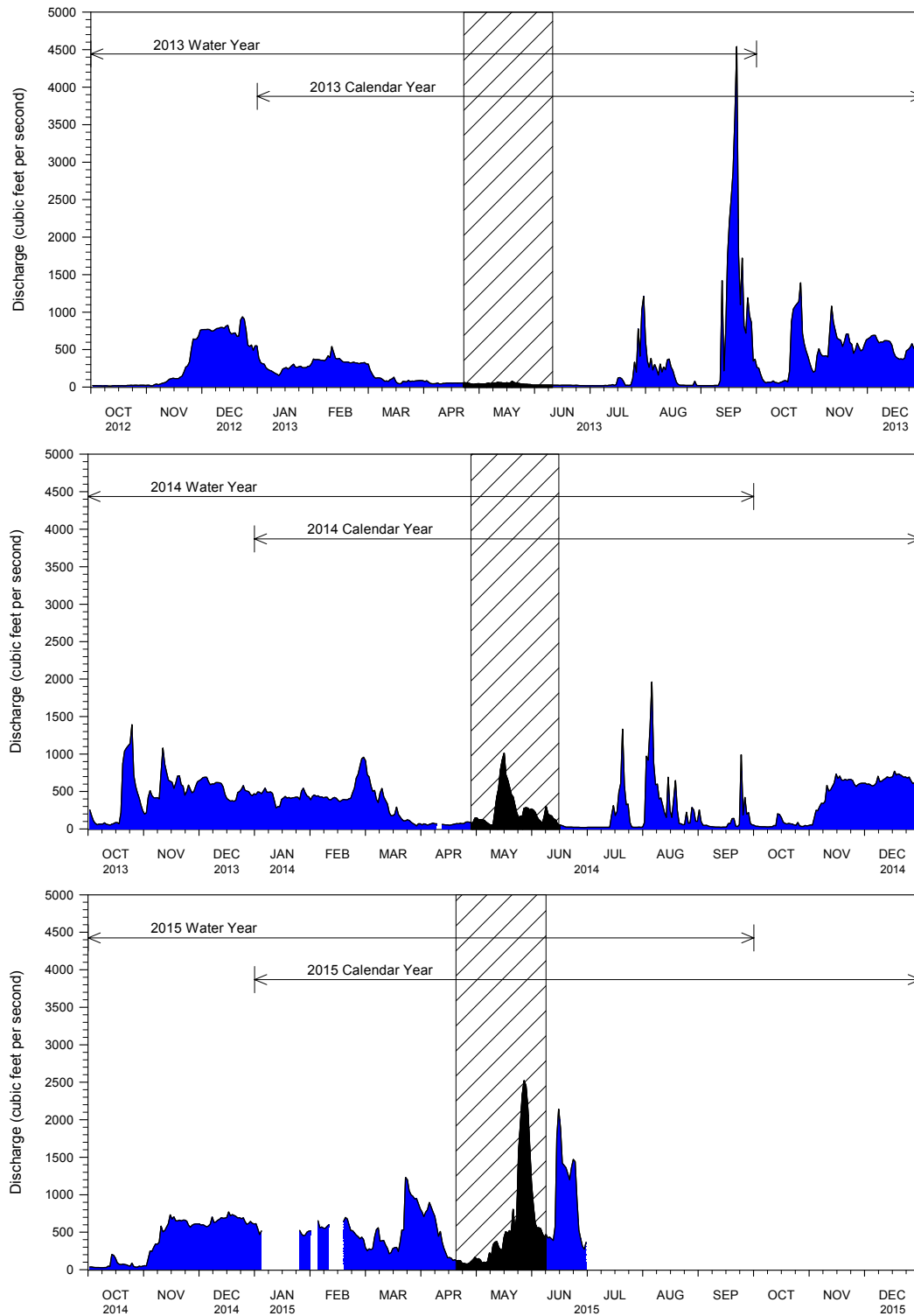


Figure 2. Annual hydrograph of the Rio Grande, New Mexico, at the San Marcial Gaging Station before, during, and after the 2013–2015 Rio Grande Silvery Minnow spawning periodicity study period. Cross-hatching indicates annual study period.

Table 1. Rio Grande Silvery Minnow spawning summary data by year and category (eggs present, eggs absent, percent frequency of occurrence, and maximum daily density) at the San Marcial Site (NS = Not sampled).

Sampling Year	Eggs Present (Number of Days)	Eggs Absent (Number of Days)	Percent (%) Frequency of Occurrence	Maximum Daily Density (# / 100 m ³)
2003	18	28	39.1	475.63
2004	3	43	6.5	0.09
2005	NS	NS	NS	NS
2006	10	36	21.7	289.33
2007	39	7	84.8	90.13
2008	3	43	6.5	5.10
2009	13	34	27.7	8.05
2010	15	32	31.9	9.47
2011	39	17	69.6	2,334.93
2012	18	30	37.5	466.71
2013	13	37	26.0	61.00
2014	25	25	50.0	560.22
2015	30	20	60.0	423.00

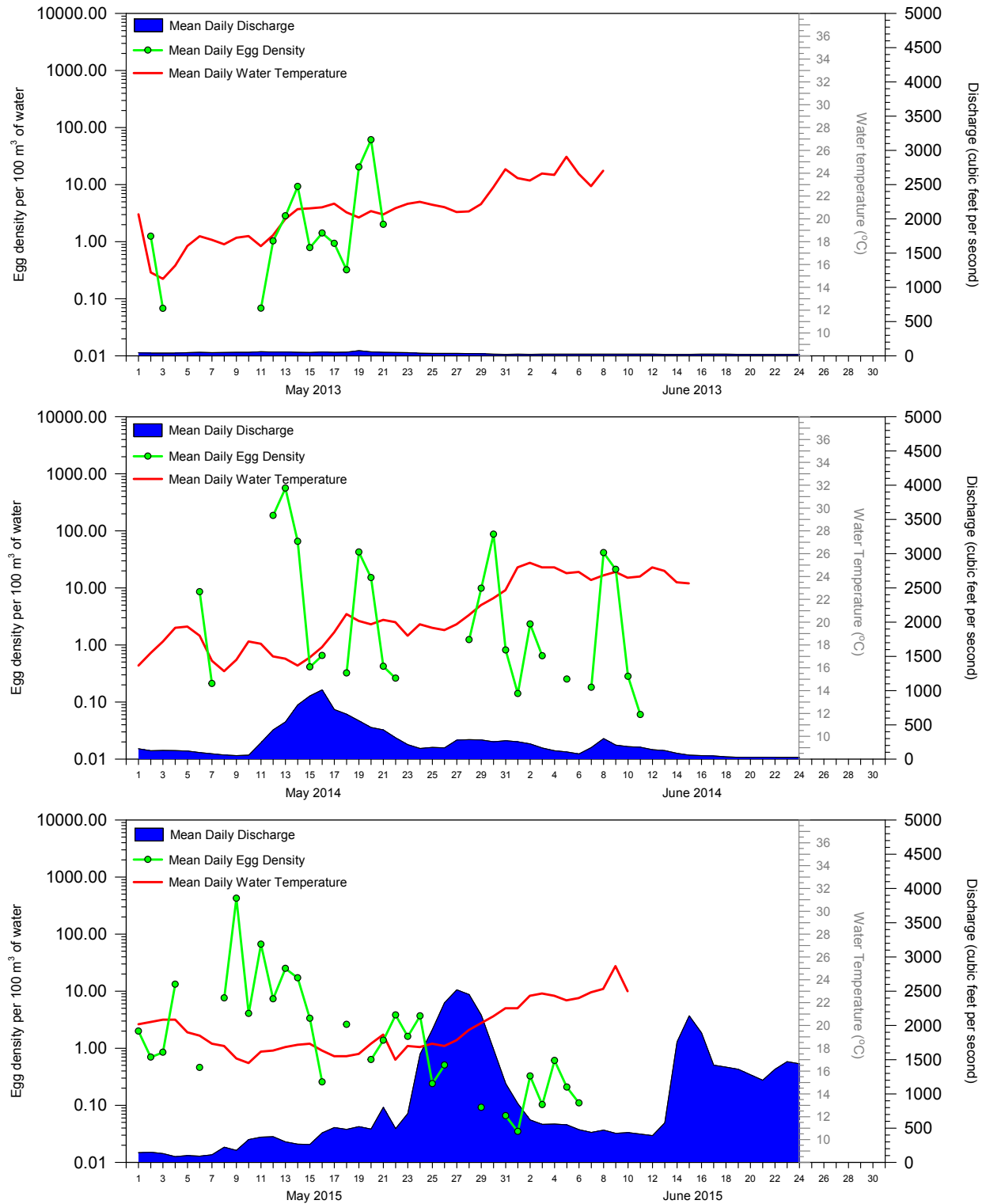


Figure 3. Mean daily discharge, mean daily egg density, and mean daily water temperature during the 2013–2015 Rio Grande Silvery Minnow spawning periodicity study period at the San Marcial Site. Note that the Y-axis for egg density is a log-scale.

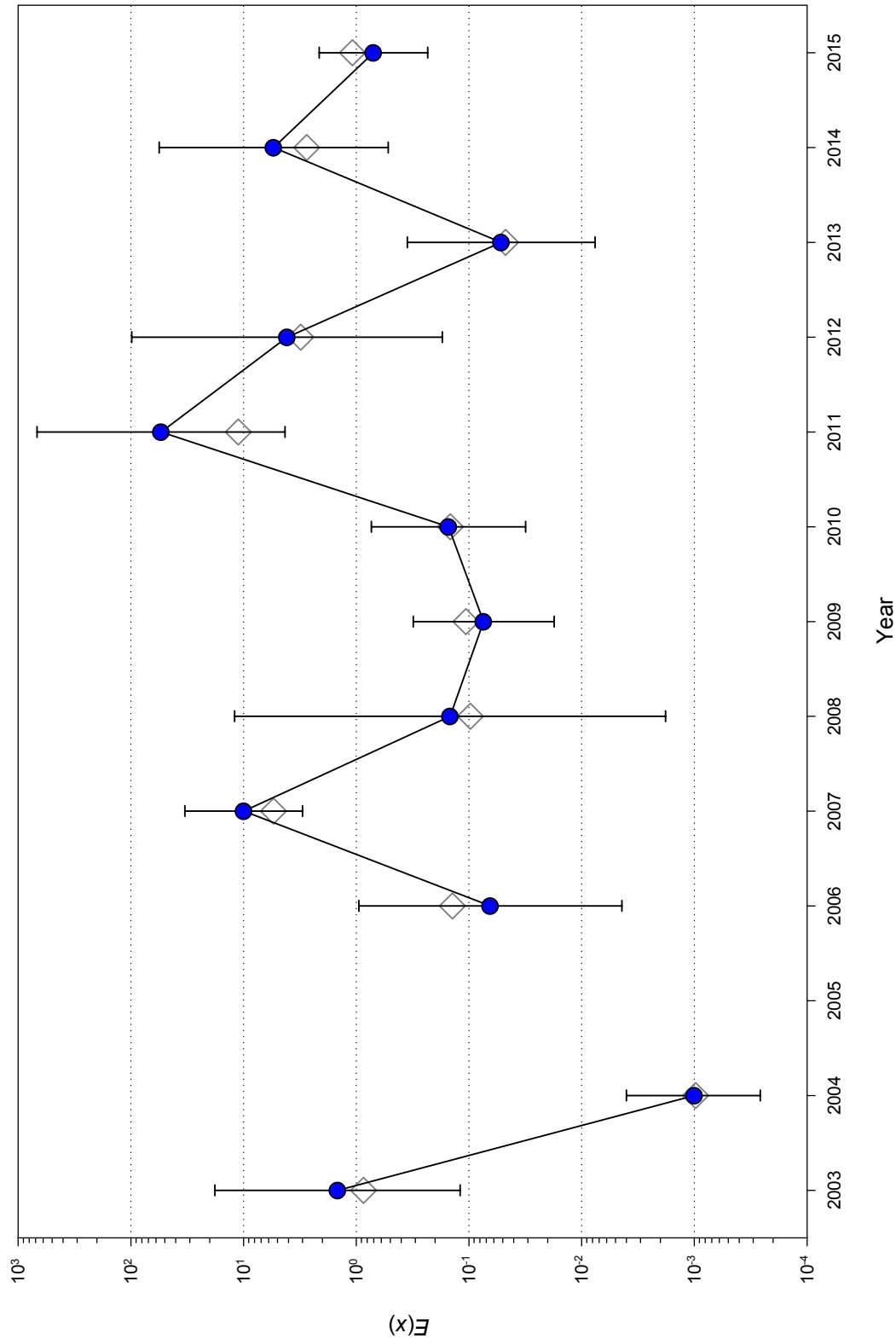


Figure 4. Rio Grande Silvery Minnow mixture-model estimates ($E(x)$), using egg passage rate data (E_p) at the San Marcial Site (2003–2015). Solid circles indicate modeled estimates and bars represent 95% confidence intervals. Dotted lines represent different orders of magnitude. Gray diamonds indicate simple estimates of mean densities using the method of moments.

Table 2. General linear models of Rio Grande Silvery Minnow mixture-model estimates (Delta (δ) and Mu (μ)), using standardized egg passage rate data (E_p) at the San Marcial Site from 2003–2015, and covariates (allowing for random effects [R]). The top ten models are ranked by Akaike's information criterion (AIC_C) and include the AIC_C weight (w_i).

Model ¹	logLike ²	K ³	AIC_C	w_i
$\delta(\text{Year}) \mu(\text{Year})$	1,068.10	36	1,146.02	0.9947
$\delta(\text{ABQmax}+R) \mu(\text{ABQ}>1,000+R)$	1,140.09	9	1,158.47	0.0020
$\delta(\text{SAN}<100+R) \mu(\text{ABQ}>1,000+R)$	1,140.58	9	1,158.96	0.0015
$\delta(\text{ABQ}>1,000+R) \mu(\text{ABQ}>2,000+R)$	1,140.62	9	1,159.00	0.0015
$\delta(\text{ABQmax}+R) \mu(\text{ABQ}>3,000+R)$	1,144.16	9	1,162.54	0.0003
$\delta(\text{SANmean}+R) \mu(\text{SAN}<200+R)$	1,152.04	9	1,170.42	<0.0001
$\delta(\text{ABQmax}+R) \mu(\text{SAN}<200+R)$	1,152.82	9	1,171.20	<0.0001
$\delta(\text{ABQ}>4,000+R) \mu(\text{ABQ}>1,000)$	1,167.56	7	1,181.79	<0.0001
$\delta(\text{ABQ}>3,000+R) \mu(\text{ABQ}>1,000)$	1,169.22	7	1,183.46	<0.0001
$\delta(R) \mu(\text{ABQ}>1,000)$	1,171.33	6	1,183.50	<0.0001

¹ = Model variables included year (2003–2015), various hydraulic variables at USGS Gages (#08330000 [ABQ; Rio Grande at Albuquerque, NM] and #08358400 [SAN; Rio Grande Floodway at San Marcial, NM]), and the estimated density of juvenile/adult Rio Grande Silvery Minnow $E(x)$ from the previous October of each sampling year

² = $-2[\log\text{-likelihood}]$ of the model

³ = Number of parameters in the model

Logistic regression modeling of Rio Grande Silvery Minnow egg presence-absence data revealed strong associations with the percentage change in mean daily discharge just prior to egg collection ($X^2 = 25.32$ and $P < 0.001$; Figure 5). Flows used to calculate Δ discharge ranged from < 50 cfs to $> 3,000$ cfs. The probability of collecting eggs ranged from 0.23 (Δ discharge = -50%) to 0.44 (Δ discharge = 0%) during periods of declining or stable flows, respectively. The probability of collecting eggs was predicted to increase rapidly up to approximately a 100% increase in mean daily discharge between days just prior to egg collection. The probability of collecting eggs during a 100% increase in flow was 0.83. A large percentage increase in flow (e.g., Δ discharge = 200%) was predicted to have a correspondingly higher probability of collecting eggs (0.97).

Spatial Spawning Patterns (2015)

Canal-monitoring sites

Sampling at the canal-monitoring sites was conducted during weekdays, excluding holidays, from 1 May through 31 May. The cumulative volume of water sampled was similar at the Peralta, Socorro, and Belen sampling sites (4,997.9 m³, 5,985.0 m³, and 4,265.2 m³). Rio Grande Silvery Minnow eggs were only collected at the Peralta canal-monitoring site during 2015 (Table 3 and Figure 6). The mixture-model was used to estimate the egg passage rate at the Peralta site ($E(x) = 0.01$; Figure 7), and the number of eggs estimated to be transported downstream of this site over the duration of the study was 11,718. The highest number of eggs estimated to be transported downstream in a single day was 8,927 (22 May 2015). The lack of documented spawning at the canal-monitoring sites in 2015 precluded any further analysis of spatial spawning patterns for these sites.

River-monitoring sites

Sampling at the Isleta and San Acacia river-monitoring sites was conducted during weekdays, excluding holidays, from 1 May through 31 May. Sampling at the San Marcial Site was conducted daily from 20 April through 8 June. The cumulative volume of water sampled at the Isleta and San Acacia sites was similar (4,410.4 m³ and 4,265.3 m³, respectively) but notably higher at the San Marcial site (73,200.0 m³). Two MECs were used at the San Marcial Site and sampling occurred over a longer duration (i.e., more days and hours/day) there as compared with the two upstream river-monitoring sites. Rio Grande Silvery Minnow spawning output was documented at all three river-monitoring sites (see Table 3). The three river-monitoring sites yielded 6,545 eggs; the vast majority was collected at the San Marcial Site ($n = 6,356$; Figure 8).

The mixture-model was used to estimate the egg passage rate at the Isleta ($E(x) = 0.43$), San Acacia ($E(x) = 0.60$), and San Marcial ($E(x) = 0.71$) sites (see Figure 7). There was no difference ($P > 0.05$) between the Isleta, San Acacia, and San Marcial estimates. The number of eggs estimated to be transported downstream over the duration of the study was 648,028 at Isleta, 937,151 at San Acacia, and 3,648,994 at San Marcial. The highest number of eggs estimated to be transported downstream in a single day was 2,297,507 (9 May 2015) at the San Marcial Site.

Comparisons among canal and river monitoring sites

Sampling at the Belen and Peralta canal-monitoring sites was longitudinally comparable to sampling at the Isleta river-monitoring site. Water temperatures were similar among these three sampling sites (range $\approx 15^\circ\text{C}$ to 21°C) with the difference in mean daily temperatures rarely exceeding 1°C . Differences in temperatures were typically $< 0.5^\circ\text{C}$ with the Belen and Isleta sites showing the most congruence. The largest temperature differences were observed when flows were very high at the Isleta site (i.e., resulting in lower temperatures at that site). While there were only three eggs collected at the Peralta Site and none from the Belen Site, the Isleta Site yielded a total of 54 eggs. The estimated egg passage rate at the Isleta Site ($E(x) = 0.43$) was significantly higher ($P < 0.05$) than at the Belen ($E(x) = 0.00$) or Peralta sites ($E(x) = 0.01$). The estimated proportion of eggs entrained into the canals ($E(x)_{EC}$) was 0.02.

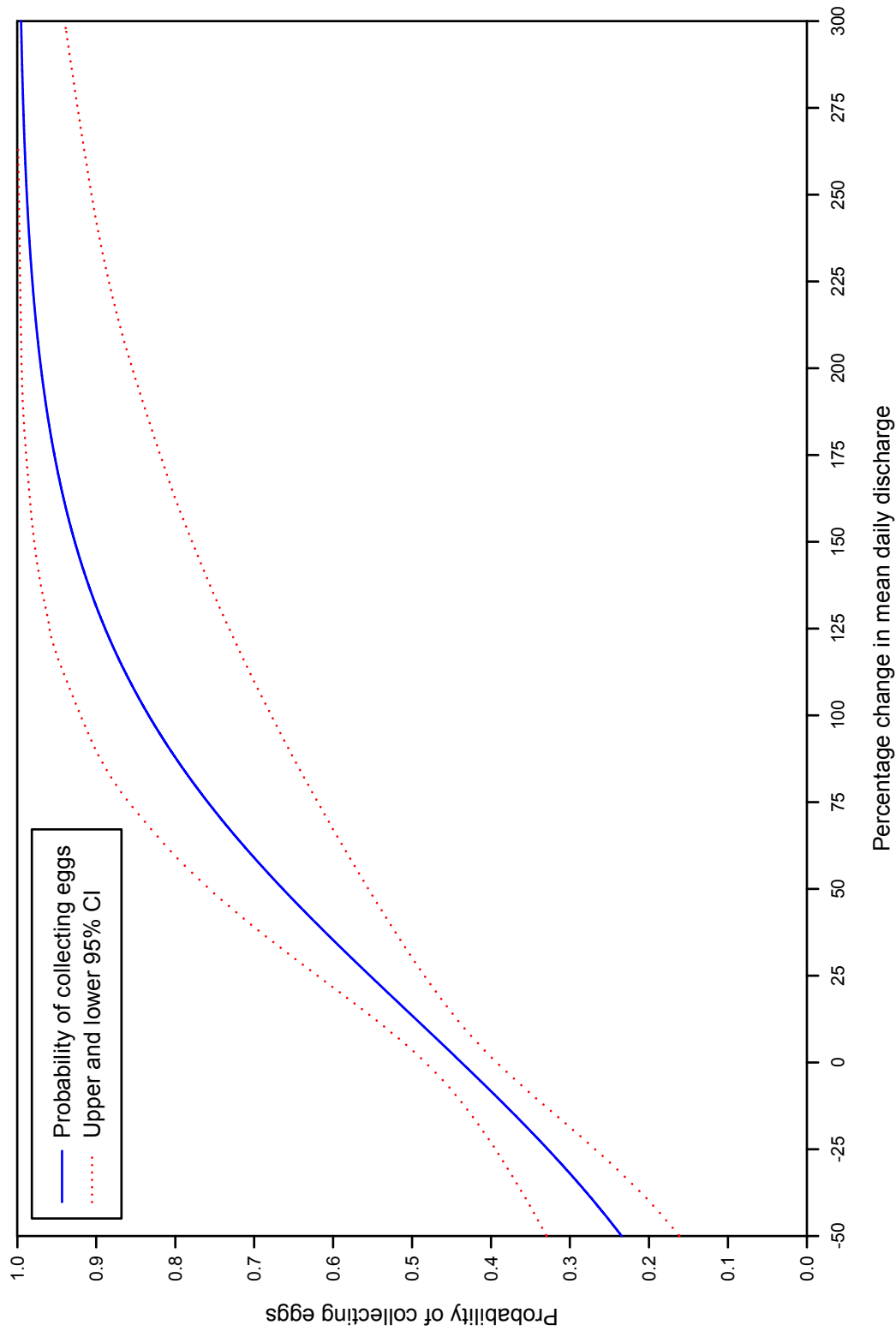


Figure 5. Logistic regression plot, using San Marcial Site data (2003–2015), illustrating the probability of collecting eggs as a function of the percentage change in mean daily discharge from two days to one day prior to egg collection. Graph shows logistic regression line (solid) and 95% confidence intervals (dotted).

Table 3. Number of Rio Grande Silvery Minnow eggs collected per day at each of the six sampling localities (canal sites are highlighted in gray). Table does not include dates that eggs were not collected at any of the sampling localities (NS = Not Sampled; only San Marcial Site sampled on weekends/holidays).

Sampling Date	Belen High Line	Peralta	Socorro Main	Isleta	San Acacia	San Marcial
1-May-15	0	0	0	0	0	21
2-May-15	NS	NS	NS	NS	NS	8
3-May-15	NS	NS	NS	NS	NS	7
4-May-15	0	0	0	0	0	120
6-May-15	0	1	0	20	0	4
7-May-15	0	0	0	0	122	0
8-May-15	0	1	0	7	0	67
9-May-15	NS	NS	NS	NS	NS	4,107
10-May-15	NS	NS	NS	NS	NS	59
11-May-15	0	0	0	2	9	883
12-May-15	0	0	0	0	2	92
13-May-15	0	0	0	0	0	408
14-May-15	0	0	0	0	0	261
15-May-15	0	0	0	0	0	54
16-May-15	NS	NS	NS	NS	NS	4
18-May-15	0	0	0	0	0	37
19-May-15	0	0	0	2	1	0
20-May-15	0	0	0	7	0	9
21-May-15	0	0	0	16	0	24
22-May-15	0	1	0	0	1	64
23-May-15	NS	NS	NS	NS	NS	31
24-May-15	NS	NS	NS	NS	NS	48
25-May-15	0	0	0	0	0	3
26-May-15	0	0	0	0	0	7
29-May-15	0	0	0	0	0	2
31-May-15	NS	NS	NS	NS	NS	2
1-Jun-15	NS	NS	NS	NS	NS	1
2-Jun-15	NS	NS	NS	NS	NS	9
3-Jun-15	NS	NS	NS	NS	NS	3
4-Jun-15	NS	NS	NS	NS	NS	16
5-Jun-15	NS	NS	NS	NS	NS	3
6-Jun-15	NS	NS	NS	NS	NS	2
Total (All Days)	0	3	0	54	135	6,356

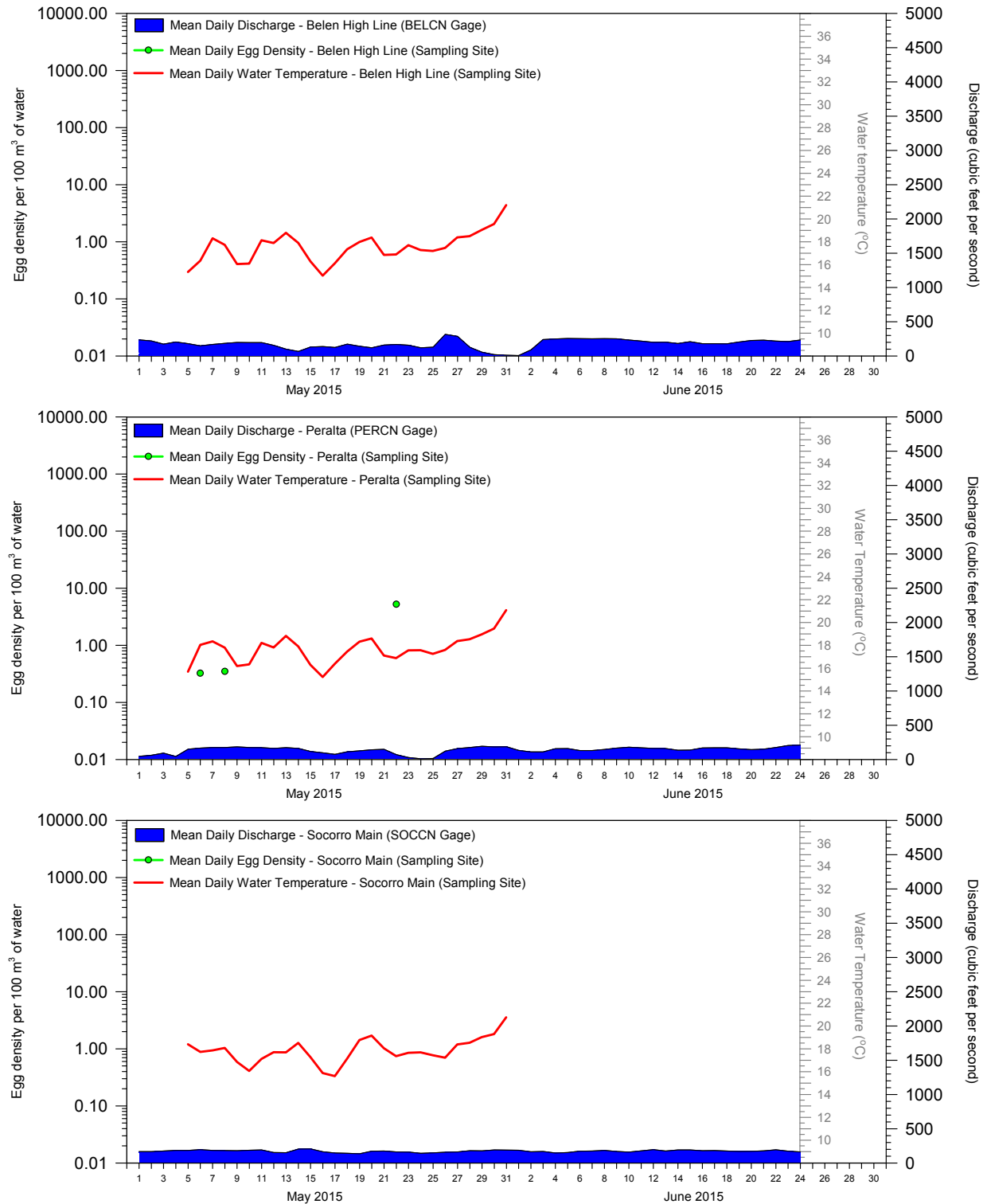


Figure 6. Mean daily discharge, mean daily egg density, and mean daily water temperature during the 2015 Rio Grande Silvery Minnow spawning periodicity study period at the canal-monitoring sites. Note that the Y-axis for egg density is a log-scale.

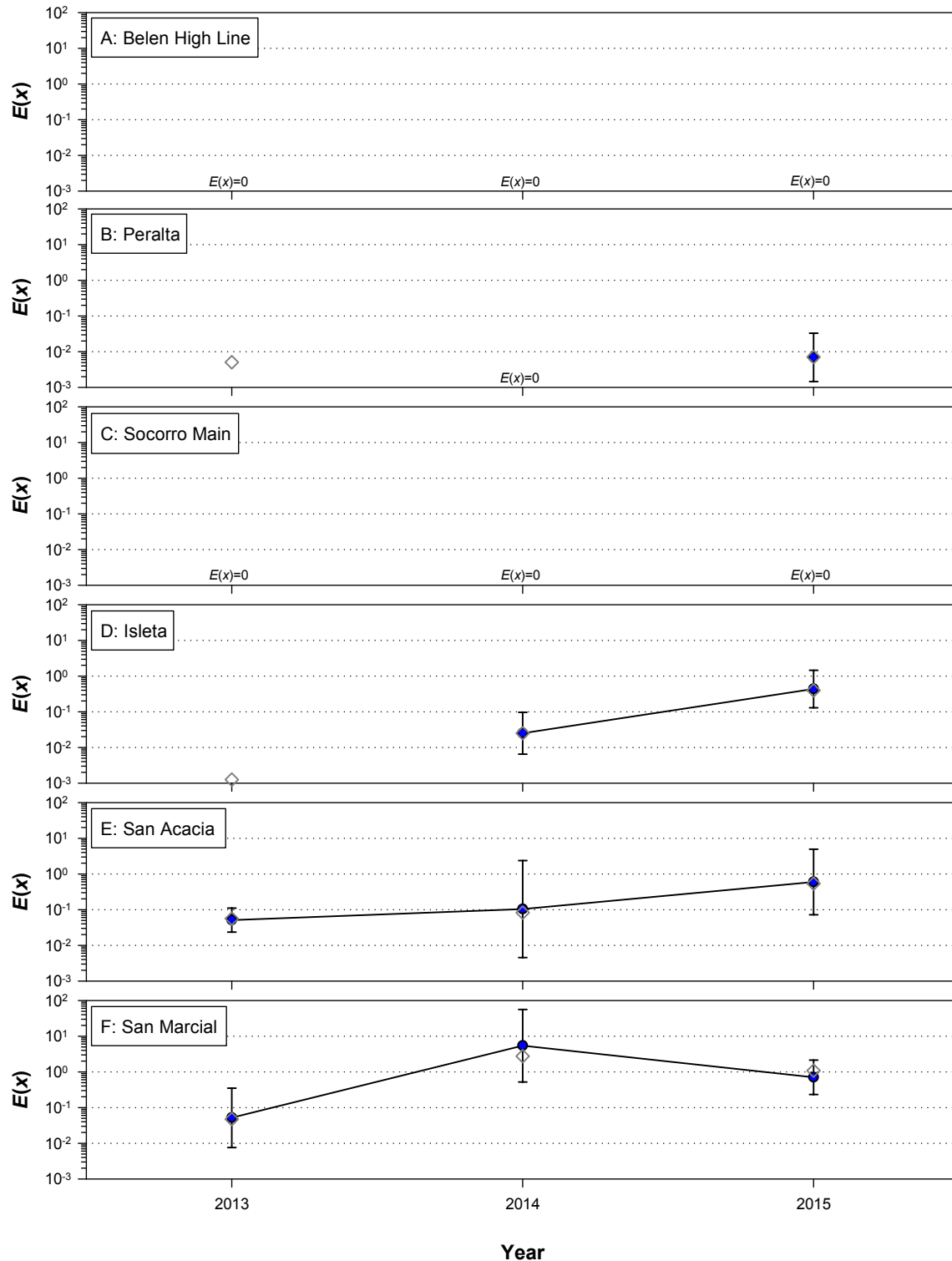


Figure 7. Rio Grande Silvery Minnow mixture-model estimates ($E(x)$), using egg passage rate data (E_p) at all sampling sites (2013–2015). Solid circles indicate modeled estimates and bars represent 95% confidence intervals. Dotted lines represent different orders of magnitude. Gray diamonds indicate simple estimates of mean densities using the method of moments.

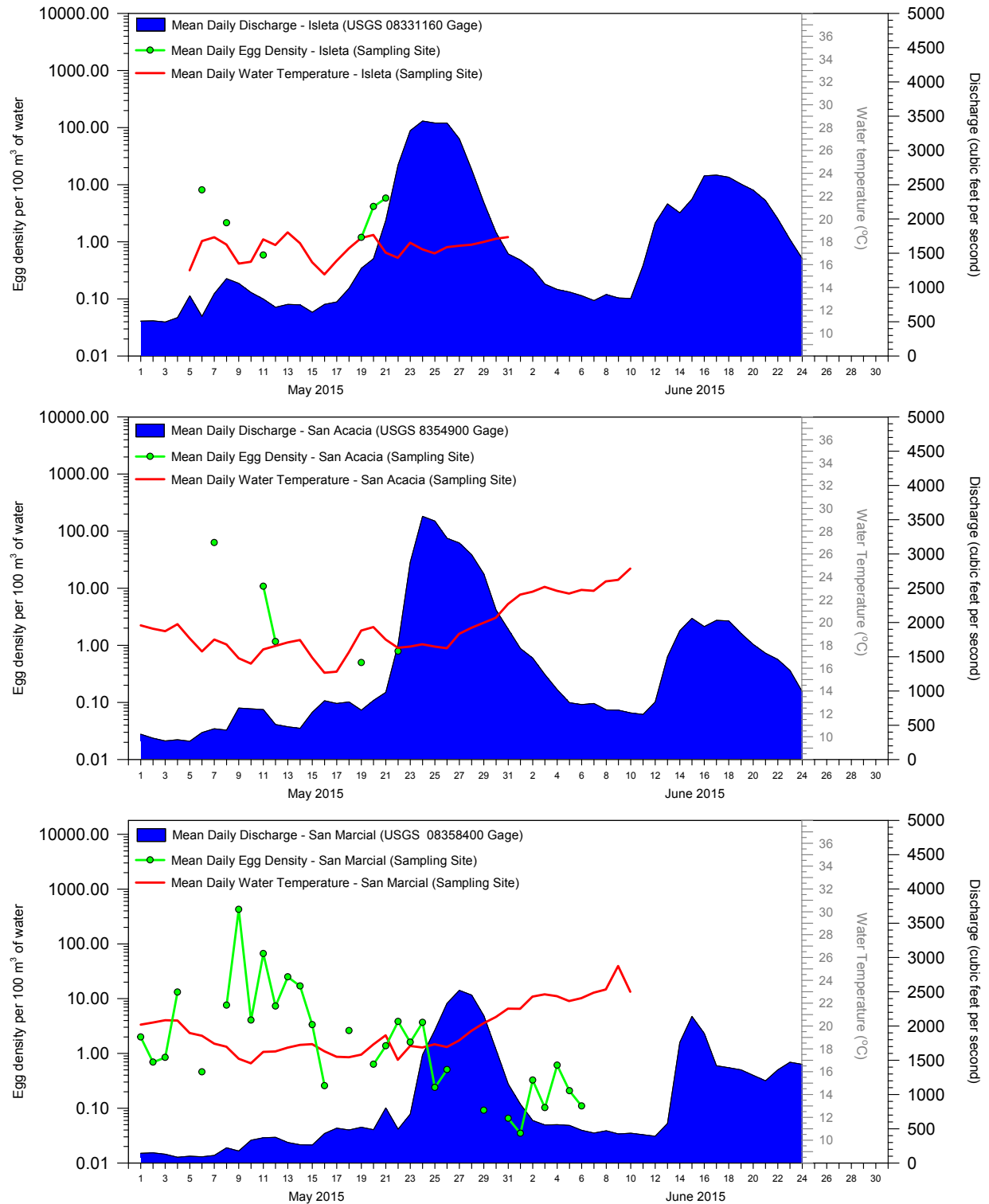


Figure 8. Mean daily discharge, mean daily egg density, and mean daily water temperature during the 2015 Rio Grande Silvery Minnow spawning periodicity study period at the river-monitoring sites. Note that the Y-axis for egg density is a log-scale.

From 1 May to 24 June, flows at the Belen Site ranged from 8 to 317 cfs (mean = 178.5 cfs). Flows at the Peralta Site were slightly lower and ranged from 10 to 210 cfs (mean = 138.3 cfs). The operating schedule of the two canal-monitoring sites periodically resulted in elevated flows at one site for an extended period with reduced flows at the other site. In contrast, flows at the Isleta Site during the same period were higher and more variable (range = 497 to 3,430 cfs; mean = 1,566.6 cfs) as compared with either of the two canal-monitoring sites. The estimated proportion of water diverted in the canals (Q_{DC}) at this longitudinal location was 0.16.

Sampling at the Socorro canal-monitoring site was longitudinally comparable to sampling at the San Acacia river-monitoring site. Water temperatures were similar between these two sites (range $\approx 16^{\circ}\text{C}$ to 22°C); the difference in mean daily temperatures was generally $< 0.5^{\circ}\text{C}$ and rarely exceeded 1°C . While there were no eggs collected at the Socorro Site, there were 135 eggs collected at the San Acacia Site. The estimated egg passage rate at the San Acacia Site ($E(x) = 0.60$) was significantly higher ($P < 0.05$) than at the Socorro Site ($E(x) = 0.00$), and the estimated proportion of eggs entrained into the canal ($E(x)_{EC}$) was 0.0.

From 1 May to 24 June, flows at the Socorro Site ranged from 135 to 207 cfs (mean = 171.9 cfs). However, no water at the Socorro Site originated from the diversion of flow at San Acacia Diversion Dam during the May study period. In contrast, flows at the San Acacia Site were higher and more variable (range = 266 to 3,550 cfs; mean = 1,302.1 cfs) as compared with the Socorro Site. The estimated proportion of water diverted into the canal (Q_{DC}) at San Acacia Diversion Dam was 0.0, and the estimated proportion of water in the canal (as compared with the river at this longitudinal location) was 0.12.

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 propagules 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 are annually transported past downstream collecting sites (Dudley and Platania, 2014). 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 of 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 output. The closure of Cochiti Dam resulted in a greatly reduced passage of fine sediments through the Middle Rio Grande that 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.

Spawning of Rio Grande Silvery Minnow and other members in its reproductive guild is triggered by specific environmental cues (Platania and Altenbach, 1998). These fishes often spawned shortly after increases in flow during spring and summer months. Egg densities for fish species in this reproductive guild in the Pecos River and Rio Grande may be more related to flow increases than 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 densities 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.

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. It is possible that Rio Grande Silvery Minnow are spawning as a result of some combination of these changing habitat and water chemistry conditions during 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 relatively wide range of mean daily water temperatures (ca. 13 to 26°C), but the majority occurs over a much narrower range of temperatures (ca. 17 to 23°C). This interaction, however, is 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. Prior to spawning, the gonadosomatic index (GSI) values of Rio Grande Silvery Minnow increase during early spring (Platania and Altenbach, 1996). The GSI value is the ratio of gonad weight to body weight and higher GSI values indicate an increased readiness to spawn. Field collections (1993–

1995) indicated that the increase in GSI values generally corresponded with the gradually increasing water temperatures of the river (Platania and Altenbach, 1996).

It is possible that the typical 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).

Multiple factors appear to affect the 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). Additionally, it is unknown if reproductive success varies among individuals according to the spawning strategy employed within a single season (i.e., single spawning vs. multiple spawning). In fact, the broad range of conditions that result in Rio Grande Silvery Minnow reproduction could indicate that there is no ideal spawning cue (i.e., combination of abiotic/biotic conditions) that would result in a consistently higher chance of early survival and recruitment. 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. Flows will sometimes 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 (Platania, 2000). 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 top general linear models (after the global model [$\delta(\text{Year}) \mu(\text{Year})$]) based on the long-term spawning data (2003–2015) indicated that spawning intensity increased with increasing spring discharge. While these spring discharge associations were relatively weak, they were more informative than models that included either the previous year's October density data or the previous year's flow during irrigation season. The physical conditions produced by prolonged and elevated flows during spring 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). Similar processes are likely 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 number of eggs collected, volume of water sampled, mean daily discharge, 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 the sampling period of a given day approximately represented the rate at which eggs were transported downstream of the site during that day, and 3) the discharge at the nearest upstream USGS station approximately represented the discharge at the sampling site. While these assumptions seem

reasonable, some non-quantified error was likely introduced into the calculations through these extrapolations. For example, the use of multiple MECs may more accurately characterize possible spatial differences in the densities of drifting fish eggs across the river channel (Worthington et al., 2013a; b). However, the number of eggs estimated to be transported downstream during peak spawning events would likely still be quite high even with modest violations of these assumptions. These results indicate a substantial downstream transport of drifting eggs at the San Marcial Site despite the seemingly modest numbers of eggs collected in individual MECs. It is unknown, however, what proportion of these eggs was viable since that was not a study objective.

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 was used for nearly all sampling days during the spawning season across years. 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 after 2002, 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 magnitude of spawning over time. 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 inaccurate egg counts (Dudley and Platania, 2011).

Since Rio Grande Silvery Minnow is the only extant species of the reproductive guild of pelagic spawners 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.

The total number of Rio Grande Silvery Minnow 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 densities among MECs and different sequential periods in a single day was minimal compared to the variation among days. The primary purpose in sampling with two MECs over an extended time period at the San Marcial Site was to filter an adequate volume of water to both detect the presence of eggs and to obtain an accurate estimate of the intensity of spawning over time.

Our long-term spawning study results indicate that substantial numbers of Rio Grande Silvery Minnow eggs, and presumably larvae, are being transported downstream every year. Additionally, the highest densities of eggs consistently occur in the San Acacia Reach. 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 following annual spawning (Dudley et al., 2015). 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 exceptionally low in the San Acacia Reach, resulting in substantial drying and loss of fish in those areas.

Despite the seemingly large number of Rio Grande Silvery Minnow propagules transported downstream every year, some portion does remain upstream (Dudley and Platania, 2007; Widmer et al., 2013). 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. The availability of floodplain habitat could be particularly important, as these areas are likely locations for the reduced transport rate and increased retention of drifting fish eggs (Dudley and Platania, 2007; Medley

and Shirey, 2013; Widmer et al., 2013; Gonzales et al., 2014). 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.

In support of this hypothesis, prolonged high flows during spring were most predictive of increased density of Rio Grande Silvery Minnow during fall based on data collected since 1993 (Dudley et al., 2015). In contrast, results from our long-term spawning study suggest that the density of eggs produced in the river during spring does not appear to be strongly related to the density of Rio Grande Silvery Minnow from the previous October. The apparent lack of a relationship between the densities of eggs and fish might not be particularly surprising, however, given the large number of unknown variables associated with collecting drifting fish eggs. For example, the number of Rio Grande Silvery Minnow eggs collected at the San Marcial sampling site is likely a result of the complex interrelationships among the spatial distribution of individuals upstream, egg development time (i.e., faster at higher water temperatures), and local egg transport efficiencies (e.g., decreased habitat complexity will generally result in increased egg transport to downstream areas (Dudley and Platania, 2007; Widmer et al., 2013)). It is yet unclear, however, whether the density of eggs tends to decline after multiple years with poor recruitment, as some time periods appear to support this observation (e.g., 2003–2004 and 2012–2013) while others do not (e.g., 2006–2007 and 2013–2014). The continuation of this long-term ecological study should further elucidate these subtle relationships and lend additional insight to the causal mechanisms resulting in the successful long-term recruitment of Rio Grande Silvery Minnow.

Sampling from multiple canal and river locations in the Isleta and San Acacia reaches provided additional insight to spatial spawning patterns during 2015. The egg passage rate at the San Marcial Site in 2015 indicated a marked increase in spawning intensity as compared with 2013. While the low numbers of eggs collected in 2015 precluded statistical analyses of spatial spawning patterns for several of the sampling sites, it was apparent that egg passage rates were lower in the Isleta Reach as compared with the San Acacia Reach. While flows were notably lower at the canal-monitoring sites as compared with the river-monitoring sites, there was no appreciable difference in water temperature between canal and river sites. There were, however, differences in the estimated egg passage rates between the Socorro (canal) and San Acacia (river) sites, which appear to be driven by the source of the canal water. Specifically, the absence of water being diverted at San Acacia Diversion Dam during river spawning events helped to minimize the entrainment of eggs into the San Acacia Reach irrigation network during 2015. In contrast, the relatively low numbers of eggs collected from the Isleta Reach river location indicate that the near absence of eggs from the Isleta Reach canal locations should be interpreted cautiously.

While Rio Grande Silvery Minnow has declined markedly throughout its range since 2009, the modest spring runoffs of 2014 and 2015 resulted in increased spawning intensity as compared with 2013. However, the recruitment success of this species during summer and fall of 2015 will likely be highly dependent on local flow conditions during spring and summer, respectively. The loss of individuals from downstream reaches during river drying events is particularly pertinent as these areas consistently support the highest occurrence and density levels of Rio Grande Silvery Minnow. The future conservation status of Rio Grande Silvery Minnow appears strongly dependent on consistently ensuring appropriate seasonal flow and habitat conditions to support the crucial spawning and early recruitment phases of this species.

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APPENDIX A (Historical Hydrographs)

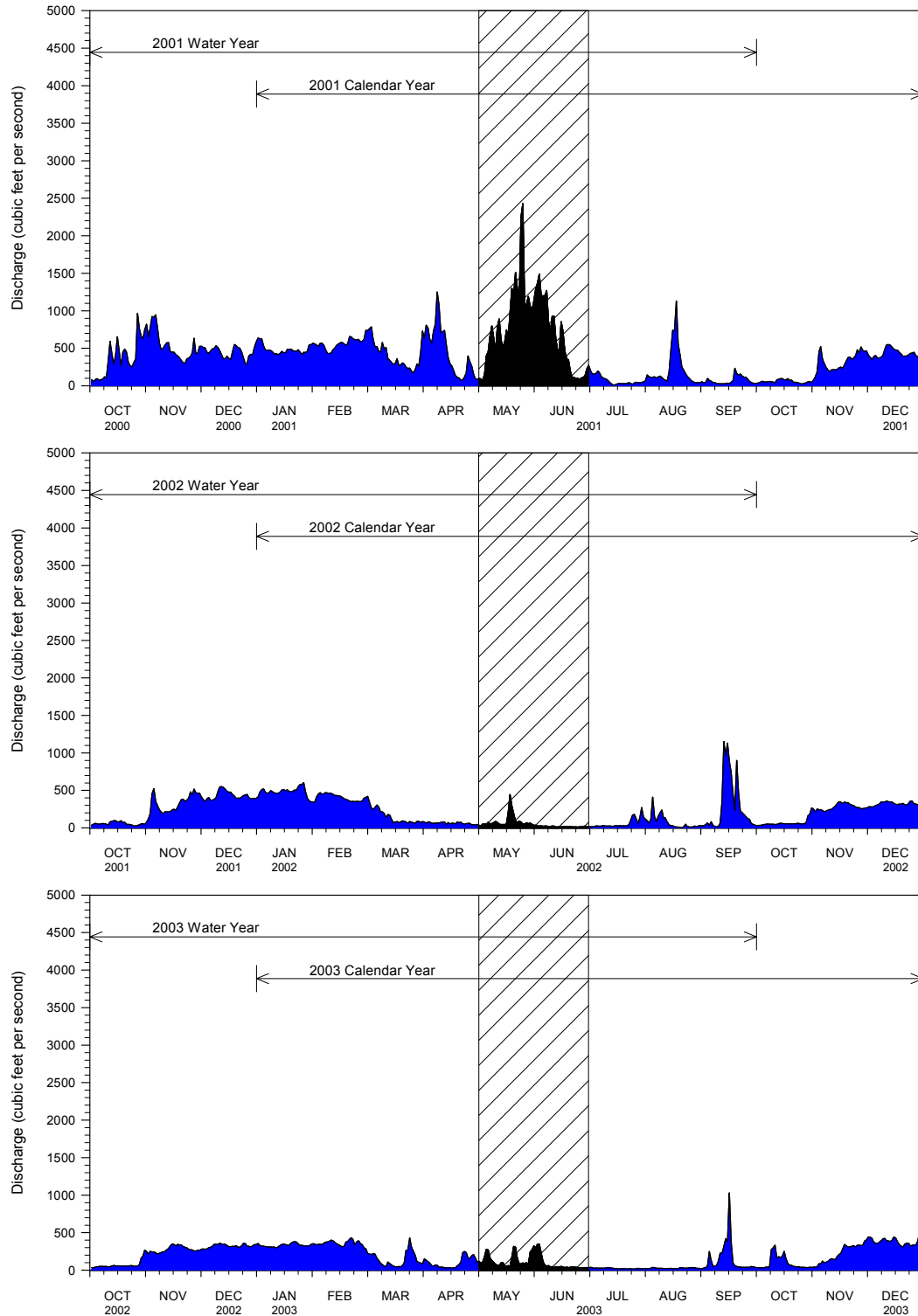


Figure A - 1. Annual hydrographs of the Rio Grande, New Mexico, at the San Marcial Gaging Station before, during, and after the 2001–2003 Rio Grande Silvery Minnow spawning periodicity study periods. Cross-hatching indicates annual study periods.

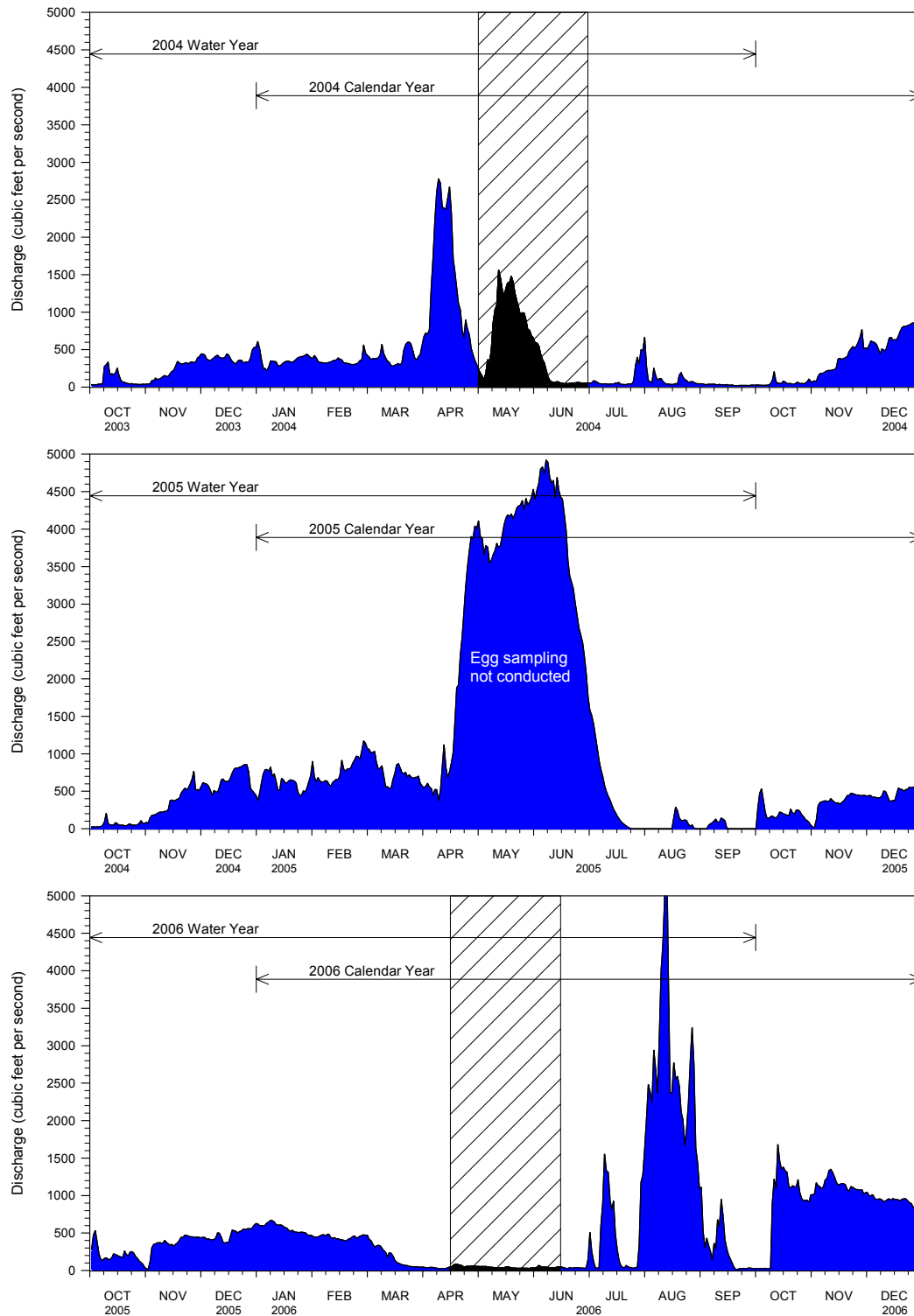


Figure A - 2. Annual hydrographs of the Rio Grande, New Mexico, at the San Marcial Gaging Station before, during, and after the 2004–2006 Rio Grande Silvery Minnow spawning periodicity study periods. Cross-hatching indicates annual study periods. Sampling was not conducted in 2005.

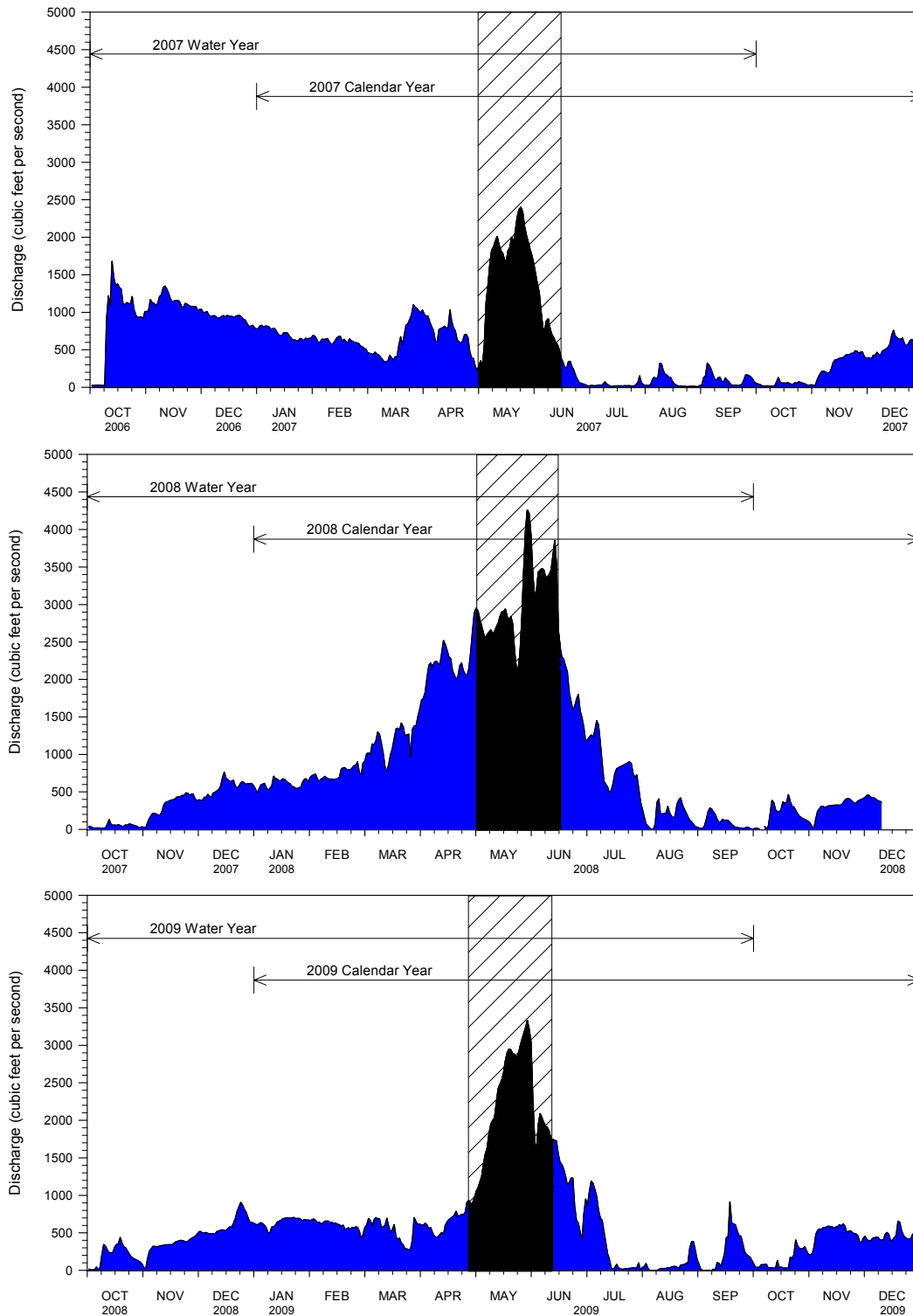


Figure A - 3. Annual hydrographs of the Rio Grande, New Mexico, at the San Marcial Gaging Station before, during, and after the 2007–2009 Rio Grande Silvery Minnow spawning periodicity study periods. Cross-hatching indicates annual study periods.

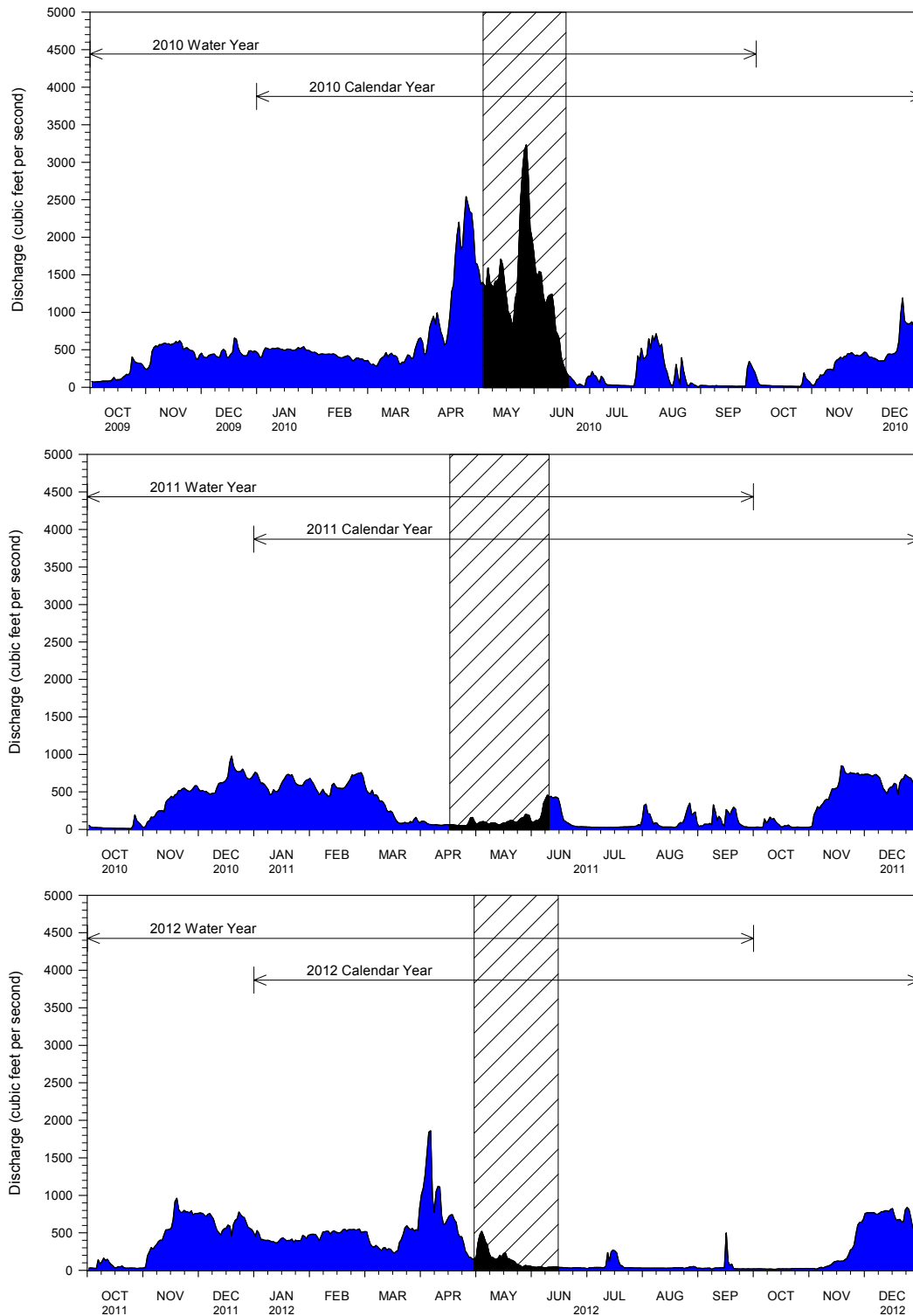


Figure A - 4. Annual hydrographs of the Rio Grande, New Mexico, at the San Marcial Gaging Station before, during, and after the 2010–2012 Rio Grande Silvery Minnow spawning periodicity study periods. Cross-hatching indicates annual study periods.

APPENDIX B (Historical Spawning Periodicity Relationships)

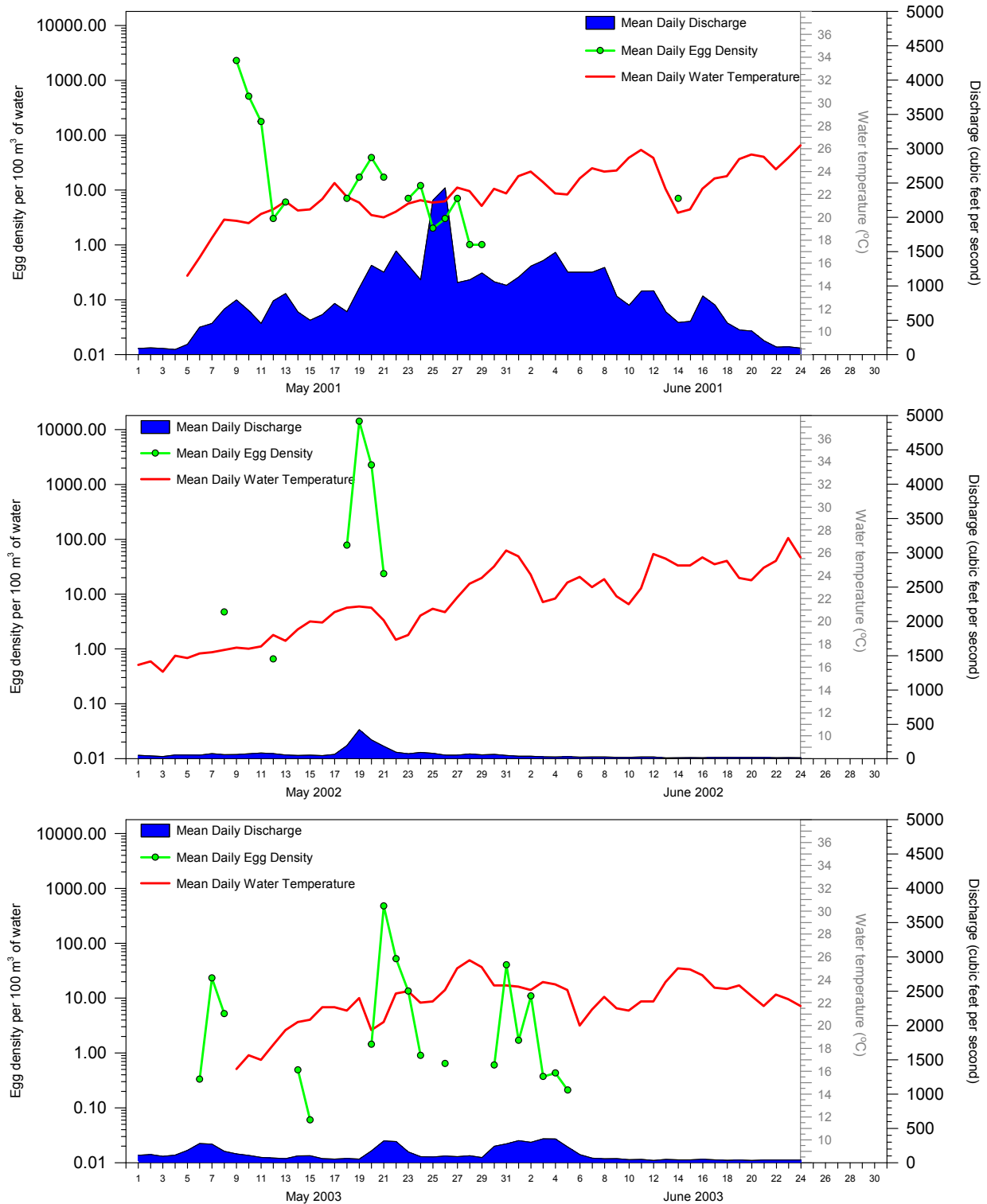


Figure B - 1. Mean daily discharge, mean daily egg density, and mean daily water temperature during the 2001–2003 Rio Grande Silvery Minnow spawning periodicity study periods at the San Marcial Site. Note that the Y-axis for egg density is a log-scale.

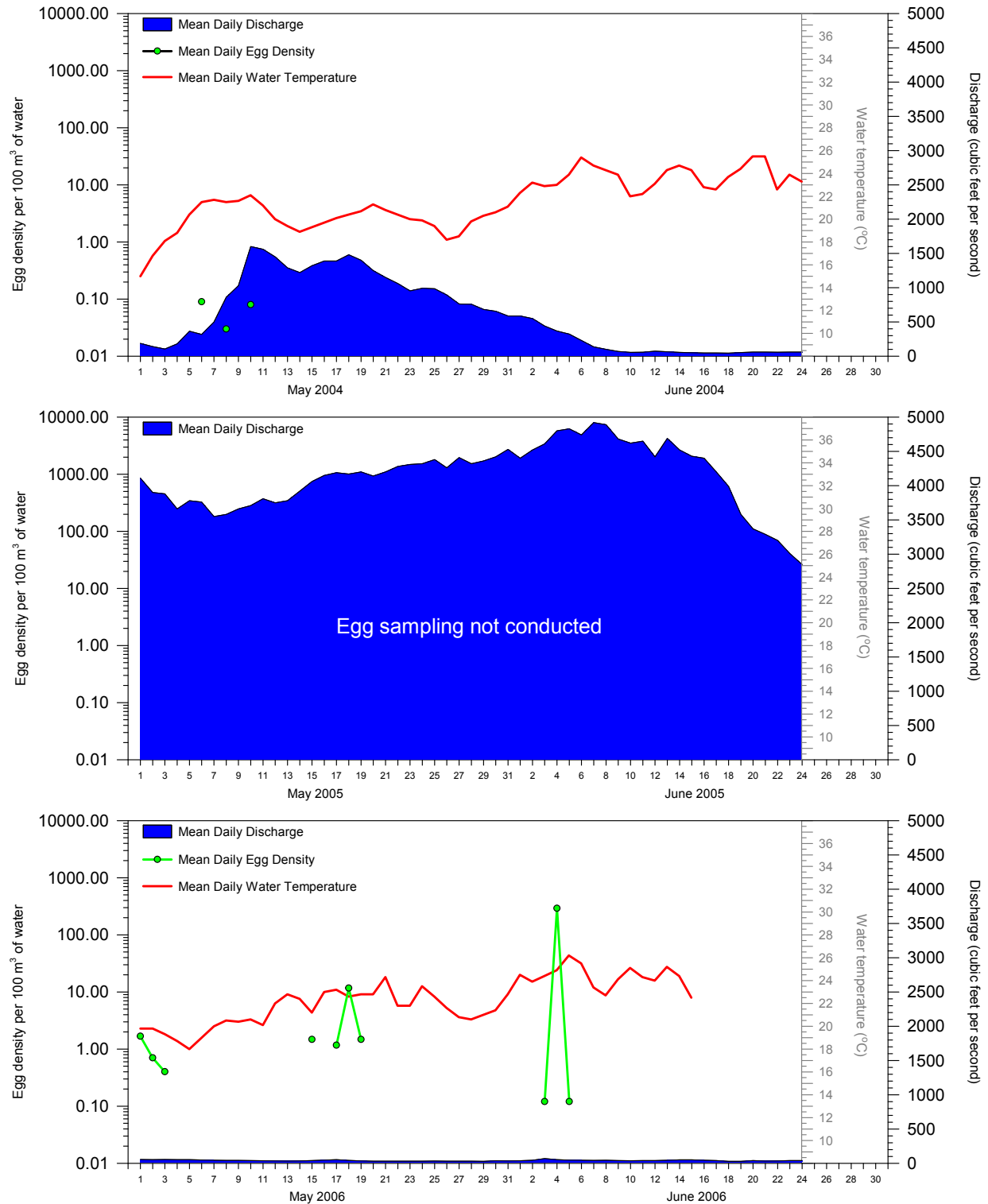


Figure B - 2. Mean daily discharge, mean daily egg density, and mean daily water temperature during the 2004–2006 Rio Grande Silvery Minnow spawning periodicity study periods at the San Marcial Site. Note that the Y-axis for egg density is a log-scale.

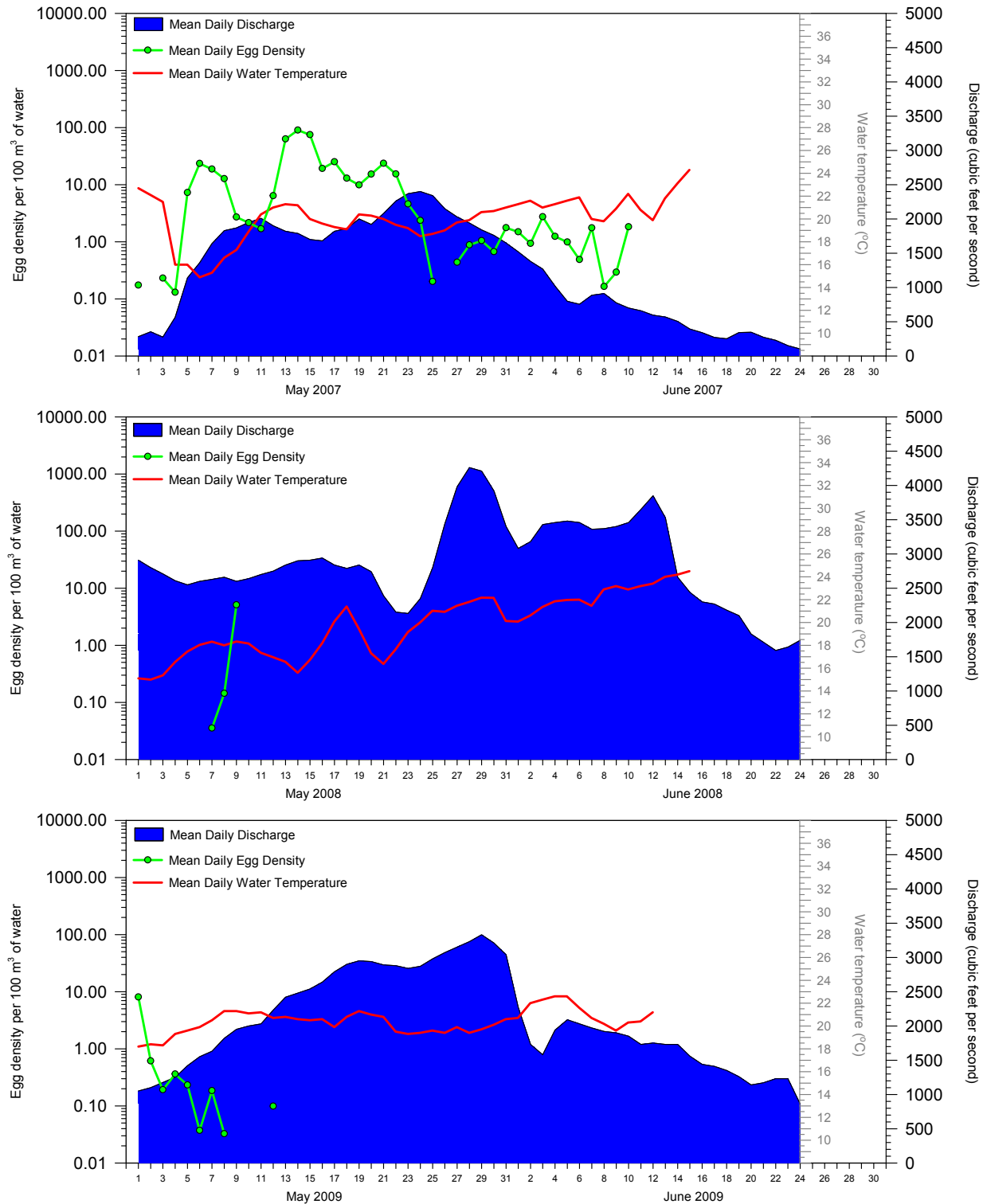


Figure B - 3. Mean daily discharge, mean daily egg density, and mean daily water temperature during the 2007–2009 Rio Grande Silvery Minnow spawning periodicity study periods at the San Marcial Site. Note that the Y-axis for egg density is a log-scale.

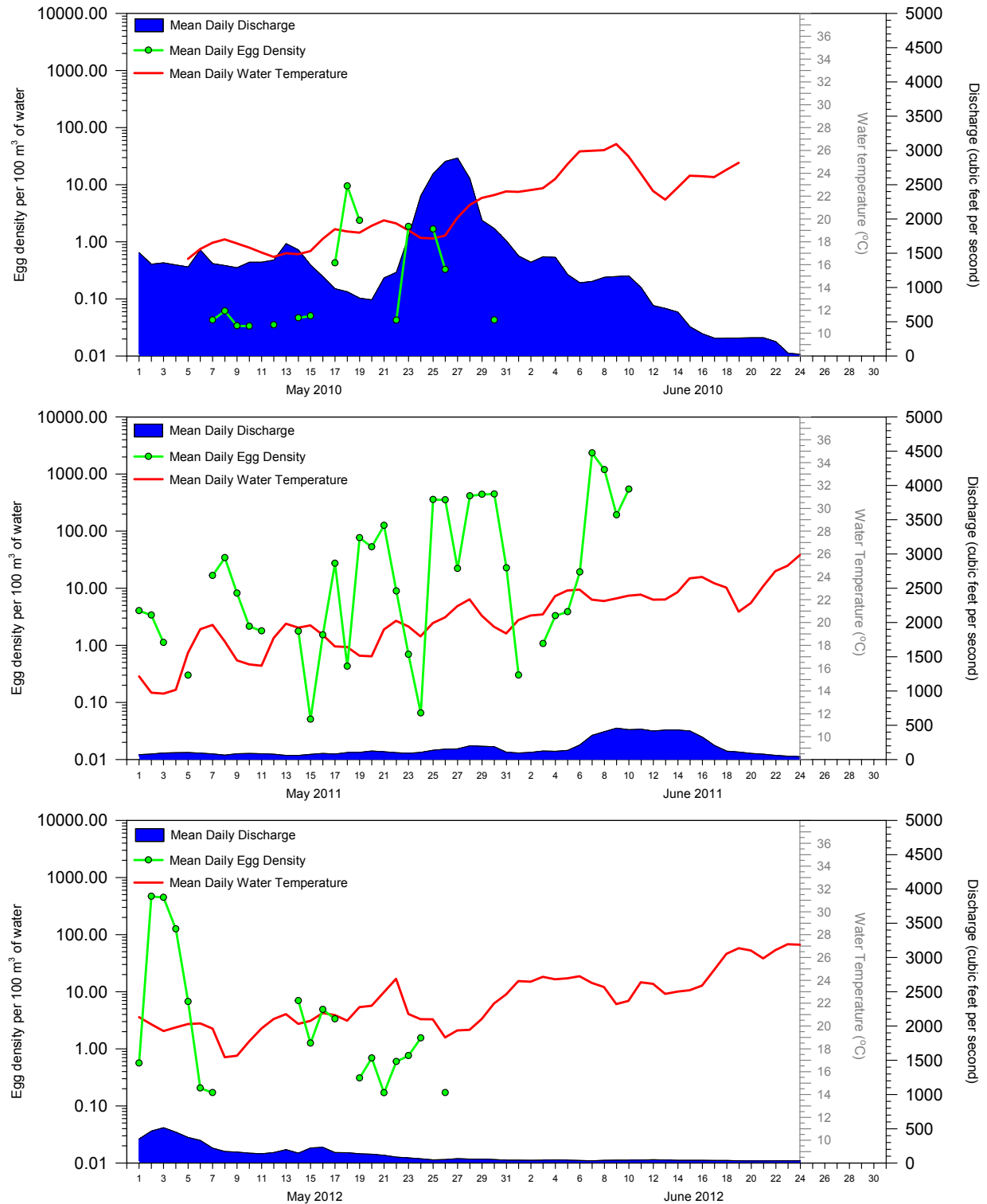


Figure B - 4. Mean daily discharge, mean daily egg density, and mean daily water temperature during the 2010–2012 Rio Grande Silvery Minnow spawning periodicity study periods at the San Marcial Site. Note that the Y-axis for egg density is a log-scale.